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PLASTIC BASED MICROFLUIDIC SYSTEMS AND
THEIR APPLICATIONS IN BIOLOGY

by

Palaniappan Sethu

A dissertation submitted in partial fulfillment
Of the requirements for the degree of
Doctor of Philosophy
(Biomedical Engineering)
in The University of Michigan
2002

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This thesis is dedicated to my parents
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1.1 Introduction

Microfabrication has been widely used over the past 20-30 years to create sensors, actuators and various other structures and devices commonly referred to as microelectromechanical systems (MEMS). Micromachining is the term that describes the processes involved in the fabrication of MEMS and takes advantage of established semiconductor fabrication processes used to fabricate integrated circuits. In many cases miniaturization can confer various advantages due to scaling down, like increased surface area to volume ratio, small volumes, high throughput, high speed and batch fabrication. These advantages may not be possible with macro scale devices. This creates opportunities to fabricate devices for a wide range of applications that exploit different aspects of scaling down to the micrometer scale.

The first application of microfabrication to the fields of biology and medicine was in the 1970s when Stephen Terry miniaturized a gas chromatography system on a silicon wafer [1.1]. But only in the 1990s after a conceptual paper on microfabricated analysis systems by Andreas Manz [1.2] did various research groups and companies begin research towards the development of integrated systems for high throughput biochemical analysis, which have led to a truly exponential growth in the field. Over the past few years various applications of miniaturization in life sciences for different areas like drug discovery in the pharmaceutical industry, molecular recognition in clinical diagnostics, cell culture and manipulation for cellular and tissue engineering and drug discovery have radically changed the way in which information is processed and experiments are performed.
Microfabricated devices for biology and medicine can be classified into three main categories based on their applications. (a) Tools for molecular biology which include microarrays and devices for capillary electrophoresis, polymerase chain reaction (PCR), reactions such as restriction digest, sequencing and detection. (b) Tools for cell biology and tissue engineering which include devices for cell patterning and culture, cell sorting, flow channels and chambers for studying cell adhesion, attachment, mechanics, dynamics and (c) Tools for chemical and biological sensors which include sensors for detecting various chemical and biological agents (glucose, pH, oxygen etc.), electrodes and probes for neural prosthesis. Of the three main categories the first two almost exclusively involve manipulation of fluids to achieve the final goal and are performed on devices, which are commonly known as microfluidic systems.

1.2 Microfluidic Systems

Microfluidics involves precise manipulation and control of fluids in micron size devices to perform a wide range of functions. The devices contain structural elements like channels and reservoirs for fluid transport and storage and control elements like valves, pumps and mixers, for manipulation of fluids within the system to perform functions like reactions, separations, isolation, detection, growth and culture. Additional components like heaters, electrodes, detectors and recording probes, can be integrated with the structural and control elements to enable the device to perform complex and multiple functions on a single chip. These devices offer several advantages over conventional macro sized systems, which include compact size, disposability, increased functionality, and they require smaller volumes of reagents and samples.

1.3 Why Microfabrication?

1.3.1 Device size

Shrinking the size of a device itself can be reason enough to minaturize. Size effects can have either enhancing or enabling characteristics. In techniques like dielectrophoresis and electrorotation, nonuniform electric fields, applied by electrodes, are used to generate forces that can manipulate cells or large molecules. The forces
generated scale with the gradient of the square of the electric field intensity. Miniaturizing the spaces between driving electrodes increases these gradients, and thus the forces, while simultaneously reducing the driving voltages necessary to generate them. This enhances the devices, allowing them to operate with a few volts instead of a few hundred volts.

The second class of size advantages is in enabling something that could not be done before. Small devices are portable and can be placed in constrained spaces (Lab-on-a-chip). Systems for point-of-care use, such as in the physician's office or the field (instead of in a centralized lab) are in high demand. Such systems need to either be hand-held or fit onto a small tabletop. Microfabricating key elements, which reduces system size, enables the application. One example is microfabricated flow cytometers, which use microfabrication to make miniature flow columns and chambers. Although it will be difficult for these devices to compete with the power and versatility of conventional flow cytometers, their portability may be useful for point-of-care hematological tests.

1.3.2 High surface-area-to-volume ratio

As devices are miniaturized, their surface area relative to their volume increases. At small enough scales, this leads to a situation where surface effects dominate volume effects. Remarkable physical enhancements result. One benefit exploited by electrophoretic channels, polymerase chain reaction chambers, and dielectrophoresis and electrorotation devices is that heat removal is enhanced as the device is miniaturized. For both dielectrophoresis and electro-rotation devices and electrophoretic channels, this means that higher electric fields than in conventional systems can be used without adverse heating effects. This gives faster and better separations for electrophoretic channels and larger forces for the dielectrophoresis and electrorotation devices. For polymerase chain reaction chambers, the high heat removal decreases thermal response times, allowing for more rapid temperature cycling. The most notorious disadvantage of increased surface-area-to-volume ratio is that surface adsorption of biomolecules increases, lowering yields. There is ongoing research into this problem [1.12].
1.3.3 Integrated electronics

The close relationship between microfabrication and conventional semiconductor fabrication allows one to integrate electronics or electrical components with microfabricated systems. The challenge lies in establishing mutually acceptable process steps to achieve this integration. One simple level of integration is fabricating piezoresistors in silicon. Piezo-resistors transduce mechanical stress into electrical resistance changes. Some blood pressure sensors integrate these onto pressure-sensitive diaphragms. Upon deflection by an applied pressure, the piezoresistors change resistance.

Thus, the electronic devices enable the integration of the transduction element (the piezoresistor) with the mechanical element (the diaphragm). Higher levels of integration include fabricating an integrated circuit with the microfabricated device. This is used by some probe-style electrode arrays that record signals from neurons in intact cortical tissue [1.11, 1.16]. These devices consist of miniature silicon probes with integrated electrodes. They are inserted into tissue and record extracellular signals from neurons. The integrated circuits generate stimulus currents, amplify recorded signals, and process the data to reduce the number of electrical leads needed.

Integration of electronics with silicon is far easier than on other materials primarily due to the excellent properties of silicon a semiconductor material. Flip chip technologies allow for the electronics to be mass fabricated on silicon and then integrated with the structural elements, which can be fabricated on cheaper materials like plastics, glass and ceramics.

1.3.4 High throughput

In many cases miniaturization can lead to high-throughput devices by either parallel or enhanced serial transfer. This is common in systems for genomic research and drug discovery. A rate-limiting step for researchers in these fields is throughput. Miniaturization of channels for capillary electrophoresis not only means faster analysis time because of the gain in surface area to volume but also the ability to array many capillaries in a small space and operate them in parallel. Both of these effects increase
Chapter I: Microfabrication and its advantages in Biology

throughput. Microfabricated nucleic acid arrays are another example. Nucleic acid arrays essentially perform a Southern or Northern blot at each active site, and so constructing arrays with tens of thousands of sites allows for that many simultaneous assays. Thus, these arrays can be used to probe the expression of many genes simultaneously or to look for mutations at many places in a genome. This results in extremely high speed devices in which the analysis time is limited only by the reactions taking place (enzyme turnover rate).

1.3.5 Small sample volumes

Decreasing the volume of sample consumed in an assay can be beneficial for several reasons. From a financial standpoint, reducing reagent volumes and waste disposal by a large factor can reduce assay costs. In addition, for drug discovery applications or some medical diagnostics, the sample materials are scarce. Reducing the needed volume can thus extend the use of each sample. Systems for monitoring cell dynamics often make use of small sample volumes. The Cytosensor Microphysiometer uses a microfabricated planar pH sensor to sense the extracellular acidification rate as a means of monitoring cell physiology [1.10]. The pH sensor can be used in a small volume, allowing it to be placed at the bottom of a small cell-culture chamber. Operating in such a small volume effectively increases the volume cell density. This increases the rate of acidification, easing detection. In this case, the small volume decreases detection requirements, enabling the system. One disadvantage of small volumes is that the detection of molecules in dilute solutions becomes more difficult. This is because, for a given solute concentration, the number of molecules scales as the cube of the volume. At small enough volumes, the number of molecules may approach detection limits.

1.3.6 Mass fabrication

Many microfabrication processes can be performed as easily on one device as on a thousand. Such batch processing can make thousands of identical devices not subject to the variations present in individually constructed objects. This is exploited by planar electrode arrays that record from and stimulate neurons in culture [1.13, 1.14]. After one electrode is made, it is only incrementally more difficult to make a large array of them.
One can then record from and stimulate many neurons. Contrasting this with conventional intracellular electrodes, where the use of multiple electrodes becomes logistically difficult. Another application that well illustrates the advantages of batch processing is nucleic acid arrays fabricated by photolithography. For an \( n \times n \) nucleic acid array, there are \( n^2 \) different oligonucleotides of length 1. Synthesizing each oligonucleotide individually would require \( n^2 / I \) chemical steps. Fodor et. al.’s method uses selectively masked photochemistry to synthesize the oligonucleotides [1.8]. It requires four chemical steps (one for each base) per unit length, or 41 steps irrespective of the number of different oligonucleotides. Thus, one can make a 4 x 4 array of octamers as easily as a 200 x 200 array. This dramatically decreases the difficulty of making large arrays.

1.3.7 Precise geometry

Geometrical control can be very important for microstructures. Photolithography allows one to pattern largely varying geometries (1 \( \mu \)m to 1 cm) in the same space with micrometer dimensional accuracy. In addition, one can vary dimensions of the same feature on a mask, instantly making tens of different but similar structures. A creative use of this concept is by Bhatia et. al. to investigate cell–cell interactions in cocultures [1.3]. Using microfabrication, they precisely control the spatial organization of hepatocytes and fibroblasts. This made it possible to eliminate variations present in random cocultures, such as amount of heterotypic interface between the two cells types, amount of homotypic inter-face, and hepatocyte : fibroblast ratio. They found that liver-specific function (as measured by albumin and urea synthesis) is dependent on the amount of heterotypic interface in the coculture and albumin production is localized to hepatocytes at this interface. Such a study would be impossible to perform without microfabrication.

1.3.8 Constrained geometries

Often all one needs is a small-constrained geometry, such as a small well. Constrained geometries can be used to confine either molecules or mechanical forces. Confining molecules prevents diffusion out of a volume, increasing a molecule’s local concentration. This is cleverly exploited by applications involving electrochemical or
optical probing of cells in small wells [1.4-1.6]. Here the microfabricated wells allow the analyte being probed to remain concentrated, instead of being diluted into a large solution volume. The advantages of confining forces are well illustrated by the work of several investigators examining the assembly of microtubules in microfabricated structures [1.7, 1.9]. Using cell-sized chambers microfabricated in glass coverslips, Holy et al examined the assembly of microtubules from artificial microtubule organizing centers consisting of tubulin-covered beads [1.9]. In these constrained geometries, results showed that microtubule polymerization alone could position the artificial microtubule-organizing centers in the middle of the well, suggesting that these forces are important when considering microtubule dynamics. Another study used shallow channels with microtubules attached to the bottom surface [1.7]. By looking at microtubule bending as it polymerized and hit the wall of the channel, they could determine its force-velocity relationship. Both of these experiments would not work in free solution; microfabricated constrained geometries enable the experiments. It is worth noting that sample evaporation can be a problem when using small wells, picoliters of fluid can evaporate in seconds. Special precautions are needed to avoid this [1.5].

1.3.9 Single-cell analysis

Shrinking devices can enable single-cell analysis for any of the aforementioned reasons (e.g. constrained geometries). The power of this lies in the heterogeneity of cell populations, which bulk measurements cannot discern. Analyzing multiple single cells can reveal the variations within populations. This can be illustrated by a device that is for measuring erythrocyte mechanics [1.15]. Microfabrication technology was used to create a device with optically accessible uniform grooves. This device, coupled with an image acquisition system, allows them to measure the volume and velocity profile of every cell (in a population) as it passes through the grooves. Thus, they can obtain individual cell data from a statistically significant population. This compares favorably to conventional methods that can measure these properties either on single cells or bulk populations but cannot measure them on many individual cells. In this case, microfabrication’s strengths (geometric control) have been used to create a device that can perform single-cell analyses.
1.4. Current state of Research in the field

Microfabrication is by itself a very broad and established field. Microfluidics is a more specific area of focus and deals with fluid manipulations for applications in the various fields of science. Our interest however is confined to the use of microfluidics to the fields of biology and biochemistry. Modern developments in the design and utilization of microfluidic devices for fluid transport have found many applications, ranging from the life sciences industries for pharmaceuticals and biomedicine (drug design, delivery and detection, diagnostic devices) to industrial applications of combinatorial synthesis (such as rapid chemical analyses and high throughput screening). In other branches of medicine, new paradigms for noninvasive diagnostics and surgery are enabled by small (possibly implanted or ingested) microdevices. As an example of the rapidly increasing demand for biomedical microdevices, the biochip market was $400M in the year 2002 and is expected to increase fivefold by 2005.

The development of mechanical structures on the nanometer length scale is another area of rapid progress. Since many of these nanofabrication processes take place in the liquid state, the understanding of “nanofluidics” is a research challenge that will likely involve contributions from continuum, statistical, and molecular mechanics. In general, the design challenge faced by the engineer is scale-down rather than the more familiar scale-up. Micro and nanodevices are useful because they allow manipulation with fast response times, they can handle small fluid volumes, sense and control flows and pattern substrates on small length scales, and, what promises to be very important, they can selectively address the cellular scale. Traditionally, silicon micromachining methods have been used to make micron-scale electrical and mechanical devices from silicon and glass. More recently, plastics and elastomeric materials have been used [1.17]. The latter systems offer potential advantages of faster design times, low cost, the ability to fabricate nanoscale features, and the possibility of deformable shapes [1.18]. Both fabrication techniques will likely continue to play important roles in future applications.

Many microfluidic devices have been developed in the past several years. These systems are rapidly being applied in the biomedical, pharmaceutical and printing
industries, to name just a few. It is natural to think that these systems will be integrated with “smart materials and devices” [1.19]. The ability to pattern substrates, implement the lab-on-a-chip concept, control and enhance chemical reactions and heat transfer, manipulate particle position, orientation and transport rates, develop mixing and separation processes, among others, will offer both research and engineering opportunities in the future and hopefully be among the successful technologies utilizing microfluidic principles and devices. The importance of scaling down devices, as well as characterizing and understanding the interplay of fluid flow, surface forces, and potentially statistical and molecular interactions, are among the research questions that will need to be addressed.

1.5. Overview of this Research Work

This research work is focused on developing plastic microfabrication techniques specifically epoxy based casting to fabrication of microfluidic platforms that can then be used for different applications in molecular and cellular biology. The second chapter is an extensive review of polymers: different types of polymers, their structures and functions are discussed. Polymer fabrication techniques particularly techniques which can and have been modified for microfabrication of polymers are also reviewed. The third chapter specifically deals with epoxies and techniques to cast them to fabricate useful microfluidic platforms and make high quality structural elements that can then be used for fabricating functional devices for various applications. Miniaturization offers the possibility to integrate multiple functions onto a single platform however integration of control elements for fluidic manipulations, detection and sensing in plastic based systems is difficult due to the material properties. Chapters 4 and 5 discuss two separate techniques for integration of control elements onto plastic based systems. The first one involves embedding active silicon micromachined devices in a plastic microsystem and the other involves surface micromachining to build from the bottom up devices that can be integrated within the system. Chapter 6 discusses the two most important biomolecules nucleic acids and proteins and also techniques for different nucleic acid and protein assays. Demonstration devices for molecular assays were designed and fabricated to perform Polymerase Chain Reaction (PCR) and Capillary electrophoresis (CE). This
chapter also explains the testing and results obtained. Chapter 7 details how microfluidics and microfabrication can be used to engineer cellular interactions with surfaces and surroundings. Cell attachment is critical to the normal functioning of the cell and requires Extra Cellular Matrix (ECM) proteins for proper attachment. An electrochemical deposition technique for patterning conductive biomolecules and proteins is explained. Laminar flows in channels are used to precisely control the dimensions of the deposited protein. Chapter 8 is a brief summary and suggestions for future work.
CHAPTER 2
Polymers

2.1 Introduction

Polymers are macromolecular substances composed of greater than 1000 monomeric units with molecular masses between 10000 to 100000 Da. A typical polymer may include tens of thousands of monomers. Because of their large size, polymers are classified as macromolecules. Unmatched in the diversity of their properties, polymers such as cotton, wool, rubber, teflon, and all plastics are used in nearly every industry. Natural and synthetic polymers can be produced with a wide range of stiffness, strength, heat resistance, density, and even price. The following section provides an introduction to the science of macromolecules and discusses techniques that can be used to fabricate parts with micro features using these polymers.

2.2 Polymer Synthesis

2.2.1 Addition Polymerization

The most common type of addition polymerization is free radical polymerization. A free radical is a molecule with an unpaired electron. The tendency for this free radical to gain an additional electron in order to form a pair makes it highly reactive so that it breaks the bond on another molecule by stealing an electron, leaving that molecule with an unpaired electron (which is another free radical). Free radicals are often created by the division of a molecule (known as an initiator) into two fragments along a single bond. The following diagram shows the formation of a radical from its initiator, in this case benzoyl peroxide.
Chapter 2: Polymers

Free radical formation

\[
\text{Location at which bond is broken} \quad \text{Active center (Free Radical)}
\]

Figure 2.1: Reaction shows the creation of an active center by breaking of a bond.

The stability of a radical refers to the molecule's tendency to react with other compounds. An unstable radical will readily combine with many different molecules. However a stable radical will not easily interact with other chemical substances. The stability of free radicals can vary widely depending on the properties of the molecule. The active center is the location of the unpaired electron on the radical because this is where the reaction takes place. In free radical polymerization, the radical attacks one monomer, and the electron migrates to another part of the molecule. This newly formed radical attacks another monomer and the process is repeated. Thus the active center moves down the chain as the polymerization occurs.

There are three significant reactions that take place in addition polymerization: initiation (birth), propagation (growth), and termination (death). These separate steps are explained below.

### 2.2.1.1 Initiation Reaction

The first step in producing polymers by free radical polymerization is initiation. This step begins when an initiator decomposes into free radicals in the presence of monomers. The instability of carbon-carbon double bonds in the monomer makes them susceptible to reaction with the unpaired electrons in the radical. In this reaction, the active center of the radical "grabs" one of the electrons from the double bond of the
monomer, leaving an unpaired electron to appear as a new active center at the end of the chain. Addition can occur at either end of the monomer. In a typical synthesis, between 60% and 100% of the free radicals undergo an initiation reaction with a monomer. The remaining radicals may join with each other or with an impurity instead of with a monomer. "Self destruction" of free radicals is a major hindrance to the initiation reaction. By controlling the monomer to radical ratio, this problem can be reduced.

2.2.1.2 Propagation Reaction

After a synthesis reaction has been initiated, the propagation reaction takes over. In the propagation stage, the process of electron transfer and consequent motion of the active center down the chain proceeds. In the diagram, (chain) refers to a chain of connected monomers, and X refers to a substituent group (a molecular fragment) specific to the monomer. For example, if X were a methyl group, the monomer would be propylene and the polymer, polypropylene.

![Propagation Reaction](image)

Figure 2.2: Chain elongation by addition of monomers.

In free radical polymerization, the entire propagation reaction usually takes place within a fraction of a second. Thousands of monomers are added to the chain within this time. The entire process stops when the termination reaction occurs.

2.2.1.3 Termination Reaction

In theory, the propagation reaction could continue until the supply of monomers is exhausted. However, this outcome is very unlikely. Most often the growth of a polymer
Chapter 2: Polymers

The polymer chain is halted by the termination reaction. Termination typically occurs in two ways: combination and disproportionation.

Combination occurs when the polymer's growth is stopped by free electrons from two growing chains that join and form a single chain. The following diagram depicts combination, with the symbol (R) representing the rest of the chain.

**Termiation Reaction**

\[
\text{(R)}\text{CH}_2\text{C}^\bullet + \text{CCH}_2\text{(R)} \rightarrow \text{(R)}\text{CH}_2\text{C} - \text{CCH}_2\text{(R)}
\]

*Figure 2.3: Termination of chain elongation.*

Disproportionation halts the propagation reaction when a free radical strips a hydrogen atom from an active chain. A carbon-carbon double bond takes the place of the missing hydrogen. Termination by disproportionation is shown in the diagram. Disproportionation can also occur when the radical reacts with an impurity. This is why it is so important that polymerization be carried out under very clean conditions.

**Disproportionation Reaction**

\[
\text{(R)}\text{CH}_2\text{C}^\bullet + \text{CCH}_2\text{(R)} \rightarrow \text{(R)}\text{CH}_2\text{C} - \text{CCH}_2\text{(R)}
\]

*Figure 2.4: Disproportionation halting chain elongation.*
2.2.2 Living Polymerization

There exists a type of addition polymerization that does not undergo a termination reaction. This so-called "living polymerization" continues until the monomer supply has been exhausted. When this happens, the free radicals become less active due to interactions with solvent molecules. If more monomers are added to the solution, the polymerization will resume. Uniform molecular weights (low polydispersity) are characteristic of living polymerization. Because the supply of monomers is controlled, the chain length can be manipulated to serve the needs of a specific application. This assumes that the initiator is 100% efficient.

2.3 Statistical Analysis of Polymers

When dealing with millions of molecules in a tiny droplet, statistical methods are employed to make generalizations about the characteristics of the polymer. It can be assumed in polymer synthesis, each chain reacts independently.

Therefore, the bulk polymer is characterized by a wide distribution of molecular weights and chain lengths. The degree of polymerization (DP) refers to the number of repeat units in the chain, and gives a measure of molecular weight. Many important properties of the final result are determined primarily from the distribution of lengths and the degree of polymerization. The following simulation allows you to examine the distribution of chain lengths under varying conditions.

In order to characterize the distribution of polymer lengths in a sample, two parameters are defined: number average and weight average molecular weight. The number average is just the sum of individual molecular weights divided by the number of polymers. The weight average is proportional to the square of the molecular weight. Therefore, the weight average is always larger than the number average. The following graph shows a typical distribution of polymers including the weight and number average molecular weights.
The molecular weight of a polymer can also be represented by the viscosity average molecular weight. This form of the molecular weight is found as a function of the viscosity of the polymer in solution (viscosity determines the rate at which the solution flows - the slower a solution moves, the more viscous it is said to be - and the polymer molecular weight influences the viscosity). The degree of polymerization has a dramatic effect on the mechanical properties of a polymer. As chain length increases, mechanical properties such as ductility, tensile strength, and hardness rise sharply and eventually level off. This is schematically illustrated by the blue curve in the Fig 2.6.

Figure 2.5: Plot shows statistical analysis of molecular weight and number of molecules in a polymer.
However, in polymer melts, for example, the flow viscosity at a given temperature rises rapidly with increasing degree of polymerization DP for all polymers, as shown by the red curve in the diagram (fig 2.6).

Figure 2.6: Plot shows change in properties with increase in degree of polymerization.

2.4 Polymer Structure

2.4.1 Chain Structure

The geometric arrangement of the bonds is not the only way the structure of a polymer can vary. A branched polymer is formed when there are "side chains" attached to a main chain. A simple example of a branched polymer is shown in the following diagram.
There are, however, many ways a branched polymer can be arranged. One of these types is called "star-branching". Star branching results when a polymerization starts with a single monomer and has branches radially outward from this point. Polymers with a high degree of branching are called dendrimers. Often in these molecules, branches themselves have branches. This tends to give the molecule an overall spherical shape in three dimensions.

A separate kind of chain structure arises when more than one type of monomer is involved in the synthesis reaction. These polymers that incorporate more than one kind of monomer into their chain are called copolymers. There are three important types of copolymers. A random copolymer contains a random arrangement of the multiple monomers. A block copolymer contains blocks of monomers of the same type. Finally, a graft copolymer contains a main chain polymer consisting of one type of monomer with branches made up of other monomers. The following diagram displays the different types of copolymers.

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An example of a common copolymer is Nylon. Nylon is an alternating copolymer with 2 monomers, a 6-carbon diacid and a 6-carbon diamine. The following picture shows one monomer of the diacid combined with one monomer of the diamine:

\[
\text{Diacid} \quad \text{Diamine}
\]

2.4.2 Cross Linking

In addition to the bonds which hold monomers together in a polymer chain, many polymers form bonds between neighboring chains. These bonds can be formed directly between the neighboring chains, or two chains may bond to a third common molecule. Though not as strong or rigid as the bonds within the chain, these cross-links have an important effect on the polymer. Polymers with a high enough degree of cross-linking have "memory." When the polymer is stretched, the cross-links prevent the individual
chains from sliding past each other. The chains may straighten out, but once the stress is removed they return to their original position and the object returns to its original shape.

One example of cross-linking is vulcanization. In vulcanization, a series of cross-links are introduced into an elastomer to give it strength. This technique is commonly used to strengthen rubber.

2.5 Classes of Polymers

Polymer science is a broad field that includes many types of materials which incorporate long chain structure of many repeat units as discussed above. The two major polymer classes are described: Elastomers and plastics

Elastomers, or rubbery materials, have a loose cross-linked structure. This type of chain structure causes elastomers to possess memory. Typically, about 1 in 100 molecules are cross-linked on average. When the average number of cross-links rises to about 1 in 30 the material becomes more rigid and brittle. Natural and synthetic rubbers are both common examples of elastomers. Plastics are polymers which, under appropriate conditions of temperature and pressure, can be molded or shaped (such as blowing to form a film). In contrast to elastomers, plastics have a greater stiffness and lack reversible elasticity. All plastics are polymers but not all polymers are plastics. Cellulose is an example of a polymeric material, which must be substantially modified before processing with the usual methods used for plastics. Some plastics, such as nylon and cellulose acetate, are formed into fibers (which are regarded by some as a separate class of polymers in spite of a considerable overlap with plastics). Every day plastics such as polyethylene and poly vinyl chloride have replaced traditional materials like paper and copper for a wide variety of applications.

Most plastics can further be classified as either thermoplastic or thermoset, a label, which describes the strength of the bonds between adjacent polymer chains within the structure. In thermoplastics, the polymer chains are only weakly bonded (van der waals forces). The chains are free to slide past one another when sufficient thermal
energy is supplied, making the plastic formable and recyclable. In thermosets, adjacent polymer chains form strong cross-links. When heated, these cross-links prevent the polymer chains from slipping past one another. As such, thermosets cannot be reflowed once they are cured (i.e. once the cross links form). Instead, thermosets can suffer chemical degradation (denaturing) if reheated excessively.

2.6 Polymer Morphology

The morphology of most polymers is semi-crystalline. That is, they form mixtures of small crystals and amorphous material and melt over a range of temperature instead of at a single melting point. The crystalline material shows a high degree of order formed by folding and stacking of the polymer chains. The amorphous or glass-like structure shows no long-range order, and the chains are tangled as illustrated below.

Figure 2.12: Crystalline structure.

Figure 2.13: Amorphous structure.
There are some polymers that are completely amorphous, but most are a combination with the tangled and disordered regions surrounding the crystalline areas. Such a combination is shown in the following diagram.

![Semi Crystalline structure](image)

*Figure 2.14: Semi Crystalline structure.*

An amorphous solid is formed when the chains have little orientation throughout the bulk polymer. The glass transition temperature is the point at which the polymer hardens into an amorphous solid. This term is used because the amorphous solid has properties similar to glass. In the crystallization process, it has been observed that relatively short chains organize themselves into crystalline structures more readily than longer molecules. Therefore, the degree of polymerization (DP) is an important factor in determining the crystallinity of a polymer. Polymers with a high DP have difficulty organizing into layers because they tend to become tangled.

The cooling rate also influences the amount of crystallinity. Slow cooling provides time for greater amounts of crystallization to occur. Fast rates, on the other hand, such as rapid quenches, yield highly amorphous materials. For a more complete discussion, see the section on thermal properties. Subsequent annealing (heating and holding at an appropriate temperature below the crystalline melting point, followed by slow cooling) will produce a significant increase in crystallinity in most polymers, as well as relieving stresses. Low molecular weight polymers (short chains) are generally weaker in strength. Although they are crystalline, only weak Van der Waals forces hold the lattice together. This allows the crystalline layers to slip past one another causing a break.
in the material. High DP (amorphous) polymers, however, have greater strength because the molecules become tangled between layers. For uses and examples of high and low DP polymers, see the section on Polymer Applications. In the case of fibers, stretching to 3 or more times their original length when in a semi-crystalline state produces increased chain alignment, crystallinity and strength. In most polymers, the combination of crystalline and amorphous structures forms a material with advantageous properties of strength and stiffness. Also influencing the polymer morphology is the size and shape of the monomers' substituent groups. If the monomers are large and irregular, it is difficult for the polymer chains to arrange themselves in an ordered manner, resulting in a more amorphous solid. Likewise, smaller monomers, and monomers that have a very regular structure (e.g. rod-like) will form more crystalline polymers.

2.7 Material Properties

2.7.1 Glass Transition Temperature $T_g$

In the study of polymers and their applications, it is important to understand the concept of the glass transition temperature, $T_g$. As the temperature of a polymer drops below $T_g$, it behaves in an increasingly brittle manner. As the temperature rises above the $T_g$, the polymer becomes more rubber-like. Thus, knowledge of $T_g$ is essential in the selection of materials for various applications. In general, values of $T_g$ well below room temperature define the domain of elastomers and values above room temperature define rigid, structural polymers.

This behavior can be understood in terms of the structure of glassy materials, which are formed typically by substances containing long chains, networks of linked atoms or those that possess a complex molecular structure. Normally such materials have a high viscosity in the liquid state. When rapid cooling occurs to a temperature at which the crystalline state is expected to be the more stable, molecular movement is too sluggish or the geometry too awkward to take up a crystalline conformation. Therefore the random arrangement characteristic of the liquid persists down to temperatures at which the viscosity is so high that the material is considered to be solid. The term glassy
has come to be synonymous with a persistent non-equilibrium state. In fact, a path to the state of lowest energy might not be available.

To become more quantitative about the characterization of the liquid-glass transition phenomenon and $T_g$, cooling an amorphous material from the liquid state, there is no abrupt change in volume such as occurs in the case of cooling of a crystalline material through its freezing point, $T_f$. Instead, at the glass transition temperature, $T_g$.

Figure 2.15: Plot shows determination of glass transition temperature in amorphous polymers and freezing temperature in crystalline polymers.

there is a change in slope of the curve of specific volume vs. temperature, moving from a low value in the glassy state to a higher value in the rubbery state over a range of temperatures. This comparison between a crystalline material (1) and an amorphous
material (2) is illustrated in the figure below. The intersections of the two straight-line segments of curve (2) defines the quantity $T_g$.

### 2.7.2 Coefficient of Linear Thermal Expansion

The CTE is defined as the change in length per unit length per unit rise in temperature. Polymer material CTE's are in general much higher than those of metals.

### 2.7.3 Creep

Creep results from the fact that the long polymer chains tend to slide over each other so that there is a time-dependency to the stress-strain diagram. A load placed on a polymer material will result in an initial deformation, but with the load remaining over time, permanent deformation will occur. Creep data is difficult to find, but is often expressed in terms of a creep modulus.

### 2.7.4 Heat Deflection Temperature

The overall definition of heat deflection temperature is a temperature at which a polymer sample deflects a certain amount under heat and load. The heat deflection temperature is a function of the temperature, strain rate, and stress.

### 2.7.5 Mold Shrinkage

Mold shrinkage refers to the amount of contraction that a part experiences after cooling to ambient after removal from a mold.

### 2.7.6 Thermal Conductivity

Thermal conductivity represents the rate at which heat is transferred by conduction through a given unit area of a given material when the temperature difference or gradient is normal to the cross sectional area. The coefficient of thermal conductivity can be defined as the quantity of heat that travels through a unit volume of a polymer in a
given time when the temperature gradient is one degree. Plastics typically have lower thermal conductivity coefficients compared to those of metals.

2.8 Polymers for Microfabrication

A wide variety of polymers are available for microfabrication and can be chosen based on the method of fabrication and desired physical and chemical properties. Commonly used materials include polyamide (PA), polycarbonate (PC), polyoxymethylene (POM), cyclo olefin copolymer (COC), polymethylmethacrylate (PMMA), polyethylene (PE), polypropylene (PP), polystyrene (PS), polydimethylsiloxane (PDMS), epoxies, acrylics and polyurethane (PU). The basic physical and chemical properties of these materials are illustrated in the tables shown below.

<table>
<thead>
<tr>
<th>Polymers for molding</th>
<th>Density ( \times 10^2 ) kg/m(^3)</th>
<th>Glass Transition Temperature ( ^\circ )C</th>
<th>Operating Temperature ( ^\circ )C</th>
<th>Thermal Conductivity ( \text{W/m} \cdot \text{K} )</th>
<th>Linear Expansion Coefficient ( \times 10^5 )</th>
<th>Heat Distortion Temperature ( ^\circ )C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyamide</td>
<td>1.13</td>
<td>60</td>
<td>80-100</td>
<td>0.29</td>
<td>80</td>
<td>180</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>1.2</td>
<td>150</td>
<td>115-130</td>
<td>0.21</td>
<td>65</td>
<td>148-150</td>
</tr>
<tr>
<td>Polyoxymethylene</td>
<td>1.41-1.42</td>
<td>-60</td>
<td>90-100</td>
<td>0.23-0.31</td>
<td>90-100</td>
<td>154-160</td>
</tr>
<tr>
<td>Cycloolefin</td>
<td>1.01</td>
<td>138</td>
<td>N/A</td>
<td>N/A</td>
<td>60</td>
<td>123</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>1.18-1.19</td>
<td>106</td>
<td>82-88</td>
<td>0.186</td>
<td>70-80</td>
<td>80-110</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>&lt; 0.92</td>
<td>-19</td>
<td>70</td>
<td>0.349</td>
<td>140</td>
<td>40</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>0.89-0.91</td>
<td>0-10</td>
<td>100</td>
<td>0.22</td>
<td>100-200</td>
<td>90-100</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>1.05</td>
<td>80-100</td>
<td>70</td>
<td>0.18</td>
<td>70</td>
<td>78-99</td>
</tr>
<tr>
<td>Polydimethylsiloxane</td>
<td>0.55</td>
<td>-10-19</td>
<td>60-70</td>
<td>0.23</td>
<td>220</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

Table 2.1: Physical properties of commonly used molding polymers.

2.9 Processing Methods

There are a variety of techniques available for processing polymers but only a few are suitable and commonly used for microfabrication purposes. These fabrication techniques can be classified into two main groups: replication methods and direct
methods. Replication methods include injection molding, hot embossing and casting and involve the use of a stamper or master from which the required part is molded or replicated. Replication technologies have been successfully implemented in the macro

<table>
<thead>
<tr>
<th>Polymers for molding</th>
<th>Solvent Resistance</th>
<th>Acid and Base Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyamide</td>
<td>Ethanol, benzene, aromatic and aliphatic hydrocarbons, mineral oils, fats, ether, ester, ketones</td>
<td>N/A</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>Water, benzene, mineral oils. Conditionally resistant against alcohols, ether, ester</td>
<td>Diluted mineral acids</td>
</tr>
<tr>
<td>Polyoxymethylene</td>
<td>Fuels, mineral oils and usual solvents</td>
<td>Diluted acids</td>
</tr>
<tr>
<td>Cycloolefin</td>
<td>Acetone, methyl ketone, methanol, isopropanol</td>
<td>Diluted and concentrated mineral acids and alkalis, formaldehyde, detergents in water</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>Water, mineral oils, fatty oils, fuel</td>
<td>20% diluted acids, diluted alkalines, Ammonia</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>Alcohols, toluene, benzene, xylene</td>
<td>Ammonia, diluted acids and alkali</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>Diluted salt solution, lubricating oils, chlorinated hydrocarbons and alcohols</td>
<td>Most diluted acids and alkali</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Alcohols and polar solvents</td>
<td>Diluted and concentrated acids and except Nitric acid</td>
</tr>
<tr>
<td>Polydimethylosiloxane</td>
<td>Water and mineral oils</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2.2: Chemical properties of commonly used molding polymers.

World for low cost, high throughput fabrication of plastic parts. The master contains a negative replica of the required part and most of the techniques faithfully replicate the master. The fabrication of the master may be expensive since care has to be taken to assure quality of the part. This can then be used to create multiple replications. Release of the master from the molded/embossed part can also be a problem but in most cases mold release agents can be used to release the part. Direct methods involve direct addition or
removal of polymer to form the final part and include 2D and 3D laser ablation, stereolithography, photolithography and polymer surface micromachining. These methods in some cases require fabrication of 2-D masks for fabrication of the required microfeatures but do not require a master or stamper. Table 2.3 lists the molding behavior of different polymers.

<table>
<thead>
<tr>
<th>Polymers for molding</th>
<th>Reproduction</th>
<th>Filling</th>
<th>Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyamide</td>
<td>excellent</td>
<td>good</td>
<td>good</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>good</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Polyoxymethylene</td>
<td>excellent</td>
<td>excellent</td>
<td>good</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>good</td>
<td>average</td>
<td>poor</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>good</td>
<td>average</td>
<td>good</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>good</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Polydimethylsiloxane</td>
<td>excellent</td>
<td>excellent</td>
<td>excellent</td>
</tr>
</tbody>
</table>

Table 2.3: Molding behavior of different polymer materials.

2.10 Master Fabrication

There are 3 commonly used techniques used to fabricate masters for plastic microfabrication. They are metal micromachining, electroplating and silicon and glass micromachining.

2.10.1 Metal micromachining

Conventional metal machining technology has undergone miniaturization and with the use of Computer Numerically Controlled (CNC) machines, techniques like milling, turning, cutting etc. are capable of producing structures of the order of tens of microns. Many materials like steel, aluminum, stainless steel and different alloys can be
used for master fabrication. These techniques are particularly useful for structures with simple geometries. These techniques have serious limitations when it comes to fabrication of high aspect ratio structures, deep holes and very small dimensions.

Another metal micromachining technique, which is capable of producing highly complex 3-D structures in conducting metals, is micro electrical discharge machining (μEDM) [2.1, 2.2]. The material is removed due to high-energy discharge between the electrode and the work piece. This technique is very recent and has a lot of potential for future development.

2.10.2 Electroplating

Electroplating is most commonly used technique for master fabrication. Different metals like Ni, Cr and alloys like Ni-Co, Ni-Fe can be electroplated to produce the master [2.3]. A conducting substrate is first processed using conventional photolithography. Photoresist is developed and the areas to be electroplated are free of resist. Conventional photoresists can be used, but SU-8 a thick negative photoresist capable of producing extremely tall high aspect ratio is commonly used. Reported heights of up to 200 μm have been made using SU-8. Electroplating is then done in an electroplating bath, which contains an electrolyte and the metal deposits itself uniformly on the exposed areas due to migration of ions from the source through the electrolyte. The free structures of the stamper (electroplated metal) can then be released by dissolving the photoresist.

2.10.3 Silicon and Glass micromachining

Silicon and glass are commonly used as substrates for microfabrication and the same techniques (a combination of photolithography and etching) can be used to fabricate stampers out of silicon and glass [2.2, 2.4-2.6]. Photolithography is a reliable technique for producing complex geometries and feature sizes of up to 1 μm. Photoresist is first patterned on the silicon or glass substrate and the features are defined. The substrate can then be etched to produce 3-D structures, which can then be used for replication purposes. Glass etching is a wet etching process and is isotropic. Tall, high aspect ratio
structures with controlled geometries and sizes are difficult to fabricate. Silicon can be wet etched or dry etched using a deep Reactive Ion Etching (RIE) process. Dry etching using RIE is more commonly used since high aspect ratio, high precision structures can be etched easily and quickly and sidewall definition is excellent due to sidewall passivation during etching. Feature sizes of up to 300 μm high and 1 μm wide are possible using this technique.

2.11 Replication Techniques

2.11.1 Injection Molding

In the injection molding process [2.7-2.9], plastic granules or pellets are heated until they are melted (typically 350 to 550 °F. depending on the type of plastic). The melted plastic is then forced under high pressure (usually 10,000 psi or more) into a rigid mold (usually metal, such as aluminum or steel) where it cools and resolidifies to produce a part of the desired shape and dimension. Injection molded parts can be as small as an item barely visible to the naked eye or as large as an exterior automobile body panel.

![Mold plates clamped at high pressure](image)

![Melt and inject plastic at high pressure using a screw injector](image)

Figure 2.16: Simplified schematic of a typical injection molding setup.
Figure 2.16 shows a typical injection molding machine setup. Plastic molds must withstand a great deal of heat and pressure as they are of heavy-duty construction and tend to be expensive. Injection molding is not suitable for jobs where only a few parts are required. Volume runs are required to justify the tooling costs and make the finished product economically viable. Injection molds cost anywhere from a few thousand dollars for a small, simple tool to hundreds of thousands of dollars for a very large complex mold.

The molds are precisely made, because today's plastic materials are produced to tight specifications and because present-day injection molding machines can closely control pressure and temperature, the injection molding process can produce hundreds of thousands of parts, which are identical in appearance, performance and integrity.

2.11.2 Hot Embossing

Hot embossing [2.10-2.12] is a commonly used technique for fabrication of thermoplastic materials. The process is straightforward. The stamper or master and a planar plastic substrate are separately mounted in a vacuum chamber in the embossing machine (figure). Both the stamper and the substrate are heated to just above the glass transition temperature $T_g$ of the polymer substrate (usually between 50 °C – 150 °C). The vacuum is necessary to ensure perfect contact between the stamper and the substrate and eliminate any cavities due to trapping of air bubbles in between. Additionally vacuum prevents corrosion of the stamping tool due to water vapor, which is driven out of the polymers at elevated temperatures. The stamper and substrate are brought under contact at elevated temperatures with controlled force. The forces are generally of the order of 0.5-2 kN/cm². The whole setup is then cooled to below the $T_g$ of the polymer and then the substrate is carefully released by controlled withdrawal of the stamper.

Thermally induced stress and replication error due to different thermal expansion coefficients can be a problem with hot embossed substrates. By keeping the thermal cycle as small as possible errors and stress can be minimized. Stamper release also plays a big role in determining the quality of the embossed substrate. Automating the withdrawal of
the stamper and controlling the withdrawal rate can play an important role in improving the quality of the microfeatures in the part. Typical cycle times are of the order of 5-10 minutes. The cost of the embossing machine and fabrication of the stamper can be expensive (though not as expensive as injection molding) but this is an excellent technique for mass production of complex microfeatures.

![Simplified schematic of a typical hot embossing setup.](image)

**Figure 2.17: Simplified schematic of a typical hot embossing setup.**

### 2.11.3 Casting

Casting [2.13-2.15] is the simplest of the replication processes and involves curing of a liquid polymer resin using a hardener or a cross linking agent and initiated by heat or light. Casting is a very flexible process and curing is usually achieved at atmospheric pressure. The first step here is the fabrication of a suitable master or stamper. The stamper surface is modified in order to facilitate easy release of the molded part. Liquid plastic resin is mixed with the cross linking agent and poured into a molding setup with the stamper. Curing is initiated or accelerated by either heat or light, which starts the polymerization process. After the resin is completely cured the molded part is released.
Chapter 2: Polymers

Figure 2.18: Simplified schematic of a typical casting setup.

The most commonly used material for casting is Polydimethylsiloxane (PDMS) [2.14, 2.15] has excellent optical and fluidic properties. This process is capable of producing sub micron size features. Other materials like epoxies [2.16], acrylics and polyurethenes are also used for casting. The tooling costs for fabrication of the mold plates are minimal and the only expensive part is the master, which can be fabricated and used for casting multiple parts. The only drawback is that casting is not truly a mass fabrication process since the time scales are of the order of a 1-3 hours and can not match the output of other replication processes.

2.12 Direct Techniques

2.12.1 Laser based techniques

Laser ablation [2.17-2.19] is used to fabricate microstructures by using high-energy laser pulse to break bonds in a polymer molecule. The ablated region contains decomposed polymer and has to be removed. A typical laser ablation setup consists of an
eximer laser, which delivers pulses with typical frequencies of 10-100 Hz to several kHz. The feature definition is achieved by moving the substrate on an x-y stage under a mask or an aperture. A wide variety of materials can be used for this process and the accuracy depends on the energy distribution and focus of the laser beam. Feature sizes in the order of a few microns can be easily achieved. Laser ablation may also cause a change in surface properties of the polymer.

2.12.2 Optical Lithography

This technique can be used to microfabricate polymers which are photosensitive. Photo curable epoxies, silicone rubbers and photoresists (SU-8) are candidates for optical lithography. Simple channel structures can be easily fabricated using simple masks and exposure to light. More complex devices can be fabricated by using a series of masks and layer-by-layer optical lithography. Feature sizes of the order of a few microns can be achieved.

2.12.3 Stereo Lithography

This is a 3-D microfabrication technique. Here again a photo curable polymer is exposed to a laser beam and the polymer at the focal point of the laser cures and forms a solid. The structure can be built by moving either the laser beam or the container with the polymer or both. Typically the laser beam is moved in the x-y direction and the container is moved in the z direction thus forming a layer-by-layer 3-D structure. Complex fluidic systems can be easily fabricated using this process however this is a time consuming process suitable for rapid prototyping rather than commercial fabrication.

2.12.4 Polymer Surface Micromachining

Polymer surface micromachining is similar to surface micromachining and thin film processing in the semiconductor industry. Sacrificial layers (usually photoresists) corresponding to the required fluidic are patterned on a substrate, then polymer thin films (like parylene [2.18, 2.19], polyimide [2.20], Teflon, PMMA, PDMS) etc can be Chemical vapor deposited or evaporated on to the patterned substrate. Access holes are

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etched and the sacrificial layer is dissolved leaving behind the required fluidic network. This process is independent of the initial substrate and integration of electrodes and other elements can be achieved easily. Highly complex fluidic systems can be fabricated using this technique.

2.13 Bonding Techniques

In order to formed sealed capillaries, the microchannels that are normally open after fabrication have to be closed. Care must be taken not to clog the channels or distort the geometry or physical parameters. This is very difficult for high volume fabrication techniques. Listed here are a few techniques that have been used successfully.

2.13.1 Lamination

In the lamination process [2.16] a thin polymer film (~ 10-50 μm thick) coated with a thin adhesive layer (~ 5-10 μm thick) is used. The lamination machine that is used also called a laminator usually consists of two rollers through which the substrates with the adhesive film on top are passed through. The rollers are maintained at elevated temperatures to help the thin film stick to the substrate. Parameters like bonding speed, pressure and temperature can be controlled.

2.13.2 Thermo-compression bonding

This technique can be used for thermo plastics which on application of heat and pressure can achieve good bonds. Only certain materials are suitable for bonding using this method. Care has to be taken however not to deform the microfeatures on the plastic substrates.

2.13.3 Adhesive bonding

This by far is the most commonly used technique for bonding plastic substrates. Various adhesives are available for bonding to create plastic-plastic, plastic-silicon and plastic-glass bonds. The adhesives can be photo defined and polymerization of the
adhesive can be initiated by heat or light. Care has to be taken to ensure that the adhesive does not seep into the channel structure.

2.13.4 Welding

Polymers can also be joined by local melting at the substrate interface. This local melting can be created by laser or by ultrasonic sound wave. These bonding techniques depend on clean environments for the quality of the bonding to be good and dust and contaminants reduce the bond quality and strength.

2.14 Summary

The first part of the chapter focused on presenting a general overview of polymers, their structure, synthesis and properties. Different types of polymers and their physical and chemical properties were extensively discussed. Then lists of polymers that can be used in microfabrication were analyzed. There are various techniques available for microfabrication of polymers but only a few are suitable for microfabrication. The second part of this chapter discussed the two major polymer microfabrication techniques. Replication and Direct techniques. Different replication and direct techniques were explained and finally a list of methods to bond substrates together was discussed.
CHAPTER 3

Epoxy Casting for Microfluidic Systems

3.1 Introduction

Miniaturized systems for biochemical analysis can significantly reduce the cycle time, reagent cost and labor intensity compared to traditional technologies [3.1–3.3]. Many of these systems are targeted for a few or even single-use assay applications where it is essential that they be fabricated using inexpensive materials and technologies. Devices for these microfluidic systems have been previously made by Injection Molding (IM) [3.4] and hot embossing [3.5]. Casting methods for microfluidic devices were initially introduced by Whitesides [3.6]. Plastic microcasting is a simple, low cost fabrication technique that uses liquid polymers, which are poured in a micromold and cured to form solid parts. Unlike conventional IM methods, microcasting offers excellent replication resolution but so far it has been limited to silicon rubber materials and non-planar substrates. This chapter discusses the extension of the microcasting process to more rigid and less permeable epoxy materials and we have developed practical methods for the production of planar plastic casted wafers that enable further lithographic processing. Since plastic microcasting uses liquid polymers, embedding of active devices in plastics can also be done relatively easily. Our process offers excellent replication resolution (< 1 μm), a high process flexibility using a wide variety of plastic materials, the ability at temperatures, good chemical resistance, and good barrier properties (second only to fluoropolymers); therefore are the most suitable polymers for microfluidic applications. For our applications, we selected low viscosity epoxies that were easily castable and provide an adequate working time before solidification. The epoxies used for fabricating the devices were EPOTEK resins (Epoxy Technologies, Billerica, MA), which are optically clear polymers for the fabrication of microfluidic systems. This method allows excellent replication to embed elements, and a low fabrication costs. High
quality sealed-channel devices are fabricated using both a lamination process on top of microcasted structures and bonding. Preliminary Injection molding results are shown at the end of the chapter.

3.1.1 Why Epoxy Casting?

The selection of a microfabrication technique for fabrication of a device depends on the desired nature of functions the device has to perform and the areas of application. Microfluidic devices for applications in biology and medicine consist primarily of a structural network of fluidic channels connected with reaction chambers and reservoirs. The channels deliver reagents from the reservoirs to the reaction chambers where different reactions take place and then the final products are detected directly in the reaction chamber or in outlet channels. These devices are required in large numbers for multiple and single use and in some cases need to be disposable. This puts a restriction on the manufacturing cost of the device. The time line from design to fabrication is also small and extremely quick turnaround times are required. The capability to modify existing designs with small modifications is also required.

<table>
<thead>
<tr>
<th>Process</th>
<th>Materials</th>
<th>Tool costs</th>
<th>Forces and temperature</th>
<th>Cycle times</th>
<th>Minimum dimensions</th>
<th>Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Molding</td>
<td>Thermoplastics, Duraplastics</td>
<td>High</td>
<td>High</td>
<td>1-3 mins</td>
<td>10 microns</td>
<td>Bulk, spherical</td>
</tr>
<tr>
<td>Hot Embossing</td>
<td>Thermoplastics, Duraplastics</td>
<td>Medium</td>
<td>High</td>
<td>3-10 mins</td>
<td>Nanometers</td>
<td>Planar</td>
</tr>
</tbody>
</table>

Table 3.1: Comparison of the three plastic replication techniques.

Due to the cost constraints in fabricating these devices, plastics seem to be the obvious choice for a material for these applications. Plastics are a lot cheaper than the other commonly used materials for microfabrication like silicon and glass and can produce fabricated structures of same or better quality. Most plastic mass fabrication techniques that meet the low cost constraint are replication techniques. The three most common replication techniques are injection molding, hot embossing and casting (Table
3.1. Of the three techniques both hot embossing and injection molding are rapid mass fabrication techniques but the equipment and overhead costs involved are very high so casting is the ideal compromise between a low cost and mass fabrication process. Casting has so far been restricted to elastomers but epoxies can be used to cast rigid substrates with excellent physical, chemical and fluidic properties.

3.2 Epoxy Chemistry

Epoxy resin is defined as a molecule containing more than one epoxide groups. The epoxide group also termed as, oxirane or ethoxyline group. is shown below.

![Figure 3.1: The structure of an epoxide group.](image)

These resins are thermosetting polymers and are used as adhesives, high performance coatings and potting and encapsulating materials. These resins have excellent electrical isolation properties, low shrinkage, good adhesion to many metals and good resistance to moisture, and both thermal and mechanical shock. Viscosity, epoxide equivalent weight and molecular weight are the important properties of epoxy resins.

3.2.1 Types of Epoxy resins

There are two main categories of epoxy resins, namely the glycidyl epoxy, and non-glycidyl epoxy resins. The glycidyl epoxies are further classified as glycidyl-ether, glycidyl-ester and glycidyl-amine. The non-glycidyl epoxies are either aliphatic or cycloaliphatic epoxy resins. Glycidyl epoxies are prepared via a condensation reaction of appropriate dihydroxy compound, dibasic acid or a diamine and epichlorohydrin. While, non-glycidyl epoxies are formed by peroxidation of olefinic double bond. Glycidyl-ether epoxies such as. diglycidyl ether of bisphenol-A (DGEBA) are most commonly used epoxies.
3.2.1.1 Diglycidyl Ether of Bisphenol-A (DGEBA)

Diglycidyl ether of bisphenol-A (DGEBA) is a typical commercial epoxy resin and is synthesized by reacting bisphenol-A with epichlorohydrin in presence of a basic catalyst.

![The structure of Diglycidyl ether of bisphenol-A.](image)

Figure 3.2: The structure of Diglycidyl ether of bisphenol-A.

Casting epoxy resins are glycidyl ethers of phenolic epoxy resins. Phenols are reacted in excess with formaldehyde in presence of acidic catalyst to produce phenolic novolac resin. Casting epoxy resins are synthesized by reacting phenolic novolac resin with epichlorohydrin in presence of sodium hydroxide as a catalyst. Casting epoxy resins generally contain multiple epoxide groups. The number of epoxide groups per molecule depends upon the number of phenolic hydroxyl groups in the starting phenolic epoxy resin, the extent to which they reacted and the degree of low molecular species being polymerized during synthesis. The multiple epoxide groups allow these resins to achieve high cross-link density resulting in excellent temperature, chemical and solvent resistance. Casting epoxy resins are widely used to formulate the molding compounds for microelectronics packaging because of their superior performance at elevated temperature, excellent moldability and mechanical properties, superior electrical properties, and heat and humidity resistance.

3.2.2 Curing agents (Hardeners)

A wide variety of curing agent for epoxy resins is available depending on the process and properties required. The commonly used curing agents for epoxies include amines, polyamides, phenolic resins, anhydrides, isocyanates and polymercaptans. The cure kinetics and the $T_s$ of cured system are dependent on the molecular structure of the
hardener. The choice of resin and hardeners depends on the application, the process selected, and the properties desired. The stoichiometry of the epoxy-hardener system also affects the properties of the cured material. Employing different types and amounts of hardener, control the cross-link density and the structure.

The amine and phenolic resin based curing agents, described below, are widely used for curing of epoxy resins.

3.2.2.1 Amine based curing agents

Amines are the most commonly used curing agents for epoxy cure. Primary and secondary amines are highly reactive with epoxy. Tertiary amines are generally used as catalysts, commonly known as accelerators for cure reactions. Use of excessive amount of catalyst achieves faster curing, but usually at the expense of working life, and thermal stability. The catalytic activity of the catalysts affects the physical properties of the final cured polymer.

3.2.2.2 Phenolic hardener

Epoxy resins when cured with phenolic hardener, gives excellent adhesion, strength, and chemical and flame resistance. Phenolic cured epoxy systems are mainly used for encapsulation because of their low water absorption, excellent heat and electrical resistance. An accelerator is necessary for the complete cure to occur.

3.2.3 Toughening of Epoxy Resins

The usefulness of epoxy resins in many engineering applications is often limited by their brittle nature and poor thermal conductivity. The term toughness is a measure of material's resistance to failure i.e. the total amount of energy required to cause failure. There are several approaches to enhance the toughness of epoxy resins which includes: chemical modification of the epoxy backbone to make it more flexible structure, increasing the molecular weight of epoxy, lowering the cross-link density of matrix, incorporation of dispersed toughener phase in the cured polymer matrix, and
incorporation of inorganic fillers into the neat resin. Amongst these approaches, toughening via dispersed toughener (flexibiliser) phase has been shown to be most effective. The flexibilisers can be reactive or non-reactive rubber.

**3.2.3.1 Toughening Agents**

Various types of thermoplastic polymers as well as reactive rubbers are employed to enhance toughness of epoxy resin. Thermoplastic polymers, such as polyetherimide, polysulphone, polyethersulphone, and polycarbonate have been studied to modify epoxy resins. These studies show significant improvement in the toughness of epoxy resins. The reactive rubbers used for toughening epoxy resins include, liquid acrylonitrile-butadiene copolymers with various terminal groups, polysiloxanes, polycaprolactone, and polyurethanes.

Although liquid acrylonitrile-butadiene copolymers with carboxyl- (CTBN) and amine- (ATBN) terminated groups have been widely used for epoxy toughening, the relatively high glass transition temperature of the copolymer limits their low-temperature applications. In addition, these copolymers also increase the CTE value of the molding compound. Also the presence of unsaturated structure of butadiene system is prone to thermal instability and thus unsuitable for long-term use at higher temperatures. Polysiloxanes have excellent thermal stability, moisture resistance, good electrical properties, low stress and lower $T_g$ values. However polysiloxanes are not compatible with epoxy resins. Addition of compatibilisers such as methylphenylsiloxane enhances the compatibility but at the same time raises the $T_g$ of polysiloxane modifier restricting its low temperature applications.

**3.3 EPOTEK casting epoxies**

Epoxy technologies (EPOTEK) are commercial suppliers, who supply optical grade epoxies for casting applications. The physical and chemical properties of the epoxies can vary significantly based on their formulation. Generally the optical grade epoxies are glycidyl ether based epoxy resins, which are cured using phenolic hardeners. They have
excellent transparency, low auto fluorescence, and good barrier properties. They can be
cured to form rigid substrates by mixing portions of the resin and hardener in the correct
ratio. They cure at atmospheric pressure and room temperature but the curing time is in
the order of 20-30 hours. The curing time can be decreased significantly by raising the
resin-hardener mixture to elevated temperatures. Care must be taken to find the optimal
curing temperature, since curing at high temperatures can compromise the quality of the
cured resin and can result in rough surface finish and poor physical and chemical
properties. Four epoxies with excellent optical and barrier properties but with varying
physical properties were chosen. Table 3.2 shows a list of epoxies used and their physical
properties. The spectral transmittance at different wavelength is illustrated in Fig 3.3

<table>
<thead>
<tr>
<th>Epoxy</th>
<th>301-2</th>
<th>301-2 FL</th>
<th>310</th>
<th>314</th>
</tr>
</thead>
<tbody>
<tr>
<td># of components</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mix ratio</td>
<td>10 : 3.5</td>
<td>10 : 3.5</td>
<td>10 : 5.5</td>
<td>10 : 0.6</td>
</tr>
<tr>
<td>Cure temperature</td>
<td>80°C (3 hrs)</td>
<td>80°C (3 hrs)</td>
<td>80°C (3 hrs)</td>
<td>80°C (3 hrs)</td>
</tr>
<tr>
<td>Viscosity</td>
<td>300-600 cPs</td>
<td>125 cPs</td>
<td>450-850 cPs</td>
<td>400-800 cPs</td>
</tr>
<tr>
<td>Refraction Index</td>
<td>1.564</td>
<td>1.514</td>
<td>1.507</td>
<td>1.494</td>
</tr>
<tr>
<td>Spectral Transmittance</td>
<td>&gt; 97% 300-900 nm</td>
<td>100% 300-900 nm</td>
<td>&gt; 96% 340-900 nm</td>
<td>&gt; 93% 480-900 nm</td>
</tr>
<tr>
<td>Color before/after cure</td>
<td>Clear/ dear</td>
<td>Clear/ dear</td>
<td>Clear/ dear</td>
<td>Clear/ dear</td>
</tr>
<tr>
<td>Lap shear strength (psiu)</td>
<td>2000</td>
<td>2600</td>
<td>570</td>
<td>800</td>
</tr>
<tr>
<td>Shore D hardness</td>
<td>82</td>
<td>75</td>
<td>--</td>
<td>80</td>
</tr>
<tr>
<td>Tg</td>
<td>&gt; 65°C</td>
<td>&gt; 60°C</td>
<td>ambient</td>
<td>&gt; 150°C</td>
</tr>
<tr>
<td>Shelf life</td>
<td>1 year</td>
<td>1 year</td>
<td>1 year</td>
<td>1 year</td>
</tr>
<tr>
<td>Pot life</td>
<td>8 hours</td>
<td>12 hours</td>
<td>2 hours</td>
<td>4 days</td>
</tr>
</tbody>
</table>

Table 3.2: Physical properties and curing schedule for the different epoxies used.
Figure 3.3: Plot showing the spectral transmittance of epoxies at various wavelengths of light.

Figure 3.4: Glass transition temperatures of the EPOTEK epoxies used.
3.4 Casting Technique

3.4.1 Stamper Fabrication

The first step involved in any replication based plastic fabrication technique is to fabricate a stamper. Replication methods like casting require the use of a stamper or master from which the required part is molded or replicated. The master contains a negative replica of the required part and most of the replication techniques faithfully replicate the master. The fabrication of the master may be expensive since care has to be taken to assure quality of the part. This can then be used to create multiple replications.

In our process we use a silicon master, which contains deep etched negative replicas of the desired features.

A blank silicon wafer is first taken and a 2.7 μm thick layer of photoresist (Microposit SC 1827) is spun on at 4000 rpm. The photoresist is then soft baked at 90 °C for 30 minutes. Then a photo mask with 2-D replica of the desired features is used to pattern the photoresist. The patterned photoresist is then hard baked at 110 °C for 10 minutes. The silicon wafer with the hard baked photoresist mask is then etched using a deep silicon reactive ion etcher (DRIE). Silicon is etched at the rate of 2.5 μm/min using a high aspect ratio etch recipe (SF₆ 120 sscm, O₂ 13 sscm, Coil power 800 W, Platen power 200 W). The process is performed using alternating etch and passivation cycles to ensure straight side wall profiles. The substrate is etched for 12 seconds and passivated for 6 seconds, and this is repeated throughout the process. The passivation cycle (C₄F₈ 85 sscm, Coil power 600 W) is performed using C₄F₈ a polymer which coats itself on the sidewalls thus preventing undercuts due to etching of sidewalls during the etching cycle thereby ensuring straight sidewalls. Channel heights of up to 300 μm with surface roughness in the order of hundreds of angstroms can be easily obtained using this process. Once the features have been etched to the required depths the substrate is stripped of the photoresist and the resulting silicon substrate is used as the stamper or master. Figure 3.5 shows a DRIE silicon stamper for replication methods.
Figure 3.5: SEMs of silicon wafer with DRIE negative replicas of desired features.
3.4.2 Release Layer

It is important to treat the surface of the master/stamper to aid release of the molded or cast part from the stamper. Molding polymers usually undergo shrinkage on curing into rigid substrates and it is important that the interaction between the molded part and the stamper surface be limited to a minimum. There are commercially available mold release agents both in solution and aerosol form which can be coated on the stamper to make the surface of the stamper inert limit interaction with the liquid polymer but the thickness of the coat is usually in the order of 10's of microns which is close to the dimensions of the features on the molded part. The only solution to this problem is to coat the surface with a extremely thin layer of an inert polymer like Teflon, parylene [3.8] or even some types of silanes to make the surface of the stamper inert.

Parylene C is a chemically inert polymer, which can be deposited as extremely thin (sub micron) layers using a chemical vapor deposition process. To improve the adhesion of Parylene C to the silicon stamper the silicon stamper is treated with a silane (AZ 174). Parylene is available as a dimer. the dimer is vaporized at 170 °C in a vaporizer and split into monomers in a furnace which is at 700 °C. The monomers are allowed to enter a chamber at high vacuum and polymerize and coat the substrate, which is also placed in the chamber. Fig 3.6 shows the structure of Parylene C. Figure 3.7 details the parylene deposition process.

![Parylene C](image)

*Figure 3.6: Figure showing the structure of the repeating Parylene C unit in the polymer.*

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3.4.2 Mold fabrication

Mold plates are required to hold the stamper and to aid in heat transfer from the outside to the liquid polymer resin. The only requirements are that the material be a cheap, strong enough to withstand working conditions and have high thermal conductivity. Aluminum satisfies all these conditions and can be easily machined into any required geometry to hold the stamper. Two aluminum blocks were cut and machined to make the surfaces smooth (roughness in the order of a few microns). A groove the shape of a silicon wafer was cut in one of the aluminum blocks to hold the stamper in place. The molds were also provided with clamping screws to clam both the plates together tightly. Fig 3.8 shows the fabricated aluminum mold plates for both open and closed mold casting.
Figure 3.8: Shown are aluminum mold plates for open and closed mold casting.
3.4.3 Backing wafer and O-rings

In most cases smooth surfaces are desired on both sides of the substrates. One side usually has microfeatures like channels and the other side is smooth and flat. In order to obtain a flat surface on the other side a smooth flat backing wafer made of an inert material like teflon or polypropylene is used. The wafer is also cut the shape of a silicon wafer and sandwiched with the stamper. There is an O-ring in between the stamper and the backing wafer, which determines the thickness of the cast part. The O-ring is also made of a material like teflon. There are cases where two stampers are sandwiched to produce substrates with features on both sides eliminating the need for a backing wafer.

3.5 The Casting Process

Epoxy casting is extremely simple replication process. A silicon stamper is used to produce substrates with cast microfeatures. Based on the assembly of the molding setup the casting process can be classified into two types (1) Open casting process and (2) Closed mold casting.

3.5.1 Open casting process

Fig 3.9 details the open casting process. A silicon stamper with the parylene C release layer is first placed on the bottom aluminum mold plate and a Teflon O-ring is placed on top of it. A second aluminum plate with an opening cut on top is placed on top of the O-ring and clamped using clamping screws to the bottom mold plate. The epoxy resin is then poured into the recess with the stamper and cured at 80 °C for 3 hours. Then the mold plates are disassembled and the molded substrate is removed. One side of the substrate faithfully replicates the stamper but the other side is not guaranteed to be perfectly flat. Fig 3.9 shows pictures of channels cast using the open casting process.
Chapter 3: Epoxy Casting for Microfluidic Systems

Silicon Stamper with etched profiles
Coated with Parylene-C

Open top Aluminum plate
Teflon O-Rings

Pour liquid plastic and heat to 80 °C

Released plastic with micro channels

Figure 3.9: Schematic of the open casting process.

Figure 3.9: Shown are microscope pictures of cast epoxy channels obtained by open casting.
3.5.2 Closed mold casting

This process is similar to the open casting process with the only difference being that a backing wafer made of polypropylene is placed on top of the O-ring to ensure a smooth surface on the other side of the substrate. A small access hole is cut in the Teflon O-ring to facilitate injection of liquid resin into the closed mold. Once the mold is filled with the resin the setup is heated to 80 °C and cured for 3 hours. The mold is then disassembled and the cured substrate is released. Fig 3.11 shows a schematic of the closed mold casting process and Fig 3.12 shows microscope and SEM pictures of microfeatures produced using the closed mold casting process.

Figure 3.11: Schematic of the closed mold casting process.
Figure 3.12: Microscope and SEM pictures of channels fabricated using the closed mold casting process.
3.6 Lamination and Bonding

Channels and reservoirs fabricated using replication technologies have to be sealed in order to provide a closed fluid circuit for transport and handling of fluids. Two techniques, lamination and bonding have been extensively used for these purposes. Channels on the cast substrate can be sealed by laminating adhesive coated thin films using a laminator under controlled temperature and pressure. Alternatively the channels can also be sealed by bonding to another substrate.

There are a large number of bonding techniques which have been demonstrated which can be used based on the type of application to produce hermetically sealed channels. Both lamination and bonding use adhesives to achieve attachment. Care must be taken to ensure that the adhesives have excellent optical and barrier properties and suitable physical and chemical properties to withstand normal working conditions. This process is capable of achieving sub micron resolution features. The surface roughnesses...
obtained were of the order of 20 nm. This is an exact replication of the roughness of the sidewalls of the silicon wafer used as a stamper. Figure 3.13 shows the sidewall of a cast epoxy channel, which is an exact replication of the rough silicon stamper, obtained after DRIE.

3.6.1 Lamination

In order to seal channels and reservoirs, a flexible transparent film is laminated [3.9] on top of the epoxy substrates. The lamination process uses a desktop roll type laminator (Kepro Circuit Systems, Inc. BLT 121-A. Fig 3.15) and a thin mylar film (2 mil. Monokote. Top-Flite. Inc.) is used to laminate the plastic substrates. The substrate is first treated with acetone, and then the film is laminated onto the substrate by passing it between two rollers. The process is carried out at room temperature and at a feed rate of 2 ft/min. Fig 3.16 shows the cross section of a laminated channel. These structures show excellent permeation barrier characteristics with water permeability $P < 1.7 \mu l/cm/day$ at room temperature. When these capillaries are filled with sample there is no seeping present at the bond interface.

![Lamination process diagram](image)

*Released plastic with micro channels + 50 um thick mylar film*

*Lamination is achieved by sending the plastic + lamination film between two hot rollers at 80 °C at 2 ft/min and a gauge thickness of 2 mm.*

*Figure 3.14: Process flow for lamination.*
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Figure 3.15: Figure shows the KEPRO desktop roll type laminator.

Figure 3.16: Cross section of a laminated channel.
3.6.2 Bonding

Another technique to seal cast epoxy channels is bonding. A flat cast epoxy substrate is bonded to the epoxy substrate with microchannels using a thin adhesive layer. The adhesive used is a Blanchard wax solution. Blanchard wax is a commercially available bonding wax for bonding dies to PC boards. It is soluble in acetone and can be dissolved completely into a bonding wax solution. Depending on the mix ratio we can get low viscosity solutions which when spun on the wafer yield sub micron thick layers. Blanchard wax and is dissolved in acetone (25:100, blanchard wax: acetone by weight) for 30 minutes and stirred continuously to ensure complete dissolution of the wax in acetone. Fig 3.17 shows the schematic for the wax bonding technique. First a thin layer of the bonding wax solution is spun on the substrate with channels then the other flat substrate is aligned with a flat substrate under a microscope.

Spin thin layer of bonding wax solution on substrate with microchannels

Bonding substrate

Lamination is achieved by sending the both substrates between two hot rollers at 80 °C at 2 ft/min and a gauge thickness of 4 mm.

Figure 3.17: Process flow for the bonding technique.
Figure 3.18: Pictures of channels bonded using bonding wax.
The microscope has an x-y stage for alignment and a z stage for vertical movement and to apply bonding pressure. When the two substrates are aligned then the z stage vertical lift is used to bring the substrates together and apply bonding pressure. The substrates are held together for 2 minutes to ensure proper bonding. Fig 3.18 shows pictures of fluid in cast epoxy channels bonded using bonding wax.

3.7 Feed throughs on Plastic substrates

Figure 3.19: Stainless steel mold for fabrication of through vias and a fabricated epoxy channel with through vias.
On-chip chemical analysis also requires reservoirs on the plastic substrate to store gels and other reagents. Currently this is done by mechanically drilling holes for this purpose. We have demonstrated a two molds single cast process that created the channels and reservoirs simultaneously Figure 3.19. A stainless steel plate, the size of a 4” silicon wafer was made and pins of height 1.5 mm and diameter 1.5mm were press fitted in the stainless steel plate. This plate was then coated with a release layer and used on one side of the mold to get feed through on the plastic substrate. The plate was uniformly coated with an aerosol based commercially available mold release.

3.8 Injection Molding

We have also fabricated plastic substrates for microfluidic applications by Injection molding. In this section the results obtained are discussed. We fabricated molds by the silicon etching technique. These molds are then used as stampers in molding inserts for the fabrication of plastic substrates. We fabricated these mold inserts at a commercial machine shop. The molds are designed to fabricate 1 mm flat structures with one side that is micromachined by introducing a silicon or glass high-resolution stamp.

Figure 3.20 shows a typical injection molded polycarbonate substrate (100 mm diameter. 2 mm thick) with channels fabricated by this method. The width of the channel is 200 μm and its depth is 5 μm. It can be seen from the pictures that the sidewalls are not very sharp. The design and manufacture of the injection mold was done in a mechanical engineering machine shop. A two-piece fixture with injection port, cavity insert, heater, cooling channels, and release mechanism was designed and manufactured in aluminum because of its machining ease and thermal properties. Figure 3.21 shows the manufactured mold.
Figure 3.20: Injection molded Polycarbonate substrate.

Figure 3.21: The molds plates used for Injection molding.
A silicon mold insert with desired microstructures is patterned on the surface of silicon wafer using bulk micromachining technique. The complete mold system was used in several mold trials with the following:

(a) Material: Polycarbonate (excellent optical, chemical, and mechanical properties)
(b) Geometry: 4" circular plate with microstructures on one surface (batch fabrication)
(c) Machine: Arburg Allrounder 221M 350-75, 40 tons (clamping force). 30 mm (screw diameter) conventional injection molding machine

Process parameters: Injection pressure, injection velocity, melt temperature, mold temperature, plate thickness

3.8.1 Molding Results

Results indicate that only low resolution replication of microstructures on polymer surface is possible. In addition the polymer melt cannot fill micro cavities completely. All of the current structures have a low aspect ratio. Finally, a flow mark is observed on the polymer surface. Figure 3.22 shows SEM photographs of low aspect ratio, injection molded structures.

Based on the results obtained and consideration of the equipment and tool making costs, it was decided that casting was a more suitable alternative. Another problem with injection molding was that the features obtained were not completely formed. The pyramid or dome shaped features in Figure 3.21 are a result of incomplete material flow into the cavities. Complete material flow would have resulted in rectangular shaped features with completely flat top surface. In order to obtain flat top surface, the mold plates have to be fitted with heaters to maintain the temperature of the mold at the melting temperature of the material. also high pressures have to be applied. Casting on the other hand, is a simpler process with much greater flexibility.
Figure 3.22: SEMs of injection molded low aspect ratio structures.
3.9 Summary

In this chapter we describe plastic (epoxy) casting as the method of choice for fabricating microfluidic systems. We compared casting to injection molding and hot embossing. We also gave a detailed review of the chemical structure of epoxies and their cross-linking and toughening agents. We then introduced the casting technique for fabricating cast epoxy structures. Both open casting and closed mold casting were discussed; fabricated substrates with micro channels and reservoirs were shown. Additional techniques for fabricating complete devices like lamination, bonding and through hole fabrication were also explained, and results were shown. Finally we looked into the preliminary work done to fabricate substrates using the injection molding technique and fabricated substrates were shown. It was seen from the results that the quality of substrates fabricated using casting was far better than the substrates fabricated by injection molding.
CHAPTER 4

System Integration: Polymer Flip Chip Process

4.1 Introduction

Plastic casting as explained in the previous chapters has shown to be an inexpensive technique for fabrication of disposable microfluidic systems for various biochemical applications [4.1]. Epoxies [4.3] apart from other materials [4.2] are ideal for microfluidic applications [4.5] and have excellent optical, thermal and chemical properties and are biologically inert. Technologies like plastic casting are suitable techniques for rapid fabrication of complex structural elements required to build a microfluidic system. Complex arrays of channels and reservoirs can be fabricated and used for various applications but many of the microfluidic systems built today require macroscopic devices like external pumps, valves, fluid controllers, microscopes, detectors and sensors. Many advantages conferred by microfabrication including portability, integration and low cost fabrication cannot be fully exploited if the micro scale device depends on macro scale components for the system to function. Many groups have been working on integrating various components for both fluidic control (pumps, valves and mixers) and for sensing and detection [4.6-4.30]. Fig 4.1 shows a schematic illustration of a completely integrated micro system. Fig 4.2 shows a typical microscope setup necessary for detection in capillary electrophoresis systems.

Integration of individual components on silicon and glass based micro systems have been reported by many researchers over the past few years but there has been little progress in plastic based systems. It is easier to integrate components especially detectors and sensors onto silicon based systems because of the excellent material properties of silicon and the established fabrication techniques available to microfabricate silicon. Glass also has established fabrication techniques and is commonly used in clean rooms for microfabrication. Hence silicon and glass fabrication affords the luxury of building
structural components like channel structures in parallel with the control elements. Most plastic microfabrication techniques are modifications or adaptations of macro fabrication techniques and are not clean room compatible. The chemical resistance of many plastic substrates also restricts the use of these substrates. Another constraint is that the structural elements have to first be fabricated using different plastic microfabrication technologies
and only after that the control elements can be added or integrated with the system. In this chapter we discuss two different techniques for integration of individual control elements with a cast epoxy microfluidic system. The first technology a Polymer flip chip process which involves mass fabrication of control elements and integrating them with the system by embedding using the polymer flip chip process and the second technology a surface micromachining process which involves the fabrication of an actuator built from bottom up entirely on a plastic substrate. The flip chip technology is more suited for integration of sensors and detectors, which exploit the material properties of silicon whereas the surface micromaching technology is useful for integrating fluidic control elements like valves, pumps and mixers. This chapter focuses on polymer flip chip technology developed for embedding silicon micromachined devices in plastic substrates and the next chapter discusses surface micromachining technology developed to fabricate control elements for fluidic manipulations.

4.2 Motivation: Why flip chip?

Plastic as a material has excellent properties for building structural elements required for fluidic systems but most plastic materials have severe limitations when it comes to electrical and thermal properties. Most plastics are dielectrics and have low $T_g$ (< 200 °C) and degradation temperatures (< 500 °C). This limits the fabrication processes that can be performed using plastic substrates. Plastics are not semiconductors and hence devices like diodes and transistors that are essential for the fabrication of detectors are impossible to fabricate. Metal polymer solutions (MPS) of materials with excellent electrical properties cannot be used due to the poor thermal properties. as the solutions have to be heated at temperature above 500 °C. The only possible processing on plastics is the deposition and patterning of metal electrodes and heaters. Modifications of electrode arrangements can be used to make pH or temperature sensors but building more complex or useful sensors and detectors is not possible. A possible solution is to design and fabricate a device that takes advantage of the material properties of a semiconductor material like silicon and the low cost plastic with desirable fluidic properties and integrate control elements made of silicon onto a plastic structural platform.
4.3 On chip detectors for Capillary Electrophoresis (CE)

A typical system to perform on-chip fluorescence detection is shown in Fig 4.3 [4]. A filtered, blue LED is used as an excitation source. Fluorescent DNA passes through a channel network on the surface of the chip. Below the channel, an on-chip optical thin film interference filter is used to filter the excitation source from the fluorescent emission. The filter is designed to reflect the blue source and pass wavelengths longer than 505nm. The fluorescence is then collected by a photodiode in the silicon substrate. A lockin amplifier measures the photocurrent from the photodiode and pulses the blue LED at a reference frequency.

![Diagram](image)

Figure 4.3: Typical setup for on-chip detection of DNA in a capillary electrophoresis device[4].

4.3.1 Detector and device fabrication

The detector is fabricated using silicon microfabrication techniques. A cross section of the device is shown in Fig 4.4. The starting substrate is a p-type silicon wafer. In order to fabricate the photodiode n-type regions are patterned on the p-type substrate by ion implantation. Once the diodes are made, an isolation layer of silicon dioxide is grown on the substrate. In order to detect light at the required wavelength a multilayer interference filter is laid down. The materials for the interference filter are based on the desired wavelength of light. Finally a transparent ground plane is laid down at the detection area. Contacts to the diodes are made using gold metal electrodes, which are
evaporated and then patterned. Once the diode fabrication is complete the channel structure can either be built on top of the diode as shown in Fig 4.3 or the diode can be embedded in another system and used for detection purposed.

4.4 Flip chip technology

In the development of packaging of electronics the aim is to lower cost, increase the packaging density and improve the performance while still maintaining or even improving the reliability of the circuits. The concept of flip-chip process where the semiconductor chip is assembled face down onto circuit board is ideal for size considerations, because there is no extra area needed for contacting on the sides of the component. The performance in high frequency applications is superior to other interconnection methods, because the length of the connection path is minimized. Also reliability is better than with packaged components due to decreased number of connections. In flip-chip joining there is only one level of connections between the chip and the circuit board.

There are many different alternative processes used for flip-chip joining. A common feature of the joined structures is that the chip is lying face down to the substrate and the connections between the chip and the substrate are made using bumps of
electrically conducting material. Cross sections of flip chip joints without and with underfill material are shown in Fig 4.5. Examples of the different types of flip chip joints are schematically shown in Fig 4.6 and 4.7. The two most commonly used techniques however are solder bumping and the use of conductive and non-conductive adhesives.

![Flip chip without underfill material](image1)

![Flip chip with underfill material](image2)

*Fig 4.5: Cross sections of different flip chip processes.*

![Solder bump](image3)

![Metal bump](image4)

*Figure 4.6: Basic flip chip joints using solder and metal.*
4.4.1 Solder joining

In flip chip soldering process solder bumped chips are soldered onto the circuit board. Solder is usually, but not always, deposited also on to the substrate pad areas. For fine pitch applications, solder can be deposited by electroplating, solder ink jet or solid solder deposition. Tacky flux is applied to the solder contact areas either by dipping the chip into a flux reservoir or by dispensing flux onto the substrate. For coarse pitch applications (>0.4 mm) solder paste is deposited on the substrate by stencil printing. The bumps of chips are placed into the tacky paste and they are refloved in an oven. After the
reflow process cleaning of the flux is preferred. The underfill material is applied by dispensing along one or two sides of the chip, from where the low viscosity epoxy is drawn by capillary forces into the space between the chip and substrate. Finally the underfill is cured by heat. Repairing of the flip chip joint is usually impossible after the underfill process. Therefore testing must be done after reflow and before the underfill application.

4.4.2 Conductive and Non-conductive adhesives

Conductive adhesives have become a viable alternative to tin-lead solders also in flip chip joining. Adhesively bonded flip chip combines the advantages of thin structures and cost efficiency. The advantages of conductive adhesives include ease of processing, low curing temperatures, and elimination of the need to clean after the bonding process. Anisotropically conductive adhesives have also the ability to connect fine pitch devices. Fig 4.8 shows a schematic drawing of flip chip bonding with isotropically and anisotropically conductive adhesives (ICAs and ACAs). Also nonconductive adhesives can be used for flip chip bonding, in this case the joint surfaces are forced into intimate contact by the adhesive between the component and substrate.

Isotropically conductive adhesives are pastes of polymer resin that are filled with conducting particles to a content that assures conductivity in all directions. Generally, the polymer resin is epoxy and conducting particles are silver. Anisotropically conductive adhesives are pastes or films of thermoplastics or b-stage epoxies. They are filled with metal particles or metal-coated polymer spheres to a content that assures electrical insulation in all directions before bonding. After bonding the adhesive becomes electrically conductive in z-direction. The metal particles are typically nickel or gold and these metals are also used to coat polymer spheres.
4.5 Polymer Flip Chip Process

The Polymer flip chip process developed is a modification of commonly available conductive adhesive based flip chip process. Two key techniques - micro-screen printing and precision die placement - along with basic microfabrication and casting techniques are used. The only major difference is thin film metal contact pads are photolithographically patterned on the plastic substrate instead of the metal bumps.

4.5.1 Chip on Flex

Plastic based microfluidic systems are being increasingly used because they offer high quality devices, which are a low cost alternative, and the turnaround time for fabricating these devices is extremely small. However due to the material properties of plastics, fabrication of detectors and sensors on plastics is not feasible. Silicon on the other hand has excellent material properties but is significantly more expensive and fabrication process is more complex. Attempts have been made to perform on chip
detection using optical, electrochemical and chemiluminescence techniques. In all these devices the detectors are built on a silicon substrate and then the channel structures are either built on top of the detectors or separately fabricated on another substrate and bonded to the substrate with the detectors. The die space required to support the channel structure is significantly larger than the area of the detectors as shown in Fig 4.9 and much of the area on the expensive silicon wafer is wasted in supporting the channel structure. Polymer flip chip technology offers a cheaper solution by integrating detector dies which can be mass fabricated on a complete silicon wafer, and then embedded in an extremely low cost plastic microfluidic system. Fig 4.10 shows pictures of some embedded silicon chips in cast epoxy substrates.

![Figure 4.9: Comparison of the detector area to the total die area [4].](image)

### 4.5.2 Polymer Flip Chip Process for embedded detectors

Polymer flip chip process involves integration of silicon micromachined chips with plastic based systems. It is important to ensure that the functions of the chip after integration are not affected by the process and continue to perform like they did before embedding. The key techniques include precise chip location on the plastic substrate to ensure that the chip is at the right position with respect to the fluidic circuit on the plastic
Chapter 4: System Integration: Polymer Flip Chip Process

Figure 4.10: Shown are silicon chips embedded in a cast epoxy substrate with fluidic circuits.

substrate, attachment of the die to the plastic substrate, ensuring perfect electrical contact to the chip and accessing the contacts to the chip from the outside.

Fig 4.11. Illustrates the process flow for integrating a silicon chip with a plastic wafer using the polymer flip chip process. A plastic substrate with channels is first laminated to seal the channels. Then a thin layer of metal is evaporated and then photolithographically patterned to form the contacts and alignment marks on the plastic wafer. Electrically conductive epoxy, which serves as both an electrical contact and glue, is micro-screen printed on the contact pads on the plastic substrate. The silicon die is patterned with backside alignment marks corresponding to the alignment marks on the plastic. A precision pick and place machine is used to precisely locate the die on the plastic wafer such that the die is aligned and the contact pads on the die are bonded to the conductive epoxy on the plastic wafer. The electrically conductive epoxy sets over a period of 12 hours but the process can be accelerated by heating. To enclose the device the plastic substrate with the attached chip is set in an aluminum mold with an O-ring and a backing wafer and liquid epoxy resin is used to embed the chips. Contact holes to the electrodes can be drilled using a drill press.

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Figure 4.11: Process flow for embedding silicon chips in plastic.

Figure 4.12: Alternative process flow for embedding chips in plastic.
An alternative approach using the same techniques like precision die placement and \( \mu \)-screen printing can also be employed to embed chip in a separate substrate and then bond that substrate to another substrate with the channels. Fig 4.12 illustrates this process flow.

### 4.6 Micro Screen Printing (\( \mu \)-Screen)

Micro-screen is a development of screen printing technology aimed at the production of very fine features for electronic or other applications. It is a simpler alternative to other more complex patterning techniques like lithography, liftoff and etching. It is particularly useful in cases where the feature sizes are fairly big (> 50 \( \mu \)m) and the feature definition is not very critical. Another advantage is the flexibility of the process: by controlling the printing parameters any liquid or gel can be screen printed on any substrate.

![Figure 4.13: A typical stencil type micro-screen for micro-screen printing applications.](image)

The \( \mu \)-Screen (Fig 4.13) can be used on a standard thick film-printing machine in the same way as a standard mesh screen, but because of its unique construction, it is capable of much greater resolution down to 50 \( \mu \)m line and space. Wider lines also can be printed. The \( \mu \)-Screen is made from a stainless steel foil, which is micro-etched with a series of ink feeder holes. The etched feeder hole pattern is computer generated from a
customer's own CAD layout file. Hence, each \( \mu \)-Screen effectively has a custom mesh which exactly matches the print pattern. This avoids the interference and poor print edge definition produced by the random alignment of a pattern to a standard wire mesh. An organic gasketing layer is applied to the underside of the foil, which allows the ink to flow beneath the metal 'bridges' between the holes and defines the sides of the printed lines and areas. The \( \mu \)-Screen is mounted in a conventional screen frame and is used in a normal off-contact mode on a standard printing machine.

The design is made as a CAD layout showing the features to be printed. Specially developed software is then used to produce artwork for etching ink feeder holes and for patterning the gasketing (emulsion) layer. Ink feeder holes are produced in the metal foil by electro-chemical etching. A gasketing layer (emulsion) is applied to the underside of etched foil and patterned to form channels that control the spread of the printing solution. The foil is then mounted onto a carrier mesh in a conventional printing frame.

### 4.6.1 Screen printing machine

*Figure 4.14: Typical screen printing machine for printing on flat surfaces.*

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Chapter 4: System Integration: Polymer Flip Chip Process

Figure 4.14 shows a typical screen printing machine. The machine contains a holder onto which the screen is mounted and locked into position. There is an x-y stage capable of angular motion also which contains a vacuum chuck to hold the substrate on which the printing is done. There is also a Teflon squeegee that is mounted in a holder located right above the screen. The squeegee is capable of x (downward) and y (along the plane of the screen) movement. The pressure applied by the squeegee on the screen (printing pressure) and the speed at which it moves across the screen can be controlled or automated. There is a microscope with alignment cross hairs located right above the chuck that holds the wafer. The process is simple. A blank substrate is loaded on the chuck and the chuck is moved underneath the screen. The screen is brought down and the material (solution) to be screen printed is applied at one end of the screen. The squeegee presses down on the screen applying printing pressure and then sweeps across the screen thus evenly coating the material through the openings on the screen. The squeegee is then withdrawn and the chuck is brought back to the original position under the microscope. The cross hairs are aligned to the printed features and the blank substrate is removed. The substrate on which printing is to be done is then placed on the chuck and the chuck is moved using the x-y stage to align areas to be screen printed to the cross hairs (alignment process). Once the alignment is complete the chuck is again moved under the screen and the squeegee is used to print the material evenly through the holes in the screen onto the substrate. The substrate is then removed with the printed features.

4.6.2 Electrically conductive epoxy

H 20E is a commercially available electrically conductive epoxy recommended for screen printing on plastic substrates. This silver filled epoxy resin adhesive is ideal for creating strong, highly conductive solderless connections and repairs. Use for circuit board repair, surface mount connections, static discharge, shielding and grounding. This epoxy is excellent for bonding heat sensitive components. The properties of H 20E are shown in the table below.
**Epoxy** | **H-20 E**
---|---
# of components | 2
Mix ratio | 1:1
Cure temperature | 60°C (3 hrs)
Viscosity | 2200-3200 cPs
Volume resistivity (ohm-cm) | < 0.00004
Thermal conductivity (W/mK) | 29
Max operating temperature | 200
Lap shear strength (psi) | 3400
Degradation temperature | 400
Shelf life | 1 year
Pot life | 4 days

_Table 4.1: Physical properties of H 20 E._

H 20 E provides quick, solderless electronic connections, which are excellent for bonding surface mount components. These epoxies offer excellent electrical conductivity while also providing high strength conductive bonds. They can be quickly cured at low temperatures or no heat cure (air-dries) in a few hours (cold solder). They are also flexible enough that even after curing the epoxy can be reworked or removed using a hot soldering iron. Figure 4.15 shows micro screen printed H 20 E bumps on a plastic substrate.
4.7 Precision die placement

To enable integration of silicon chips with plastics a second key technique is required. This technique involves die placement at precise locations on the plastic substrate. A die pick and place instrument (Royce instruments) is used to accomplish this. The semi-automatic die pick and place system is an elegantly simple low cost machine for picking die from sawn wafers mounted on adhesive film or waffle packs and placing them on plastic wafers. The die pick-and-place system is designed for die handling where flexibility and rapid setup are of great importance. Capabilities include picking and testing laser diodes, handling die as small as 0.008 in. sq., picking pressure sensitive die, CCD imager chips with Non-Surface Contact option. The instrument (shown in Fig 4.16) is modified to improve accuracy. The microscope setup is removed and replaced by two microscopes with CCD cameras connected to TV screens. The cameras are at right angles to each other thus help minimizing alignment error. The alignment marks on the plastic wafer are accomplished by using metal lines, which can be patterned along with the
electrical contacts. The silicon wafer with the chips for embedding is patterned on the backside to provide backside alignment marks which are necessary since the silicon chips have to be flipped before being placed on the plastic substrate. The plastic wafer is placed on a vacuum chuck, which is placed on a moving table. The table is capable of movement along the x direction. The dies are picked using a vacuum tip (Fig 4.17), which is capable of movement along the z direction and rotational movement. The dies are picked and then the table moves bringing the plastic substrate under the tip. The table movement can be programmed since the system is provided with a computer. Once the die is approximately above the given location precise alignment can be done using a puck which controls the fine motion of the table. The puck moves the table both in the x and y directions and also controls the rotation of the tip. The die is brought in close proximity with the plastic substrate and the alignment is completed. Then the tip is brought down and the chip is released. The chip bonds to the conductive epoxy on the bonding pads. Entire wafers can easily be processed using this machine. Die placement locations can be programmed which improves the process speed and offers a degree of automation.

Figure 4.16: The Royce 110 die pick and place system.
4.8 Embedding silicon chips with heaters on plastic

After developing the techniques for precision silicon die placement onto plastic substrates we decided to pick and place chips with simple devices to establish proof of concept. First metal heaters were mass fabricated on silicon wafers. A blank silicon wafer was taken and a 1 μm layer of thermal oxide isolation layer was grown. Then a layer of Aluminum (200 nm) was deposited using an evaporation process. The Aluminum was photolithographically patterned into resistive heaters with 20 μm wide heater lines. The substrate was then flipped over and backside alignment marks were patterned photolithographically using photoresist. The wafer was diced and individual dies were transferred to waffle packs.

A cast epoxy wafer with channels was sealed using the lamination process. Then metal layers of Chrome (adhesion layer) and Aluminum were evaporated on the laminated surface (Cr/Al, 50/200 nm). The metal layers were then photolithographically...
patterned to form the metal contacts, interconnects and alignment marks. The contact pads on the plastic wafer were screen printed with silver filled electrically conductive epoxy bumps using the μ-screen printing process. A stainless steel emulsion mask with 50 μm square openings was used. The heights of the features obtained was approximately 30 μm. The plastic wafer with the contacts printed with silver epoxy is transferred to the pick and place system and the dies are picked and precisely placed by aligning the alignment marks on the back side of the silicon chip to the alignment metal lines on the plastic substrate. Fig 4.18 shows pictures of the back side and front side of a chip with a heater which has been placed using the above process. Fig 4.19 shows a plastic substrate with embedded silicon chips with heaters.

![Patterned metal Contacts on plastic](image)

*Figure 4.18: Microscope pictures of the back and front side of a precision placed silicon chip on a plastic substrate.*

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4.9. Summary

In this chapter we first discussed the need for integration of complexity in the form of control elements like valves and pumps and detection elements. There are two ways system integration can be accomplished. One way is to mass fabricate the control elements on silicon or glass which have excellent material properties and then embed them in plastic. Embedding can be done using Flip Chip technologies. In order to embed active silicon micromachined devices in plastic a Polymer flip chip process was developed. The two key technologies used are screen-printing and precision die placement. Both techniques are explained and the embedding of silicon micromachined heaters in cast epoxy systems is detailed.
CHAPTER 5

System Integration: Surface Micromachining

5.1 Introduction

The future of epoxy based microfluidic systems depends on the ability to build in more complexity along with the channel structures to help perform complex functions like valving, pumping, mixing etc. In the previous chapter we discussed ways to integrate pre-fabricated components into plastic systems using the polymer flip chip process. Another way to accomplish this goal is to make use of current fabrication techniques to build from the bottom up devices capable of performing different function within the microfluidic system. In this chapter we discuss technology developed to fabricate a polymer-based actuator that can easily be built bottom up on epoxy substrates and integrated within the microfluidic circuit. The actuator is then used to power a nozzle-diffuser pump that can be used to control fluid flow in microchannels. Pumps are critical for microfluidic systems, as they are needed for a wide variety of applications including transport, mixing, cooling and creating pressure differences. We discuss in detail the fabrication of a valve-less nozzle-diffuser pump integrated within a plastic microfluidic system. The pump uses thermal actuation, making use of the large volumetric expansion of a wax patch when it transitions from solid to liquid phase. The wax used is polyethylene glycol. MW 8000 (PEG 8000) a carbowax with a volume expansion of up to 30%. The melting point of the PEG 8000 is 60 °C and thermal modeling shows that pump operates with fluid temperatures of less than 100 °C at the interface which is critical in order to prevent sample loss due to evaporation. Testing of fabricated devices show maximum flow rates of 70 nl/min and generation of pressure heads of up to 1400 Pa.
5.2 Micro Actuators

Scaling not only has consequences for mechanical design but also for the actuation of microactuators. The right choice of the actuation principle is dependent on the structural dimensions, the technology, the response time, the force and torque as a function of displacement and the maximum power consumption, as well [5.1].

Force can be generated following two main principles: (1) External forces which are generated in the space between stationary and moving parts using thermopneumatic [5.2] or electrochemical [5.3] effects and electrostatic [5.4] or magnetic fields [5.5]. (2) Internal forces, which use special materials having intrinsic actuation capabilities including piezoelectric [5.6], thermomechanical [5.7], shape memory [5.8], electro- and magneto-strictive effects [5.9]. Table 5.1 shows typical MEMS devices together with their activation principle, which have been successfully realized and implemented in industrial applications or prototypes.

<table>
<thead>
<tr>
<th>Actuation principle</th>
<th>Typical MEMS devices</th>
</tr>
</thead>
<tbody>
<tr>
<td>piezoelectric</td>
<td>Micropump, microvalve, HDD servo system</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>Micromotor, micromirror, microshutter, microscanner, microrelay</td>
</tr>
<tr>
<td>Electromagnetic</td>
<td>Microrotor, micropump, microvalve</td>
</tr>
<tr>
<td>Thermomechanic</td>
<td>Microvalve, microgripper</td>
</tr>
<tr>
<td>Thermopneumatic</td>
<td>Micropump, microvalve, inkjet printhead</td>
</tr>
<tr>
<td>Shape memory alloys</td>
<td>Microvalve, fiber optic switch</td>
</tr>
<tr>
<td>Electrothermal (Phase change)</td>
<td>Microvalve, micropump, micromixer</td>
</tr>
</tbody>
</table>

Table 5.1: Illustration of the use of different types of actuators.

Electrostatic actuation is the most frequently applied principle combining versatility and simple technology. It needs neither additional elements like coils or cores, nor special materials like shape-memory-alloys or piezoelectric ceramics. Above that, the electrostatic actuation draws its force from the relation of surface to spacing and not
from the relation of volume to spacing, therefore it is less affected by scaling and more favorable for Very Large Scale Integration (VLSI) actuators [5.10].

Rotational and linear micromotors are often found to be a key part of micromechanical systems allowing them to perform physical functions. They can be used in x-y-stages, for aperture controlling in microphotronics, driving forces for micro-relays, micro-mirrors and micro-grippers. They also initialise mechanical systems, carry out on-chip assembling and rise pop-up structures. The most commonly used activation principle for micromotors is the electrostatic field between the plates of capacitors including comb drives [5.11], curved electrodes [5.12], scratch [5.13], wobble [5.14], linear stepping [5.15] and side drives with synchronous and asynchronous operation [5.16].

Actuator selection is critical to the success of the application. Two microfluidic applications that invariably require actuators are pumping and valving. An ideal actuator for pumps and valves in microfluidic systems has a simple structure and is capable of high forces (~1N), large deflections (~20μm) while operating at low voltages (~5V).

<table>
<thead>
<tr>
<th>Actuator type</th>
<th>( P_a \ (J m^{-3}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape Memory Alloy</td>
<td>( 10^7 )</td>
</tr>
<tr>
<td>Solid-Liquid Phase Change (paraffin)</td>
<td>( 10^7 )</td>
</tr>
<tr>
<td>Solid-Liquid Phase Change (PEG)</td>
<td>( 10^7 )</td>
</tr>
<tr>
<td>Thermo-pneumatic</td>
<td>( 10^6 )</td>
</tr>
<tr>
<td>Thermal Expansion</td>
<td>( 10^5 )</td>
</tr>
<tr>
<td>Electromagnetic</td>
<td>( 10^5 )</td>
</tr>
<tr>
<td>Electrostatic</td>
<td>( 10^3 )</td>
</tr>
<tr>
<td>Piezoelectric</td>
<td>( 10^5 )</td>
</tr>
</tbody>
</table>

*Table 5.2: Comparison of actuation power for different actuation mechanisms.*
The factor that determines the performance of the actuator is the actuation power. The actuator we have fabricated is a polyethylene glycol based electro thermal actuator and the actuation power is calculated below. Table 5.2. illustrates the actuation power obtained using other actuation mechanisms.

\[ P_a = \frac{F_a e_a}{V_a} = \frac{(1N)(20\mu m)}{(15\mu m)(3.14)(1000\mu m)^2} \]

\[ \text{Volume} \]

5.3 Pumps for microfluidic systems

Pumps are essential in microfluidic systems for fluid transport but also have other applications like mixing, cooling and pressure generation. Pumping can be achieved using different actuation mechanisms such as electrohydrodynamic, electrokinetic, mechanical displacement and bubble generation. All of these have been miniaturized and used for pumping liquids in mostly glass and silicon based microsystems. Ideally pumps should be inexpensive, easy to fabricate and easy to integrate within a plastic microfluidic system with high flow rates and pressure generation at relatively low power. For most bioassay applications bubble generation and local heating of the liquid are undesirable. We have developed a wax-based actuator similar to the actuator developed by Carlen et al. [5.16] that makes use of the large volumetric expansion of wax when it transitions from solid to liquid phase to generate large volume displacement that can be used to pump liquids. The volumetric expansion of PEG 8000 is significant and volume changes of up to 30% can be obtained. The fabrication technique is simple and inexpensive and these actuators can be mass fabricated on cast epoxy substrates. The actuator is used in a nozzle-diffuser [5.17] arrangement in series to generate a net fluid flow. The pump uses relatively low power and there is no bubble generation because it operates at low temperatures (< 100 °C at the surface of the wax patch). The device is fabricated using epoxy casting. The channel structure is cast on one substrate and the wax actuator is
fabricated using a screen-printing technique on another substrate. Both the substrates are then bonded together using an adhesive bonding technique.

5.4 Nozzle-diffuser pumps theory

Nozzle-diffuser pumps are built by coupling a nozzle, pumping chamber and diffuser together in series. The volume of fluid in the pumping chamber is periodically varied using an oscillating pressure (actuator). The kinetic energy of the fluid (velocity) is converted to potential energy (pressure) in both the nozzle and diffuser but the efficiency of this process is much greater in the diffuser direction thus discharging more fluid through the diffuser. If \( Q_d \) is the discharge through the diffuser and \( Q_n \) the discharge through the nozzle then an increase in the chamber volume causes \( |Q_d| > |Q_n| \) and a decrease in the chamber volume causes \( |Q_d| < |Q_n| \) resulting in a net pumping action (Fig 5.1.).

Figure 5.1: Principle of operation of nozzle-diffuser pumps and fluid flow with increasing and decreasing chamber volume.

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The pressure differences across the nozzle and diffuser are given by:

\[
\Delta P_d = \frac{\rho v_d^2 \xi_d}{2} \quad \text{and} \quad \Delta P_n = \frac{\rho v_n^2 \xi_n}{2}
\]

where:

\( \Delta P_d \) and \( \Delta P_n \) = Pressure difference across the diffuser and nozzle
\( \xi_d \) and \( \xi_n \) = Pressure loss coefficients for the diffuser and nozzle
\( v_d \) and \( v_n \) = Average velocities at the diffuser and nozzle

If \( \xi_d \) is less than \( \xi_n \) then pumping will occur in the direction of the diffuser [5.18]. These pumps cannot generate large pressure and are suitable for low-pressure fluidic systems.

And the volume flow rate is given by:

\[
Q = 2Vf \frac{[(\xi_n / \xi_d)^{1.2} - 1]}{(\xi_n / \xi_d)^{1.2} + 1}
\]

where:

\( Q \) = Volume flow rate
\( V \) = Volume variation per cycle
\( f \) = pulse frequency

5.5 Fabrication

The device is fabricated using plastic casting as shown in Fig 5.2. The substrate with the channels, reservoirs, pumping chamber and the nozzle-diffuser geometry is made using epoxy EPOTEK 301-2 FL (Fig 5.3.a.) which is a clear optical grade epoxy (> 97% light transmission), low viscosity (125 cpS at 100 rpm) and glass transition temperature.
Figure 5.2 (a) and (b). Shown is the process flow for fabrication of the channel structure and wax actuator respectively.

$T_s = 65 \, ^\circ C$. A stamper with 50 $\mu$m tall negative replicas of the channels, chamber and nozzle-diffuser geometry is formed by etching a silicon wafer using a deep RIE process ($SF_6$ 160 sccm, $C_4F_8$ 80 sccm $O_2$ 13 sccm, coil power 800W, platen power 600W). The stamper is then vapor deposited with a 1 $\mu$m thick parylene layer, which serves as a release layer to aid separation of the cured plastic from the stamper. The silicon stamper is sandwiched with a Teflon O-ring (2 mm thick) and flat polypropylene disc in an aluminum mold. The epoxy resin is injected into the sandwich and maintained at 80 $^\circ$C.
for 3 hours and then the cured plastic is released. Access holes for channels and electrodes are drilled using a micro drill press. A second epoxy substrate is made by casting using EPOTEK -314 a clear, high temperature epoxy ($T_g = 150\,^\circ C$). This epoxy is essential since metal heaters and contacts. The two substrates are then bonded using an adhesive are patterned on the surface using conventional photolithography techniques and the plastic must be able to withstand temperatures of up to $110\,^\circ C$. This epoxy is made using the same technique but a blank silicon wafer with a $1\,\mu m$ thick parylene layer is used instead of the etched stamper. The released substrate is then vapor deposited with a $0.5\,\mu m$ thick parylene layer to improve adhesion of metals to the surface. Then metal layers Cr/Al ($50\,nm/200\,nm$) are evaporated onto the substrate and heaters and contacts are patterned using photolithography.

The wax patches are made using a screen-printing process. The screen-printing process uses a $15\,\mu m$ thick stencil screen made of aluminum with precision cut openings for wax printing. The plastic substrate (EPOTEK 314) with the patterned heaters is then mounted on the screen printer and the openings on the screen and the heaters on the substrate are aligned and the screen is locked in position. The wax used is Poly ethylene Glycol, MW 8000 (PEG 8000), which is a water-soluble carbowax with a melting point of $65\,^\circ C$ and a volumetric expansion of $30\%$ on transition from solid to liquid. The wax is melted by heating to $80\,^\circ C$ and then screen-printed using a teflon squeegee (2kPa pressure, 8 ft/min speed). The substrate is released and placed in an oven at $65\,^\circ C$ for 3 minutes for the wax to re-flow and to obtain a smooth surface. The contacts for the heaters are masked using masking tape and the substrate is coated with a $4\,\mu m$ thick parylene C layer. Fig 5.3.b. shows a picture of $15\,\mu m$ tall wax actuator.

The EPOTEK 301-2 FL substrate with the channels and nozzle-diffuser geometry is spin coated with a $0.5\,\mu m$ thick layer of bonding wax solution (Blanchard bonding wax dissolved 1:1 by weight in acetone) and then aligned and bonded to the EPOTEK 314 substrate with the wax actuator using a laminator with the rollers at $60\,^\circ C$, speed of 2 ft/min and gauge of 4 mm. The access holes for the electrodes are filled with conductive epoxy and cured at room temperature for 18 hours. Figure 3.c. shows a picture of the
bonded device. The pumping chamber is 500 μm in diameter with diffuser angle of 30°, and channel widths of 400 μm.

Figure 5.3: (a) Pictures show SEM of EPOTEK 301-2 FL substrate with chamber, channel and nozzle-diffuser geometry. (b) wax actuator on EPOTEK 314 substrate and (c) bonded nozzle-diffuser pump respectively.
5.6 Thermal Modeling

Actuation methods like bubble based actuation makes use of bubble generation in the liquid to create a volume change in the pumping chamber and cause a net flow in one direction. Bubble generation occurs at elevated temperatures and is not ideal for transport of biofluids, which are sensitive to high temperatures. The wax-based actuator operates at lower temperatures and the thermal modeling, which was performed to determine the heater temperature and the heat transferred to the fluid in the channels, shows that the temperature at the wax-liquid interface at the maximum operating power is less than 100°C. This is essential to avoid generation of bubbles and to prevent sample loss due to evaporation and sample damage due to high temperatures.

A one dimensional steady state heat transfer model was developed to first model the temperature of the heater with increasing power. The heater is fabricated on top of an epoxy substrate by evaporation of 200 nm of Aluminum. The resistance of the fabricated heater, which is 0.01 m long, 100 μm wide was determined and found to be 80 ohms. The temperature of the heater can be determined by modeling the system using a steady state model with constant thermal properties and ignoring the losses due to radiation. By the first law of thermodynamics we know:

\[ E_g - E_{out} = E_{st} \]

where \( E_g \) , \( E_{out} \) , \( E_{st} \) are the energy generated, energy outflow and the energy stored per unit volume respectively and are given by:

\[ E_g = I^2 R_e \cdot L \]

\[ E_{out} = h(\pi DL) (T - T_\infty) \]

\[ E_{st} = \frac{d}{dt} (\rho V c T) \]

where \( I \) is the applied current, \( R_e \) is the electrical resistance per unit length, \( L \) is the length of the wire, \( D \) is the diameter of the wire, \( V \) is the total wire volume, \( T \) is the
temperature of the wire. $T_\infty$ is the ambient temperature, $\rho$ is the density of aluminum and $c$ is the specific heat capacity of aluminum. Since we assume steady state the term $E_{st}$ goes to zero. The equation reduces to:

$$\pi D h (T - T_\infty) = I^2 R_e.$$ 

The data for an aluminum wire with the given dimensions the temperature of the heater for different applied current was calculated and plotted in Figure 5.4. The maximum operating current was approximately 30 mA (5V AC).

![Figure 5.4](image)

Figure 5.4. The graph shows the calculated change in heater temperature with increasing current.

The entire actuator can also be modeled using a one dimensional steady state heat transfer model. Figure 5.5 shows a layer-by-layer representation of the actuator along with the thickness of each layer. The actuator that was modeled for test devices with a 2 mm diameter, the height of the actuators are only 20um and therefore the heat transfer through the sides is negligible. The convection heat transfer coefficient of water can be calculated using the equation $h = (Re)^{1/2} (Pr)^{1/2}/L$ where $Re$ and $Pr$ are the Reynolds number and Prandlt number respectively and $L$ is the length traveled by the fluid. The value of $h$ was 45 W/ m² K. and a convective heat transfer coefficient of air 100 W/m² K.
was assumed. The thermal conductivity of epoxy, PEG 8000 and parylene C are shown in table 5.3. The thermal resistances of each layer are calculated and since we know the ambient temperature and the heater temperature for different applied voltages the fluid temperature $T_w$ can be calculated.

$$q_c = q_1 + q_2$$

where $q_c$, $q_1$, and $q_2$ are equal to the power generated and dissipated in the upward and downward direction of the actuator.

$$q_1 = \frac{(T_c - T_w)}{R_1}$$
$$q_2 = \frac{(T_c - T_\infty)}{R_2}$$

Figure 5.5: Layer by layer representation of the actuator.
where $T_c$, $T_w$ and $T_x$ are the heater, fluid and ambient temperatures. $R_1$ is the sum of thermal resistances in the upward direction and $R_2$ the sum of the thermal resistances in the downward direction. The values of $T_c$ for different values of applied current have been previously calculated, therefore $T_w$ can also be calculated for different values of current and the plot is shown in Fig 5.6. From the figure it can be seen that the temperature of the fluid at the interface at maximum operating current 30 mA (5V AC) is below 100 °C.

<table>
<thead>
<tr>
<th>Material</th>
<th>Thermal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG</td>
<td>0.23 W/m K</td>
</tr>
<tr>
<td>Epoxv</td>
<td>6.92 W/m K</td>
</tr>
<tr>
<td>Parlylene</td>
<td>0.86 W/m K</td>
</tr>
</tbody>
</table>

*Table 5.3: Thermal conductivity of materials used in fabricating the PEG 8000 actuator.*

5.7 Measured Results

The surface profiles obtained due to deflection of the membrane due to expansion of wax at different applied voltages is shown in figure 5.7. Voltages up to 5V were applied and a maximum deflection of 23 μm was obtained at 5V. The deflecting 4 μm parylene C membrane containing the wax fails at higher voltages due to high stress obtained when the wax expand which results in the liquid wax escaping from the actuator.
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Figure 5.6: The graph shows the change in fluid temperature with increasing current. The pump usually operates below 30 milliamps (2.5V DC).

Deflection heights at different applied voltages

Figure 5.7: Optical profiles of the deflection heights of the wax actuator surface obtained at different voltages using a ZYGO optical interferometer.
Flow rate measurements were made using the setup as shown in figure 5.8(a) and the particle flow velocities were measured for different applied voltages and pulse frequencies. Volume flow rates were calculated and plotted (figure 5.9) and maximum flow.
Volume Flow Rate Vs. Frequency

Figure 5.9: Volume flow rates obtained at different pulse frequencies for different applied voltages.

Pumping Pressure vs Frequency

Figure 5.10. Pumping pressure generated at 5V plotted for different pulse frequencies.
rates of 70 nl/min was obtained at an applied voltage of 5V AC and pulse frequency of 1 Hz. The wax-based actuator operates best at lower frequencies (0.5 Hz - 1.5 Hz). This is due to the slow response time of the carbowax. High frequencies result in low volumetric expansion and hence low volume displacement because the heat generated due to the pulse is not sufficient to melt the entire wax patch.

Experiments were performed to calculate the pumping pressure generated using the nozzle-diffuser pump. The setup used was as shown in figure 5.7(b). The channels were filled with fluid and air was trapped in a column, which is located just past the diffuser. The pump was operated at 5V AC and the pulse frequency was varied. The pressure generated was calculated from the data obtained for height of the trapped air column. Maximum pumping pressure of 1400 Pa was obtained at a frequency of 1 Hz. Results are plotted in figure 5.10.

5.8 Summary

This chapter looks at another approach to integrating different functional components with a plastic microfluidic system. The approach involves building components on an already existing platform (plastic substrates) and using conventional surface micromachining techniques to create functional components. Actuators are the basic building blocks for active pumps, valves and mixer. The second section is an in depth review on different types of actuators and actuation mechanisms. Then we specifically looked at a polymer-based actuator which can be used for nozzle-diffuser pumps in microfluidic systems. The next half of the chapter goes into discussion on design, fabrication, modeling and testing of the fabricated PEG based actuator for nozzle-diffuser pumps in cast epoxy microfluidic systems.
CHAPTER 6

Tools for Molecular Biology

6.1 Introduction

The ability of microfabricated devices to interrogate and manipulate biomolecules is rapidly emerging (Table 6.1). Applications relating to genome sequencing and genomics have received widespread attention because of their use in clinical diagnostics and human disease. These applications are among the most mature (>10 years) and have already entered the commercial sector. Coupled with the bioinformatics revolution, they are poised to have a significant impact on people's everyday lives.

<table>
<thead>
<tr>
<th>Application</th>
<th>Advantage conferred by microfabrication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channels for molecular separations and sequencing</td>
<td>Small volumes, high throughput, high surface to volume ratio, higher resolution</td>
</tr>
<tr>
<td>On-chip PCR reactions</td>
<td>Small volumes, high throughput, high surface to volume ratio, faster heat transfer</td>
</tr>
<tr>
<td>Nucleic acid and Protein micro arrays</td>
<td>Small volumes, high throughput, batch processing, increased automation</td>
</tr>
<tr>
<td>Reactions</td>
<td>Small volumes, high throughput</td>
</tr>
</tbody>
</table>

Table 6.1: Advantages conferred by microfabrication to commonly used molecular biology assays.

Genomics is operationally defined as investigations into the structure and function of very large numbers of genes undertaken in a simultaneous fashion. After the early
demonstrations of the usefulness of electrophoresis microchips by Manz et al. [6.1], other
groups applied microfabricated device technology to the analysis of amino acids [6.2].
DNA restriction fragments [6.3-6.5], PCR products [6.6, 6.7] and DNA sequencing [6.8].
Electrophoresis microchip-based separation devices, like microreactor technology, were
based on techniques elaborated by the semiconductor industry, suggesting that channels
and other functional elements can be fabricated in glass substrates by microlithography.
With the advent of the so-called cross channel and double T injector setup, well-defined
sample quantities could be analyzed on electrophoresis microchips [6.9]. Samples are
typically loaded electrokinetically into the injector region, and the analyte molecules are
separated by applying the electric field, not only across the separation channel but also, to
a lesser extent, to the sample and waste reservoirs, preventing bleeding of the sample into
the separation channel [6.10]. The separated solute molecules are then most frequently
visualized by confocal microscopy with laser-induced fluorescence detection. Short
injection plugs, a high-strength electric field and short effective separation lengths result
in rapid separations in seconds and extremely high separation efficiencies because of the
minimized extra-column broadening effects [6.11].

The field of proteomics focuses on protein profiling, which is the identification
and ideally the quantitation of proteins of interest in a cell. Another part of the proteomics
effort involves the large-scale study of protein-protein interactions. 'Lab-on-a-chip'
technology is the method of choice to integrate processes and reactions and scale them
down from conventional glassware to microfluidics, involving micron-sized channels in
glass or polymer chips. A typical proteomics-based drug-discovery effort involves a first
phase in which cell lysates are obtained and analyzed by two-dimensional (2D) gel
electrophoresis. SDS PAGE is the most commonly used method to separate and size
protein mixtures. This gel method typically requires several hours for completion and is
fairly labor intensive. Several authors have investigated protein separations in
microchannels [6.12-6.24] to speed up separation and lay the building blocks toward
automation of protein sizing.

Protein profiling relies almost exclusively on MS to identify and possibly
quantitate proteins. A large body of work has recently emerged to integrate and speed up
the required pre-MS steps by using lab-on-a chip approaches. Some groups have focused on the upstream sample preparation [6.25-6.31], such as sample access and trypsin digestion, whereas other groups focused on separation and the chip/MS interface [6.32-6.38]. These devices show potential for high-throughput quality control of purified proteins. Rapid digestion in reduced volume [6.39-6.43] is a clear technological advance and chip/MS interface is being well characterized by many groups. The technical challenges that must be overcome to perform real proteomics on a chip include improving the separation resolution and the sensitivity of MS detection. Both these areas are particularly critical to a viable chip-based 2D separation.

Tools for molecular biology and biochemistry can be classified into two types based on the type of molecules, nucleic acids and proteins. Both these molecules play important functions in maintenance of cells and ultimately organisms. This chapter details both these molecules and discuses various techniques used to study, understand and analyze them. The techniques described can be miniaturized and integrated together to our advantage. Demonstration devices for Polymerase chain reaction (PCR) and Capillary electrophoresis of DNA have been fabricated and tested. Methods of fabrication and device characterization are discussed and results are illustrated.

6.2 Genetic Information and Analysis techniques [6.51]

The genetic information in all cellular and some viral life is contained in genes, which are made up of DNA (deoxy ribonucleic acid). A DNA chain is a long unbranched polymer composed of four different deoxy ribonucleotides containing the four bases Adenine (A), Cytosine (C), Guanine (G) and Thymine (T). Nucleotides are linked together by phospodiester bonds that join the 5' carbon of one deoxyribose group to the 3' carbon of the next. Figure shows the arrangement of the bases and sugars to form a DNA strand.

The DNA molecule is a helical polymer composed of 2 strands of DNA. The bases are arranged on the inside and the sugars on the outside of the helix making the core of the helix non-polar and the exterior of the helix polar. Adjacent bases from
opposite strands are very close and base pairing occurs between the bases through weak hydrogen bonds. An A always base pairs with a T and a C always base pairs with a G which results in the two strands of DNA being complementary to each other.

The double stranded DNA in eukaryotes is very long and is packaged into chromosomes. The double stranded DNA is bound around proteins called histones like beads on a string. DNA that is associated with histones is called chromatin. Chromatin fibers are packed together to form packed nucleosomes. The nucleosomes fold into loops, which fold again to give the metaphase chromosome. Different organisms have different number of chromosomes, humans have 46 chromosomes and yeast has 34 chromosomes.

Only certain portions of the DNA code contain the coding information and these DNA are called genes or coding DNA. One of the strands of DNA is the coding strand and the other is the template strand. RNA (ribo nucleic acid), which is similar to DNA except that it has a ribose instead of a deoxy ribose and one of the bases Thymine is replaced by a Uracil is synthesized from the template strand by a process called Transcription. The RNA produced is called messenger RNA or mRNA. Proteins (chain of Amino Acids linked by peptide bonds) are synthesized from the mRNA by a process called translation. Every three bases code for an amino acid and are called codons. There are start and stop sequences that initiate and terminate the process. The proteins produced are further modified, packaged and sorted and sent to different organelles where they perform their specific functions, which control the activities of the cell.

Therefore essentially all information related to cell growth and regulation is stored in the DNA in the nucleus of the cell. One of the ways to understand how the cell functions is to identify the genes that code for different functions and study them. This involves extraction and purification of DNA from the nucleus, which involves lysis of the cell membrane using heat or detergents to release the DNA from the cell. The other cellular impurities can be removed by methods like centrifugation, filtration or chromatography finally leaving the DNA in a solution. The purified can then be amplified and then sequenced to know the entire sequence. other assays like restriction
Chapter 6: Tools for Molecular Biology

digest, hybridization or expression assays can be done to further understand the function of that particular gene.

6.2.1 Chemical Amplification

DNA extracted from cells is usually present at concentration levels that are too low for any assay or detection. There are a few enzymatic methods for amplification of DNA like strand displacement amplification (SDA) and polymerase chain reaction (PCR). However PCR has been used as the method of choice because of its extremely quick cycle time and amplification factors as large as $2^{30}$. To amplify a sequence of DNA, two short oligonucleotide primers are annealed to the denatured (strand-separated) DNA under hybridization conditions ensuring that only primers perfectly complementary (or nearly so) with the desired sequence will anneal. The two primers are complementary to two 3' ends of the DNA segments to be amplified. The primers are then extended using DNA polymerase and the 4 deoxy nucleotide triphosphates (dNTPs), generating two duplex DNA copies of the targeted region. After a period of time long enough to allow DNA replication of the desired region the reaction is terminated and the DNA strands are separated using heat. This yields variable length fragments with the average length slightly larger than the distance between the primers. The three steps (annealing, elongation and denaturing) constitute one cycle of a PCR reaction. The process is repeated over many cycles and an almost exponential amplification is obtained. The three steps are accomplished by altering the reaction temperature. The annealing is usually done at 55°C, which is good for hybridization of perfectly annealed strands of DNA. The elongation temperature depends on the type of polymerase used. The most commonly used polymerase is Taq polymerase which is extracted from Thermus aquaticus which lives in hot springs at temperatures of 90-100°C. This enzyme works best at 70°C. The denaturing temperature is usually accomplished at 95°C. Thus the reactants have to be thermally cycled repeatedly to achieve the required amplification.
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Figure 6.1: The different steps involved in the PCR reaction.

The success of the reaction depends on the correct composition of components, precise temperature control, type of reaction chamber and presence of contaminants, which inhibit the reaction. After completion of n cycles of the reaction the concentration of the fragment of DNA amplified increases by a factor $F$ where $F$ is given by

$$F = (1 + E(n))^n$$

where $E$ is the efficiency factor depends on the number of cycles $n$ and it value ~ 1 when $n < 20$ and for values of $n > 20$ the efficiency drops. The losses in amplification cannot be exactly quantified so PCR cannot be used as a quantitative analysis tool. Conventional macro scale PCR usually takes about 90 minutes. A significant amount of time is spent waiting for heating and cooling to the required temperatures. Micro scale devices due to the sub microliter volumes and increased contact area, have the capability of heating and cooling at rates of a few degrees per second which make these devices extremely fast and
the reaction time is dependent more on the time taken for the enzyme to perform its function rather than the equipment heating or cooling time.

### 6.2.2 Detection Techniques

The presence of DNA fragments can be detected by tagging the DNA strand with suitable labeling molecules. Some labeling molecules can be end tagged (i.e.) attached to either the 5' or 3' ends of the DNA and other labeling molecules are intercalating and are attached between the two strands of DNA. End tagging usually has only one molecule per strand of DNA and is suitable for both single and double stranded DNA. Intercalating labeling molecules are present in multiple copies and are suitable only for double stranded DNA.

Earliest methods used radioactive $^{32}$P incorporated with the nucleotide. A single nucleotide has 3 phosphate groups (α,β and γ). When one nucleotide is attached to another the α phosphate is lost during the formation of the bond. Only the base at the 5' end has the α phosphate so replacing the α phosphate with a radioactive $^{32}$P would result in 1 tag per strand and replacing β or γ phosphates with radioactive $^{32}$P would result in multiple tags per strand.

Most commonly used methods are optical detection methods which involve attaching fluorescent molecules to the DNA and exciting them with either UV light or laser and observing the emitted light using epi-fluorescence microscopes with Photon multiplier tubes or cooled Charge coupled cameras. A commonly used tagging molecule is ethidium bromide an intercalating dye which when excited by an UV light source fluoresces only when it is bound between two nucleotides. Since one molecule is accommodated between two nucleotides the number of dye molecules is equal to the number of base pairs in the DNA strand thus giving a very strong signal. This again is used only for double stranded DNA. Single stranded DNA cannot use intercalating dyes and hence single stranded DNA detection techniques make use of end tagging using fluorescent molecules like fluorescin which can be attached by chemical reaction to the 5' end of the single stranded DNA. The signal however is weak and hence the
concentration of ssDNA has to be large or the sensitivity of the detector has to be high. Electro chemiluminescence is another technique, that uses end labeled Ru(bpy) \textsuperscript{3+-} a large bright molecule that emits light in the presence of an electrochemical reaction. This technique is a highly sensitive tagging method and is becoming increasingly important.

Electrochemical detection techniques, which make use of electrochemical tags, which produce signals when they pass over electrode, are also used. The electrochemical tags can either be end tagged or intercalating and can be used for both single and double stranded DNA detection

6.2.3 Restriction Digestion

The analysis of replicated DNA often involves fragmentation or digestion of the molecule. Double stranded DNA may be specifically cleaved at particular sequences by the action of enzymes known as restriction endonucleases. The cleavage by a restriction endonuclease depends on the DNA sequence. A given DNA molecule will have a characteristic pattern of cleavage when treated with several different restriction endonucleases. This property allows us to distinguish or identify different DNA molecules of the same length, but with different sequences without sequencing them. The process of determining the location of restriction endonuclease cleavage sites within a piece of DNA is known as restriction mapping. The fragments of DNA that result from restriction digest are commonly referred to as restriction fragments.

6.2.4 Electrophoretic Separation

Electrophoresis is a frequently used technique to separate and identify DNA molecules of different sizes. At alkaline pH DNA and RNA are negatively charged and therefore migrate in an electric field. For smaller DNA fragments polyacrylamide gel electrophoresis (PAGE) is the method of choice and for larger DNA molecules agarose gel electrophoresis is used.
All nucleic acids are of similar chemical composition and have a high charge to mass ratio at physiological pH. Therefore the mobility of various DNA and RNA molecules in agarose and polyacrylamide gels depends primarily on the frictional coefficient, which is related to the length and conformation of the nucleic acid. Most linear double stranded DNA fragments will be separated by electrophoresis on basis of length (molecular mass). However supercoiling and circular DNA can affect mobilities. Single stranded molecules present a more complex phenomenon since they have considerable secondary structures such as loops. To avoid the effects of secondary structures in electrophoresis, single stranded nucleic acid molecules are separated in the presence of strong denaturants. Markers, which contain different fragments of known lengths, can be run side by side and the lengths of the unknown fragments can be deduced.

Figure 6.2: Electrophoresis separation of nucleic acids.
The nucleic acid molecules drift under the influence of an electric field $E$ toward the anode with a velocity $v$, which is given by:

\[ v = \mu_i(N_i)E \]

where $\mu$ is the fragment mobility. The mobility depends on the type of mobile phase and fragment size $N_i$. When the fragments of different sizes are introduced in the gel and the field is applied they drift towards the anode and the fragment separation is $\Delta L = \Delta \mu E t$ where $\Delta L$ is the distance between two bands and $t$ is the drift time. The resolution for separation is measured by the number of theoretical plates, $N$, which is equal to the square of the number of distinguishable bands that fit within a given length of the gel. If the band width is determined by the diffusional spreading with a diffusion coefficient then $N$ is given by:

\[ N = \frac{\mu V}{2D} \]

Therefore high resolutions are obtained at high voltages but high voltages also mean high temperatures, which can cause damage to the nucleic acid molecules. This relationship is also independent of length and it only holds good for uniform fields.

Electrophoresis is usually done on slab gels over distances of 20 – 100 cm at fields of 5-40 V/cm, which usually takes about 30-60 min. Electrophoresis can also be done on capillaries of diameters 10-300 μm diameter and 50 cm long. High-resolution separations can be obtained with applied fields of about 1200 V/cm in less than 10 min.

6.2.5 Sequencing

A great deal of information can be obtained by knowing the exact base pair sequence of the gene. Genes with long DNA sequences first have to be cut using restriction endonucleases into smaller fragments (~ 1000 bp) and then the smaller
fragments can then be sequenced using either of the two methods available the Maxam-Gilbert technique or the Sanger sequencing technique.

The Maxam-Gilbert technique is a chemical cleavage method. The strategy involves specific cleavage of end labeled DNA at only one type of nucleotide under conditions such that each molecule is broken at an average of one at a randomly located nucleotide (i.e.) Four reactions are carried out using chemical reagents that cleave after only one particular base. And the concentrations of the reagent are at levels that only one random cleavage reaction at that particular base occurs per molecule, which leaves a set of radioactive fragments which extend from the end labeled 5’ end to the position occupied by that chosen base. The fragments obtained using each of these four reactions can then be electrophoresed on a gel side by side and the sequence can be determined by looking at the bands on the gel.

Figure 6.3: Sanger reaction for sequencing of DNA.

An alternative method is the Sanger sequencing technique that can easily be automated and the automated sequencers can be used for sequencing any given DNA
sequence. This method combines replication techniques along with electrophoretic separation. The technique utilizes DNA polymerase to make complementary copies of the DNA that is to be sequenced and deoxy nucleotide triphosphates (dNTPs) are added to accomplish this. Four reactions are done side by side and in each one of those reactions one of the four Dideoxy nucleotide triphosphates (ddNTPs) are also added which when incorporated into a strand instead of a dNTP causes chain termination. The ddNTPs are added in quantities that are small enough that the replication results in strands of varying length which have been terminated at that places where that particular dNTP would have been incorporated. These fragments of DNA can then be electrophoresed and the sequence can be read by looking at the position of the bands on the gel.

6.2.6 Hybridization

![Figure 6.4: Illustration of hybridization in an 8 X 10 microarray well.](image)

Hybridization is the technique used to describe the renaturing or base pairing between complementary base pairs on two strands of nucleic acid thus resulting in a double strand through the formation of hydrogen bonds. The hybridization between two strands occurs only at specific temperatures and pH conditions and is highly specific.
since base pairing can occur only between complementary bases. Hybridization assays usually occur between immobilized probe, which is a tethered nucleic acid with a known sequence and a target, which is the free nucleic acid whose identity/abundance is being detected.

Hybridization experiments can be done using microarrays. Microarrays are arrays of micrometer sized reaction chambers usually made out of glass or plastic where different probes are laid down and then the sample that is being tested is eluted over the microarray and hybridization occurs between complementary strands in the sample and the probe. The unbound excess is washed away leaving only the bound nucleic acids which can be detected using methods like fluorescence detection. Microarrays can be used to perform DNA-DNA hybridization, DNA-RNA hybridization, Protein-Nucleic acid interactions and used for variety of applications including gene identification, gene expression, polymorphism detection, drug discovery and pathogen analysis. The advantage of using microarrays is that they allow multiple samples to be analyzed in parallel. Microarrays provide a high throughput large-scale technology that is necessary for the study of interaction of individual genes in an organism and allow the study of multiple genes simultaneously.

Probes can be immobilized to the microarray by covalently attaching the probe to the glass or plastic surface or by using liquid or gel to suspend the probe to the chamber. The two prominently used techniques that are used to lay down probes are the Stanford technique and Affymetrix technique. The Stanford technique uses a device, which is similar to an inkjet printer, which can be programmed to place the probes at precise locations on the array. The Affymetrix technique is a photolithographic method, which uses a series of masks, and the nucleic acid probe is built base by base. Light is used to activate the sites where a base is to be laid down and when the nucleotides are washed over the array then attach themselves at the activated locations and then a new mask is used and another set of bases are laid down. The differences - apart from the way the probe is laid down - are in the fact that only small probes of the order of 10-20 bp can be laid down using the Affymetrix technique, whereas the Stanford technique can be used to lay down larger probes.
6.3 Proteins and Analysis Techniques [6.52]

Proteins are the machines that drive cells and, ultimately, organisms. Proteins are composed of individual units called amino acids. Amino acids all share a similar structure. The difference between them is the so-called "R" group. The "R" group is the cluster of atoms that give an amino acid its particular characteristics.

Amino acids are strung together in particular sequences: a given sequence will fold up into a specific structure. If each amino acid is given a code letter (for example, leucine is "L"), then a protein sequence resembles a written word or sentence.

While an amino acid is a letter in the sequence of the protein, in the structure each amino acid letter is actually a piece of a three-dimensional jigsaw puzzle. The relationship between amino acids and protein structure can be seen by looking at a picture of two amino acids connected to form a dipeptide. Each protein is not just a sequence and a structure, but is also a nanomachine that can perform a particular task. The letters determine the sequence, the sequence determines the structure, and the structure determines the task. For example, some proteins latch onto biochemical compounds and transform them into different compounds. These proteins are called enzymes. One such enzyme, lysozyme, is found in mucus and helps fight bacterial infections by selectively chopping bacterial cell walls into smaller fragments. Other proteins, such as insulin, are hormones. Insulin is secreted by the pancreas to stimulate the uptake of excess glucose by liver cells for conversion to glycogen.

By understanding how different proteins fold up and how they work, we can begin to understand how they work together to make up a cell. The diversity of protein structure underlies the very large range of their function. The functions performed by proteins are detailed below.
6.3.1 Protein Functions

6.3.1.1 Enzymes—biological catalysts

Most of the chemical reactions that occur in biological systems are catalyzed by enzymes, which are proteins. Some relatively straightforward reactions such as hydration of carbon dioxide and the modification of small organic molecules are enzyme-catalyzed; at the other end of the scale are complex transformations of large molecules. Enzymes are involved in the reading of genetic information stored in DNA, the first step in the synthesis of proteins themselves. In addition, enzymes synthesize the non-protein components of cells. Thus, enzymes are a central component of "cellular machinery". The rates of the reactions they catalyze are generally increased by the order of at least a million-fold.

6.3.1.2 Storage

Various ions, small molecules and other metabolites are stored by complexing with proteins: for example hemoglobin carries oxygen and iron is stored by ferritin in the liver.

6.3.1.3 Transport

Proteins are involved in the transportation of particles ranging from electrons to macromolecules. Iron is transported by transferrin; hemoglobin occurs in red blood cells, and so delivers oxygen from lungs to other tissues, and also plays a role in the transport of carbon dioxide to the lungs. Some proteins form pores in cellular membranes through which ions pass; the transport of proteins themselves across membranes also depends on other proteins.

6.3.1.4 Messengers

Proteins are involved in the transmission of nervous impulses, by acting as receptors of small molecules that cross junctions separating nerve cells.
organism, biological processes must be coordinated between cells in tissues and indeed between different organs. This is achieved by the signaling molecules called hormones: a number of hormones are proteins (for example insulin). Proteins also act as hormone receptors.

6.3.1.5 Antibodies

The immune system depends on the production of antibodies: proteins that bind to specific foreign particles such as bacteria and viruses.

6.3.1.6 Regulation

The information required to synthesize proteins is stored in genes (sequences of DNA). The precise orchestration of cellular activity requires that the various gene products (proteins) are present in appropriate quantities at the correct times. Enzymes synthesize proteins by translating sequences of DNA, and this production can be promoted or repressed by other proteins, in complex feedback mechanisms.

6.3.1.7 Structural proteins

Some proteins have a structural role, providing mechanical support. The "skeleton" of a cell consists of a complex network of protein filaments. On a larger scale, muscle contraction depends on the action of large protein assemblies. Other organic material such as hair and bone are also based on protein. Collagen is found in all multicellular animals, occurring in almost every tissue. It is the most abundant vertebrate protein: approximately a quarter of mammalian protein is collagen.

6.3.2 Protein Structure

Proteins are linear heteropolymers of fixed length: i.e. a single type of protein always has the same number and composition of monomers, but different proteins have a range of monomer units, from a few tens to approximately a thousand. The monomers are amino acids, and there are 20 types, which themselves have a range of chemical properties. There is therefore a great diversity of possible protein sequences. The linear
chains fold into specific three-dimensional conformations, which are determined by the sequence of amino acids: proteins are generally self-folding. The three-dimensional structures of proteins are therefore also extremely diverse, ranging from completely fibrous to globular.

Protein structures can be determined to an atomic level by X-ray diffraction and neutron-diffraction studies of crystallized proteins, and more recently by nuclear magnetic resonance (NMR) spectroscopy of proteins in solution. However there are many proteins whose structures cannot yet be solved.

Protein sequences are encoded in DNA, the holder of genetic information, which is itself a linear molecule composed of four types of "base" (monomers that act as "letters" of the genetic alphabet. In principle, it should therefore be possible to translate a gene sequence into an amino acid sequence, and to predict the three-dimensional structure of the resulting chain from this amino acid sequence. However, there are numerous problems that make this very difficult, as we shall see later on.

The field of research dealing with the prediction of structure from sequence is generally known as bioinformatics. The reason that this field is so important is that the structure of proteins is intrinsically related to its function. Experimental structure determination, or structure prediction, aids the elucidation of protein function: conversely, synthetic protein sequences might be designed so that the protein performs a desired function.

The study of protein structure is therefore not only of fundamental scientific interest in terms of understanding biochemical processes, but also produces very valuable practical benefits. The field of proteomics focuses on protein profiling, which is the identification and ideally the quantitation of proteins of interest in a cell. Another part of the proteomics effort involves the large-scale study of protein-protein interactions. Microfluidics can play an important role in elucidation of protein structure and determination of the amino acid sequences of different proteins. Two commonly used techniques for these purposes are electrophoresis and mass spectroscopy.
6.3.3 Protein analysis methods

Proteins carry both positive and negative charges with their net charge being dependant on pH. The pH that gives a zero net charge is the isoelectric point or pI. The pI is a very important property of a protein. Proteins are generally least soluble at their pI and most readily crystallized. In addition they are frequently least stable at their pI.

Knowledge of the pI of a protein assists in the design of purification protocols for that protein. Most proteins have a pI value of between 5 and 9 depending on the number of positive and negative charges exposed to solvent. An important property of proteins is that many of them can exist as charge isomers. These have essentially the same molecular weight but mutations affecting charged residues result in isomers with different charges and hence different pI.

6.3.3.1 Isoelectric Focusing

![Isoelectric Focusing of Proteins](image)

*Figure 6.5: Isoelectric focusing of proteins.*

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In IEF proteins are electrophoresed in a gel containing a pH gradient. This gradient is generated by compounds known as ampholytes. These are mixtures of amphoteric buffers with a range of pI values. During electrophoresis they migrate according to charge and form a pH gradient. Ampholytes are commercially available to generate pH gradients over defined ranges such as 2.5-11, 5-7 or 8-10. To perform an IEF analysis proteins are applied to a gel (polyacrylamide or agarose) and current is applied. The proteins in the mixture will then migrate according to charge density until they reach that part of the gel with a pH corresponding to their pI. At this point, their net charge is zero and migration stops.

6.3.3.2 SDS Poly Acrylamide Gel Electrophoresis (PAGE)

SDS PAGE is the most commonly used method to separate and size protein mixtures. This gel method typically requires several hours for completion and is fairly labor intensive. Protein separations in microchannels are performed to speed up
separation and enable automation of protein sizing. The sensitivity must be at least comparable to existing gel methodologies. The two requirements combined make fluorescence the method of choice for chip-based protein separation.

Capillary electrophoresis is an automated high-resolution approach to electrophoresis. Separation is carried out in a capillary, around 25-75 μm internal diameters. The separation takes place in free solution and convection currents are controlled by the capillary. CE is more versatile than gel electrophoresis and it can be used to analyze proteins, nucleic acids, peptides, carbohydrates, anions, cations, vitamins, organic acids, amino acids, pesticides, even whole cells and viruses. CE is not yet a mature technique and applications are developing all the while. In addition, as the cost of instrumentation falls, the technique will become more widely available.

6.3.3.3 Gel filtration chromatography

Figure 6.7: Gel filtration chromatography of proteins.
Gel-filtration chromatography separates proteins on the basis of size. The technique measures the relative rates of passage through a molecular sieve. This molecular sieve is in the form of a polysaccharide gel in the shape of spherical beads. The column is eluted with buffer and the protein concentration in the elute is measured.

\[ V_0 = \text{Void volume: volume of buffer outside the beads. This is the volume needed to elute the largest, completely excluded proteins.} \]

\[ V_e = \text{Elution volume: volume needed to elute any given protein.} \]

\[ V_t = \text{Total volume: volume of buffer in the column, both inside and outside the beads.} \]

Determination of the elution volumes for proteins allows us to calculate partition coefficients \( K_d \)

\[ K_d = \frac{V_e - V_o}{V_t - V_o} \]

\( K_d \) is dependant on the average solution volume of the protein as described by the Stokes Radius.

### 6.3.3.4 Mass Spectroscopy

A mass spectrometer is an instrument that produces ions and separates them in the gas phase according to their mass-to-charge ratio (m/z). Today a wide variety of mass spectrometers is available, ranging from bench top detectors for gas chromatography to warehouse sized instruments such as accelerator mass spectrometers. All of these share the capability to assign mass-to-charge values to ions, although the principles of operation and the types of experiments that can be done on these instruments differ greatly.
Basically, a mass spectrometric analysis can be envisioned to be made up of the following steps:

**Sample Introduction → Ionization → Mass Analysis → Ion Detection/Data Analysis**

Samples may be introduced in gas, liquid or solid states. In the latter two cases volatilization must be accomplished either prior to, or accompanying ionization. Many ionization techniques are available to produce charged molecules in the gas phase, ranging from simple electron (impact) ionization (EI) and chemical ionization (CI) to a variety of desorption ionization techniques.

Mass spectrometers are operated at reduced pressure in order to prevent collisions of ions with residual gas molecules in the analyzer during the flight from the ion source to the detector. The vacuum should be such that the mean free path length of an ion, i.e., the average distance an ion travels before colliding with another gas molecule, is longer than the distance from the source to the detector. For example, at a pressure of $5 \times 10^{-5}$ torr for instance, the mean free path length of an ion is approximately one meter, i.e., about twice the length of a quadrupole instrument. Thus, the introduction of a sample into a mass spectrometer usually requires crossing of a rather large pressure drop, and several means have been devised to accomplish this. Gas samples may be directly connected to the instrument and metered into the instrument via a needle valve. Liquid and solid samples can be introduced through a septum inlet or a vacuum-lock system. However, when connecting continuous introduction techniques like gas chromatography (GC), high performance liquid chromatography (HPLC) or capillary electrophoresis (CE), special interfacing becomes imperative to prevent excessive gas load.
6.4 Demonstration Devices

6.4.1 Introduction

The integration of multiple analysis functions on a single device, and the integration of off-chip device control elements, will provide advantages in miniaturization, system portability, and operational automation. Microfabricated devices with multi-analytical functions for molecular and biochemical analysis have been reported on glass [6.44], silicon-glass [6.45] and silicon-plastic [6.46]. The use of plastics as substrates for these kinds of devices is becoming a feasible alternative approach as a result of the versatile material properties and the ease of batch fabrication through molding and embossing processes [6.47-6.49]. However, function or active component integration is difficult to achieve with these fabrication technologies. Plastic casting as an alternative technology presents unique advantages. While high-resolution microfluidic features can be fabricated by curing casting material against a microfabricated mold [6.50, 6.51], this technique also allows complex devices with integrated functional components is cast formed through embedding. In this part of the chapter we discuss the first cast formed miniature plastic micro thermal reactor with integrated heating, cooling and temperature sensing elements.

6.5 PCR Device

6.5.1 Device Fabrication

Materials for plastic casting are liquid monomers or pre-polymers that are stable under ambient temperature or in the absence of UV light. Once mixed with hardeners, or heated to a higher temperature, or brought under UV radiation, they start to polymerize or cross-link, becoming solids. Unlike other plastic replication techniques, casting process can be carried out under near atmospheric pressure at relatively low temperature ranges. This makes it possible to incorporate functional elements out of various materials. The monomers or the pre-polymers are in relatively low viscosity fluidic state during the casting process, which leads to intimate mold contact, and as a result, high-resolution micro features.
Figure 6.8 shows the structure of the thermal chemical reactor. The cylindrical reaction chamber (glass capillary, Laboratory Devices, INC, 1mm ID) is surrounded by a resistive heater coil (Scientific Instrument Services, W73). The reaction mixture temperature is sensed through a thermocouple (Omega Engineering, Inc. OD 250 μm). A thermal electric device (Melcor, 4mmx4mmx2.2mm) is positioned at the bottom of the chamber to facilitate cooling and to assist uniform heating. The entire assembly is embedded in a transparent polymer matrix (Epotek, 301-2Fl) with the normal heating side of the TE device exposed for better heat conduction.

Figure 6.8: Device design and fabricated PCR device.

Figure 6.9 shows the schematics of a casting mold assembly. Device components were first pre-assembled on a release-assisting surface at one side of the metal mold. A circular Teflon O-ring with a sample injection opening was placed around the component
assembly. The mold was closed by placing a second release assisting surface. After the mold assembly was tightened to avoid leakage, casting resin was injected and cured at 110 °C for 50 minutes.

Figure 6.9: Process flow for fabrication of the PCR device.

The fabrication scheme presented here allows achieving the 3-D nature of the structure. This would be very difficult to accomplish through other fabrication technologies that are based on surface micromachining or multilayer stacking.
6.5.2 Thermal Modelling

Temperature Field Near the Sample, Single Well

Figure 6.10: Meshing and Heat Distribution around the PCR chamber.
Modeling was carried out to understand the thermal performance of the device using ACE+ software package from CFD Research Corporation. The computational domain includes the plastics, sample, liquid wax and air regions as in a normal testing situation (Figure 6.10). The governing equation is the energy conservation equation in 2D asymmetry form. The boundary conditions assumed here were natural convection on the top of the cover as well as the sidewall of the device. At the bottom, the device is in contact with a thermoelectric (TE) device, and does have some heat loss. The computed steady-state temperature distribution is showed in Figure 4. The input power was varied until the maximum temperature of the sample was 94°C. At a heater power setting of approx. 0.12 W the maximum temperature inside the sample was 94°C. As seen here, the temperature in the sample is maximum inside the mid-height of the sample. A temperature variation of ~1°C was seen over the entire sample volume.

### 6.5.3 Device characterization

A controller (Physical Interface Device (PID) loop) (MOD30ML from ABB) was programmed to allow flexible automation. The temperature control point was taken from the thermocouple located at the bottom of the chamber. The output of the PID was sent to a HP power supply directly to provide heating via the coils. TE device when turned on was running at both heating and cooling modes, which are controlled via an exponential relationship based on the temperature difference between the set point and measured temperatures. The controller was connected to a PC that is running under a custom version of graphical user interface.

A two-step temperature cycle (94°C for 30 s and 70°C for 60 s) was used (Figure 6.11) to evaluate the thermal performance of the device. Figure 6.11 (a) shows the measured temperature when the device has active heating/cooling control. (i.e., heating coils and TE device are turned on). The heating rate is ~2.4°C /s while the cooling rate is ~2.0°C /s. When the device is controlled by heating coils only. (i.e., TE device turned off), the heating rate is ~1.0°C /s while the cooling rate is ~1.6°C /s (Figure 6.12 (b)).
6.5.4 On chip thermal lysis and PCR reaction

*Escherichia coli* cell lysis and the subsequent amplification of the released genomic DNA segments were carried out using the described micro-chip. Approximately two-thirds of an *E. coli* bacterial colony was collected and resuspended in 50 µL of sterile H₂O. This suspension was diluted 1:10 in a PCR reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 250 g/mL bovine serum albumin, 200µM each deoxynucleotide triphosphate, 1.0 µM each primer, 2.5 units/100µl AmpliTaq DNA polymerase. The cell and PCR mixture 1.5 µL was loaded in the micro reaction chamber. Chill-out™ liquid wax was topped on the solution to prevent sample evaporation. The temperature program was initiated at 94 °C for 1 min to induce cell lysis. The analyte was then cycled between 50 °C for 30 sec, 72 °C for 30 sec. The primer set used to amplify a 346-bp segment of the *E. coli* lamB gene was 5'-CTG ATC GAA TGG CTG CCA GGC TCC-3' and 5'-CAA CCA GAC GAT AGT TAT CAC GCA-3'. The PCR product was visualized and compared with control reaction through gel electrophoresis (Figure 6.12).
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Lane 1: DNA Ladder
Lane 2: Micro PCR in plastic device
Lane 3: Control PCR reaction in a conventional PCR instrument

Figure 6.12: PCR product compared with control reaction through gel electrophoresis.

6.6 Capillary Electrophoresis Device

6.6.1 Device Fabrication

Figure 6.13 shows the simplified microcasting process. First, patterns for channels, reservoirs, chambers, and reactors are etched in a silicon wafer using a deep RIE system (Specialty Technology Systems). (SF₆:130 sscm. O₂:30 sscm. C₄F₈: 80 sscm. coil power: 800 Watts and platen power: 600 Watts. 30 mins.) Next, a 1 μm-thick p-xylylene (parylene-C) layer is vapor deposited on the patterned silicon substrate [6.55]. This thin film serves as a release layer, which facilitates the separation between the casted material and the wafer surface. The patterned silicon substrate is then used to create a mold by joining it with a supporting plastic plate and teflon ring spacer. The mold assembly is then placed between two aluminum molds as shown in Figure 15. The mold is then heated to 45 °C for 8 hrs and filled with a low viscosity catalysed epoxy (EPOTEK 301-2. EPOTEK 301-2 FL). In order to seal channels and reservoirs, a flexible transparent film is laminated [6.56] on top of the epoxy substrates.
Figure 6.13: Process flow for fabricating the CE device.

The lamination process uses a desktop roll type laminator (Kepro Circuit Systems, Inc. BLT 121-A) and a thin mylar film (2 mil. Monokote, Top-Flite, Inc.) is used to laminate the plastic substrates. The substrate is first treated with acetone, and then the film is laminated onto the substrate by passing it between two rollers. The process is carried out at room temperature and at a feed rate of 2 ft/min. Figure 4 shows the cross section of a laminated channel. These structures show excellent permeation barrier characteristics with water permeabilities $P << 1.7 \mu l/cm^2/day$ at room temperature. When these capillaries are filled with sample there is no seeping present at the bond interface. The separation channel is 3 cm long and the reservoir dimensions are 5 x 5 mm$^2$. The channels for sample introduction are 0.5 cm long. Figure 6.14 shows a fabricated capillary electrophoresis device.
6.6.2 Separation Results (HEC)

The fabricated capillary electrophoresis devices were used to perform separations using a 0.5\%(w/v) hydroxyethylcellulose (HEC) (MW 90,000-105,000) sieving matrix. Approximately 3\(\mu\)l of the electrophoresis buffer (0.2g HEC, 4 ml of 1x TBE buffer, and 36 ml of distilled DI water) was introduced in the analyte waste reservoir using a stiff needle syringe. After the channels have filled completely due to capillary action, the other reservoirs are filled with the buffer and the analyte reservoir is filled with 0.5x TBE (1X Tris-borate EDTA). Pre-electrophoresis was done by applying 300 V/cm across the separation channel for 10 minutes and keeping the analyte and analyte waste reservoirs at a potential of 0 V. This is done to produce a concentration gradient in the HEC while increasing the concentration of HEC in the column above 0.5\%. Approximately 2 \(\mu\)l of a DNA sample labeled with SYBR Green I at an intercalating ratio of greater than 1:5 dye:DNA bp is loaded into the injection reservoir. Samples were then separated under an electric field of 110 V/cm in a distance of 3 cm. Figure 6.15 shows the DNA plug and separations obtained using the plastic capillary electrophoresis devices. The plastic has
very low background fluorescence and does not interfere with the fluorescence of the DNA bands.

![DNA plug and separated bands (HEC)](image)

**Figure 6.15: DNA plug and separated bands (HEC).**

### 6.6.3 Separations results (UV gels)

Though satisfactory results were obtained and it was shown that electrophoretic separations can be done in devices made of cast epoxy, the resolution of the bands obtained needs to be improved. In order to achieve this, separation was done again using the same devices but the sieving matrix was replaced by polyacrylamide. Controlled polymerization only in the separation channel was obtained using UV initiated cross linking, once the gel in the separation channels is polymerized the unpolymerized gel can be removed and filled with 0.5X TBE. The DNA is loaded in the injection channel and it diffuses everywhere except into the separation channel. Voltage (60 V/cm) is applied across the separation channel, and DNA slowly moves into the separation channel. Once there is a sufficient DNA plug in the separation the voltage is reduced to zero and the
remaining DNA in the injection channel is removed and 0.5X TBE is added. Separation is now done at 120 V/cm with the small DNA plug captured during the initial procedure. The resolution of bands obtained using UV initiated poly acrylamide is of better quality than the HEC sieving matrix. The bands however are not very sharp and tend to diffuse but resolution is good enough to identify 100 bp bands.

Figure 6.16: DNA plug and separated bands (UV gels).
6.7 Summary

In this chapter we look at the various applications of microfabricated devices in the field of molecular biology. The two major biomolecules are nucleic acids and proteins. This chapter details the structure and function of both these molecules and the techniques used to extract, isolate, purify and characterize these molecules. These techniques can all be miniaturized and done sequentially on microchips. To illustrate this devices for PCR of DNA molecules with integrated heating and cooling elements and a plastic capillary electrophoresis device were fabricated and tested. The device design, fabrication and testing are explained and the results obtained are shown.
CHAPTER 7

Tools for Cell Biology

7.1 Introduction

In the field of clinical diagnostics and pharmacology, the use of living cells for fast specific and non-specific chemical sensing is an area of increasing importance [7.1-7.3]. For example, cells in a liquid environment can be transported within microchannels by using electro-osmotic flow by applying high voltages across the microchannel [7.4, 7.5]. A cell or cell layer is a complex system with appropriate response to a variety of external physical and chemical excitations. Experiments on such living biological systems (e.g. using electrical resistance or fluorescence measurements [7.6]) can lead to the study of diffusion and transport of biological or pharmacological molecules through the cell or cell layer [7.7, 7.8]. Until now, all these experiments were done in macroscopic tissue resistance measurement chambers [7.9, 7.10]. Individual cell culture chambers, with a diameter ranging from a few millimetres up to some centimetres, are realised in arrays in standard plate compatible formats. In these devices, a cell tissue layer is grown on a nano-porous membrane placed at the bottom of the chamber and measured with two pairs of electrodes, which are mechanically inserted in the system after cell culture. Despite the functionality of these devices, there are several drawbacks. The relatively large size of the cell chamber requires relatively large amounts of cells and biological fluids. Also, the requirement of a perfectly closed cell culture layer (to prevent electrical short-circuiting events) is a condition, which is much harder to fulfill for the larger membrane surfaces. Moreover, the non-integrated and mechanically positioned electrodes are a source of uncertainty in determining the correct cell layer resistance, as varying contact geometries can give rise to large resistance contributions of the physiological cell medium. Evidently, the application of external electrical contacts also can be a source of cell culture contamination. The progresses in the field of
microfabrication technology for the fabrication of three-dimensional microsystems have opened new ways in miniaturization. Evidently, small millimeter-size or smaller cell culture chambers and membranes in a microsystem configuration with integrated electrical contacts and microfluidic feed-throughs do not suffer from all problems mentioned above.

Cellular mechanisms such as adhesion, migration, growth, secretion, and gene expression are triggered, controlled, or influenced by the biomolecular three-dimensional organization of neighboring surfaces. This organization is difficult to achieve since cells respond to local concentrations of a variety of molecules that may be dissolved in the extracellular medium (e.g. enzymes, nutrients, and small ions), present on the underlying surface (extracellular matrix (ECM) proteins etc.) or on the surfaces of adjacent cells (membrane receptors). In conventional cell culture, these factors are distributed evenly on the substrate. Microfabrication techniques enable the researcher to design, with precision control, the biochemical composition and topology of the substrate (otherwise homogeneously adherent to cells), the medium composition, and the types of cells in the vicinity of each cell. Over the years cell biologists have resorted to different approaches to recreate different degrees of cellular organization in the laboratory. Researchers have studied cell migration on spiderwebs as early as 1912 [7.11]. Others studied cell behavior on surface features such as milled grooves on mica [7.12], polystyrene replicas of diffraction gratings [7.13], polyvinyl chloride music records [7.14], dried protein spots [7.15, 7.16], crystals [7.17], and scratches in agar [7.18], in phospholipid films [7.19], or in ECM protein [7.20]. The technology utilized in these studies is not mature enough to address the structural dimensions, chemical heterogeneity or precise repeatability over large areas found in live tissue. Microfabrication technology offers the potential to control cell-surface, cell-cell, and cell-medium interactions on a micro and nano scales. There has been a lot of research on protein patterning, which is pertinent to cellular micropatterning [7.21].
7.2 Extracellular Matrix

The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells that are found within mammalian tissues. The ECM is often referred to as the connective tissue. The ECM is composed of 3 major classes of biomolecules:

- Structural proteins: collagen and elastin.

- Specialized proteins: e.g. fibrillin, fibronectin, and laminin.

- Proteoglycans: these are composed of a protein core to which are attached long chains of repeating disaccharide units termed of glycosaminoglycans (GAGs) forming extremely complex high molecular weight components of the ECM.

7.2.1 Collagen

Collagens are the most abundant proteins found in the animal kingdom. It is the major protein comprising the ECM. There are at least 12 types of collagen. Types I, II and III are the most abundant and form fibrils of similar structure. Type IV collagen forms a two-dimensional reticulum and is a major component of the basal lamina. Collagens are predominantly synthesized by fibroblasts but epithelial cells also synthesize these proteins.

7.2.2 Fibronectin

The role of fibronectins is to attach cells to a variety of extracellular matrices. Fibronectin attaches cells to all matrices except type IV that involves laminin as the adhesive molecule. Fibronectins are dimers of 2 similar peptides. Each chain is 60-70nm long and 2-3nm thick. At least 20 different fibronectin chains have been identified that arise by alternative RNA splicing of the primary transcript from a single fibronectin gene.

Fibronectins contain at least 6 tightly folded domains each with a high affinity for a different substrate such as heparan sulfate, collagen (separate domains for types I, II
and III). Fibrin and cell-surface receptors. The cell-surface receptor-binding domain contains a consensus amino acid sequence, RGDS.

### 7.2.3 Laminin

All basal laminae contain a common set of proteins and GAGs. These are type IV collagen, heparan sulfate proteoglycans, entactin and laminin. The basal lamina is often referred to as the type IV matrix. Each of the components of the basal lamina is synthesized by the cells that rest upon it. Laminin anchors cell surfaces to the basal lamina.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Collagen</th>
<th>Anchor</th>
<th>Proteoglycan</th>
<th>Cell-Surface Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibroblasts</td>
<td>I</td>
<td>fibronectin</td>
<td>chondroitin and dermatan sulfates</td>
<td>integrin</td>
</tr>
<tr>
<td>chondrocytes</td>
<td>II</td>
<td>fibronectin</td>
<td>chondroitin sulfate</td>
<td>integrin</td>
</tr>
<tr>
<td>quiescent hepatocytes, epithelial; assoc. fibroblasts</td>
<td>III</td>
<td>fibronectin</td>
<td>heparan sulfate and heparin</td>
<td>integrin</td>
</tr>
<tr>
<td>all epithelial cells, endothelial cells, regenerating hepatocytes</td>
<td>IV</td>
<td>laminin</td>
<td>heparan sulfate and heparin</td>
<td>laminin receptors</td>
</tr>
<tr>
<td>quiescent fibroblasts</td>
<td>V</td>
<td>fibronectin</td>
<td>heparan sulfate and heparin</td>
<td>integrin</td>
</tr>
<tr>
<td>quiescent fibroblasts</td>
<td>VI</td>
<td>fibronectin</td>
<td>heparan sulfate</td>
<td>integrin</td>
</tr>
</tbody>
</table>

*Table 7.1: Cells and respective ECM proteins*
7.3 Cell Attachment

Mechanisms by which cells recognize certain substrates as suitable for attachment and growth has been an important topic of study to understand cell proliferation and differentiation. Cell adhesion is mediated by cell membrane bound receptors. In particular, integrins, a family of heterodimeric transmembrane proteins that are linked to the cytoskeleton on the cytoplasmic side of the membrane, recognize specific peptide sequences present in the fibrillar protein meshwork found in vivo and known as the ECM. Integrins establish mechanical links between the membrane and the ECM substrate and also between the ECM and the cytoskeleton. Integrins aggregate in organized structures termed focal contacts [7.22–7.24]. In most cell types biochemical signals essential for cell growth, function, and survival are initiated by integrins upon attachment. Failure of integrins to attach causes the cell cycle to end resulting in apoptosis [7.25]. Since many cell types secrete ECM, an artificial substrate is capable of supporting cell adhesion even if it is not initially coated with an ECM protein. Success in creating cellular micropatterns depends on the ability to control the size, geometry, and chemical nature of the adherent layer. Cells are sensitive to other physical parameters of the substrate, such as local temperature, which may be used for selective attachment of cells to surfaces [7.26]. Materials other than physiological biomolecules were the first to be explored owing to the constraints imposed by early micropatterning techniques. Initially micropatterning techniques involved micro patterns of metals and polymers. With the emergence of more flexible microfabrication techniques ECM proteins, peptides with RGD sequences for cell attachment and bioactive molecules have been micropatterned. The following section is a detailed review about the existing techniques for micropatterning proteins.

7.4 Micropatterning proteins for Cell Attachment

Many researchers have tried a variety of different techniques to create protein micro patterns for cell culture. Physisorbed protein layers are not stable in aqueous solutions. Also, proteins present in the medium or secreted by the cells after attachment...
may displace an underlying protein layer [7.27]. Many researchers have focused on the
chemical immobilization of proteins via cross-linkers or photoreaction schemes [7.28].
Even though chemical (or physical) immobilization of proteins is likely to induce partial
denaturation of the protein structure, which can affect cell function [7.29, 7.30], the
peptide sequences necessary for attachment seem to remain largely exposed.
Denaturation is to be expected when the micropattern is exposed to solvents during the
micropatterning step. It is still found that cells attach, spread, grow, and function on
denatured ECM proteins, either physisorbed [7.31, 7.32] or chemisorbed [7.33, 7.34]
because denaturation does not affect integrin binding. It is believed that protein
adhesiveness correlates with the hydrophobicity of the surface. But neither very
hydrophilic surfaces such as agarose gels nor very hydrophobic surfaces such as teflon
support protein adhesion. Even though physisorbed protein layers may elute from the
surface as a function of time, physisorption procedures promoting cell attachment are
simple and have been used in cell culture on a variety of materials. Protein physisorption
onto polymers may be enhanced by plasma polymerization [7.35] or plasma glow
discharge (e.g. the tissue culture-grade polystyrene petri dishes) of the polymer surface.
These treatments introduce a rich variety of chemical functionalities that attract proteins
through electrostatic or dipolar interactions.

7.4.1 Lithography and Stencil Patterning

Letourneau and colleagues created the first biomolecular micropatterns [7.36]
based on the fact that cell adhesiveness of laminin could be inactivated by selective UV
irradiation through a metal stencil. Most existing microfabrication and micropatterning
techniques use photolithography. Photoresist is first patterned using by exposure to light
and then developed leaving patterned areas. Metals like gold, which are excellent for
protein attachment, are then evaporated on the substrate and dissolving the photoresist
results in a liftoff to create islands or features with metals. The whole process involves
several steps especially treatment with an alkaline or organic solution to dissolve the
photoresist which is incompatible with patterning proteins or cells. Photolithography
involves the use of solvents. Elastomeric stencils may be used to mask the physisorption
of protein or the direct attachment of cells on a surface [7.37]. The elastomeric
membranes can be used to provide patterned access to the surface. After patterning, the membranes can be physically lifted off the substrate. Stencils are convenient for patterning cell types, such as fibroblasts, which feature poor adhesiveness selectivity and for patterning cells on homogeneous surfaces. Multiple materials can be patterned using multiple. stacked membranes.

7.4.2 Micro-contact Printing

Another way to eliminate the problems associated with photoresist removal is to use metal [7.38] and elastomeric [7.39] microstamps for micropatterning ECM proteins followed by cell attachment and culture. The general procedure for micro-contact printing involves inking the stamp with a solution of the substance to be printed. As the solvent (ethanol) evaporates, the protein is deposited on the relief structure. The stamp is then brought into conformal contact with the surface for a predetermined period of time. On removing the stamp from the surface, a pattern is left that is defined by the features on the stamp. Rudolph [7.40] fabricated microtextured surfaces with deep trenches in a variety of biomedical polymers and selectively physisorbed proteins onto the mesas by carefully dipping the microstructures in a protein solution. As a result, only the mesas, and not the trenches, were coated with protein solution. With this method, cells to fibronectin coated PDMS mesas.

7.4.3 Patterning using laminar flows

In microfluidic systems with micrometer sized channels it is almost impossible not to have laminar flows. Reynolds number is a dimensionless parameter that determines the type of flow of the liquid. Laminar conditions in a fluid are obtained at low values of the velocity, the diameter of the capillary, and the density of the liquid and high values of the viscosity. Fluids are said to flow in the laminar regime if the Reynolds number is < 2000. Because the diameter of the channels is small for microfluidic systems, Reynolds numbers are typically 0.1–1 for flow rates commonly used. Channel systems, in combination with laminar flow, can be used to pattern material onto a surface from solution. Delamarche used multiple, adjacent channels to pattern proteins onto a surface.
and to direct chemical reactions on surfaces [7.41, 7.42]. The system uses small volumes of reagents and may be useful in immunoassays. Laminar flow of liquids can be used to generate simple patterns of proteins and cells inside a channel [7.43, 7.44].

### 7.5 Electrochemical deposition of conductive polymers

#### 7.5.1 Electrochemical deposition (ECD)

The abbreviation ECD applies to both electrochemical and chemical plating processes. Although in chemical plating (usually referred to as electroless plating) an external supply of "electrons" is not necessary, it is still an electrochemical process. For all types of ECD batch processing is possible, and the electrolytes are usually operated at temperatures from room temperature to about 90°C. also helps to reduce equipment needs as well as costs in general. Operating temperatures close to room temperature will reduce the problems originating from differences in thermal expansion (between Si and most metals), that are known from other deposition techniques.

#### 7.5.2 Basic Electrochemistry

An electrochemical reaction is a chemical reaction in which transfer of electrons from one species (called Red for reduced) generates another (called Ox for oxidised). If the number of electrons involved in the reaction is called $z$, this can be expressed as:

$$\text{Red} \leftrightarrow \text{Ox} + ze^-$$

Such a reaction is called a half-cell reaction, because the electron "donor" reaction must have an "acceptor" counterpart - since free electrons can not exist in the electrolyte. Consider an electrochemical cell consisting of two electrodes submerged in an electrolyte. If $z$ electrons are transferred, the two half-cell reactions would be:

**Left:** $A^0 \leftrightarrow A^{z+} + z e^- (E_{\text{left}})$  
**Right:** $B^{z+} + z e^- \leftrightarrow B^0 (E_{\text{right}})$
As indicated by the double arrows used in all the equations, the electrochemical reactions can move in both directions depending on the activity (concentration) of the species involved, the temperature and the standard electrode potentials. When an external potential is applied (outside power supply or battery) the "concentration" of electrons will increase. If we look at the right half-cell reaction above, it will move towards $B^0$ (to the right) as the number of available electrons is increased. When no external potential is applied to the cell, the electromotive force, $E$, depends on the activity of the different species as expressed in the Nernst equation:

$$E = E_{right} - E_{left} = E^0 + \frac{RT}{zF} \ln \left( \frac{[ox]}{[red]} \right)$$

The electromotive force will tell us the direction of spontaneous reaction. When $E$ is larger than 0 the reaction will move to the left ($B$ will dissolve) and when $E$ is less than 0 the reaction will move to the right ($A$ will dissolve). $E^0$ is the standard electromotive force for the entire system (two half-cells). $E_{left}$ and $E_{right}$ are the electromotive forces for each of the two half-cells.

In order to calculate the electromotive force of a cell, it is usually more convenient to focus on the difference between the half-cells. That is:

$$E = E_{right} - E_{left} = E^0_{right} + \frac{RT}{zF} \ln \left( \frac{[ox]_{right}}{[red]_{right}} \right) - E^0_{left} + \frac{RT}{zF} \ln \left( \frac{[ox]_{left}}{[red]_{left}} \right)$$

Applying the general reactions from the two half-cells above we get that:

$$E = E_{right} - E_{left} = E^0_{right} + \frac{RT}{zF} \ln \left( \frac{[B^{z+}]}{[B^0]} \right) - E^0_{left} + \frac{RT}{zF} \ln \left( \frac{[A^{z+}]}{[A^0]} \right)$$

The activity of solid metals and other pure compounds (like water, etc.) is 1 by definition. When $R$ is 8.3144 J/mole-K, $T$ is 298 K (room temperature), $F$ is 96487 C/mole and we use that $\ln(x) = \ln(10) \cdot \log(x) = 2.3026 \cdot \log(x)$ we get that:
In most diluted solutions the activity of an ion is equal to the concentration, and when this is true \([x]\) can replace \(\{x\}\) above. The two standard electrode potentials, \(E^0_{right}\) and \(E^0_{left}\), can be found in the literature for most half-cell reactions. They have been measure experimentally at standard conditions versus the Standard Hydrogen Electrode (SHE). By definition the standard electrode potential of this half-cell reaction:

\[
E = E_{right} - E_{left} = E^0_{right} + \frac{0.0592V \ ln \ \{B^{z+}\}}{z} - \frac{0.0592V \ ln \ \{A^{z+}\}}{z}
\]

is set to zero. Using this electrode (and a rather complicated set-up) as the left electrode, it is possible to measure a relative potential of any electrode on the right versus SHE.

### 7.5.3 Deposition of conductive polymers (Polypyrrole)

Polypyrrole is an electrically conductive polymer that can be polymerized electrochemically and deposited onto electrodes. The ease of preparation, inherent electrical conductivity, controllability of surface properties, and compatibility with mammalian cells make polypyrrole an attractive candidate for biomedical applications [7.45]. Additionally, when polypyrrole is in its oxidized state, it exists as a polycation with delocalized positive charges along its conjugated backbone. In order to neutralize this charge, counter ions in the solution are incorporated into the polypyrrole film during electrochemical deposition. The conductivity, morphology, and stability of polypyrrole vary significantly when the counter ions are changed [7.46, 7.47]. When a polyelectrolyte having negative charges serves as the counter ion, it is incorporated into the polymer through the following reaction, where \(PE^-\) stands for polyelectrolyte. Based on this mechanism, bioactive species, such as DNA, heparin, poly (hyaluronic acid), proteins, nerve growth factors, and even red blood cells have been patterned onto electrode sites together with the electrically conductive polypyrrole [7.48-7.51].
Electrochemical polymerization has many advantages over other methods in the surface modification for biomedical applications. The method can be used on many different electrically conductive surfaces whereas chemical attachment techniques are usually limited to a few substrate choices. The coating can be precisely patterned specifically onto the active site of the electrode without covering the nonfunctional areas of the device. The deposition of organic and biologic species does not compromise the electrical functionality of the electrode since the coating material is electrically conductive. Actually the signal transport can be enhanced because of the unique surface morphology of the coatings.

### 7.6 ECD of Polypyrrole doped with proteins

Any polyelectrolyte with negative charge can serve as a counter ion and will be incorporated into polypyrrole by the reaction illustrated in the reaction shown earlier. Bioactive species like proteins serve as excellent polyelectrolytes. However the conductivity, morphology and stability of the polypyrrole varies significantly with the counter ion. Most ECM proteins are good polyelectrolytes and can be incorporated into polypyrrole. In this section we discuss the incorporation of ECM proteins like collagen by electrochemically polymerizing polypyrrole on gold electrodes in microfluidic channels. Laminar flows can be used to pattern only specific sections of the electrode. This technique will be particularly useful in cell culture applications where precise patterning of proteins is essential for cell attachment. Proteins can be specifically patterned and then the top channel structure, which is made of a soft elastomeric epoxy, is lifted off and the bottom substrate with the patterned protein can be used for applications like cell culture.
7.6.1 Cyclic Voltametry

Figure 7.1: Cyclic Voltammetry of PPy/PSS and PPy/BSA. PPy/Collagen swept between 0.5 and -0.9V.
During deposition there is a reversible redox reaction in the polypyrrole film. The redox reaction involves the charging and discharging of polypyrrole and also is accompanied by movement of ions. Because the oxidized state and reduced state have very different electrical properties, this redox reaction provides a switching ability to the doped polypyrrole films. Cyclic voltammetry shows the intrinsic redox reaction of the electrode material as the potential of the electrode is swept in a cyclic manner. No redox reactions occur at the gold electrode, but the switching ability of polypyrrole was demonstrated. Within a cycle of voltage sweeping, there are two peaks (one anodic and one cathodic) at potentials of -0.14 and -0.56 V, which are indications of oxidation and a reduction reaction of PPy/PSS respectively. The redox reaction was accompanied by the movement of small cations in and out of the film. Additionally, the interaction between the polypyrrole and the large dopant molecules were changed upon each reaction, as were the electronic properties of the film. CV was also performed to determine if PPy/Collagen and PPy/BSA were electrochemically active. PPy/BSA did not show redox peaks which is an indication that PPy/BSA is not electrochemically active on the other hand PPy/Collagen is electrochemically active and shows two redox peaks at -0.2 and 1 V respectively. The electrodes were swept between 0.5 to -0.9 V, the electrode area was 0.002 mm² and the scan rate was 100 mV/sec. The results are detailed in figure 7.1

7.7 Device for laminar flow patterning of protein doped conductive polymers

7.7.1 Electrode fabrication

The devices were fabricated using the epoxy casting technique to provide a low cost but high quality platform for cell culture applications. These substrates are extremely cheap and easy to fabricate and can be used for single assay use. The device consists of 2 substrates bonded to each other. The bottom substrate is made using EPOTEK 301-2 FL (Epoxy Technologies). This epoxy is optically clear and has no background auto fluorescence. A blank epoxy substrate is fabricated using the casting technique and then a thin layer of parylene C is deposited to improve gold adhesion. Then a 200 nm thick layer of gold is evaporated onto the substrate and then photolithographically patterned into
electrodes. Fig 7.2 shows a picture of the fabricated electrodes. The working electrode is 300 μm wide and has a working length (area for deposition) of 900 μm. The counter electrode is 1 mm wide and 900 μm long. The counter electrode is made significantly larger than the working electrode to aid the electro polymerization process.

Figure 7.2: Fabricated electrodes

The substrate processing is similar to conventional photolithography techniques except that the soft bake is done for 40 minutes at 60 °C, which is the T_g for this epoxy.

7.7.2 Channels for laminar flow patterning

The channel structures are made on a separate substrate, which is also made using the epoxy casting technique. The epoxy used is EPOTEK 310 (Epoxy technologies). This epoxy is an elastomer and has a T_g below room temperature. The fabricated (Fig 7.3) channels have three inlet channels, which feed into one wider channel that enables the
Figure 7.3: Fabricated device for laminar flow patterning of protein doped conductive polymers

Figure 7.4: Laminar flow demonstration in fabricated channel structure
flow of 3 different liquids through the 3 inlet channels resulting in laminar flow of 3 streams in the main channel. Mixing of different fluids in the laminar flow regime is only due to diffusion, which is significantly small. By creating a stream of polyelectrolyte flanked on either side by an inert liquid ECD can be achieved on an area defined by the stream of polyelectrolyte thus enabling precise patterning and incorporation of proteins into PPy. The smaller channels are 300 µm wide and the large channel is 900 µm wide. The soft epoxy substrate seals reversibly to the other substrate with electrodes without the use of any adhesive resulting in a complete bonded device. Colored liquids are used to demonstrate laminar flow (Fig 7.4).

7.7.3 Deposition of PPy/PSS and PPy/Collagen

Initial experiments were done to characterize the deposition on PPy/PSS (0.3 mg/ml. 0.3 mg/ml. pH 7 buffer), PPy/BSA (0.3 mg/ml. 0.3 mg/ml. pH 7 buffer) and PPy/Collagen (0.3 mg/ml. 0.3 mg/ml. pH 7 buffer), which were supplied to the electrodes through microchannels. The reservoirs were filled with the polyelectrolyte, which then completely filled the channel structure. A pump was used to ensure continuous flow over the electrodes and avoid bubble formation due to the electrochemical reaction. From the cyclic voltammetry plots the minimum deposition current was determined. The area of the working electrode is 0.0045 sq. cm. For PPy/PSS the minimum deposition current is 0.5 mA/ sq. cm and for PPy/Collagen the minimum deposition current is 1.5 mA/ sq. cm. A femtostat setup is then used to maintain a constant current supply of 0.9 mA/ sq. cm for PPy/PSS and 2 mA/ sq. cm for PPy/Collagen respectively. Deposition on the working electrode is observed. Fig 7.5 shows deposited PPy/PSS and PPy/Collagen layers using the fabricated device. The channel was continuously flowed with the poly electrolyte at 20 µl/min to avoid bubble formation. The flow was controlled by the use of a syringe pump.
Figure 7.5: ECD of PPy PSS and PPy:Collagen on fabricated device
7.7.4 Patterning using laminar flows

Precise patterning of the area of deposition can be achieved using multiple streams, which flow in the laminar flow regime. As explained earlier the streams do not mix and are well defined. The first experiment done was to achieve precise deposition of PPy/Collagen by supplying the 2nd of the three inlet reservoirs with the polyelectrolyte and supplying the other two reservoirs with PPy/BSA (electrochemically inactive). This results in three streams in the main channel BSA/Collagen/BSA. Deposition occurs only on parts of the electrode which is in contact with the PSS stream. Deposition was again done at 2 mA/ sq. cm. And Fig 7.6 shows the precisely patterned ECD of PPy/Collagen. The experiment was done again but the order of the streams was modifies to be Collagen/BSA/Collagen and deposition was achieved at the two ends of the electrode. Fig 7.6 shows the results obtained.
Figure 7.6: Patterned ECD of protein doped conductive polymers using laminar flow patterning

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7.8 Summary

This chapter discusses the use of microfabrication in cell biology. Cells require attachment to the extracellular matrix to survive and maintain their differentiated state. The attachment to the ECM is through ECM proteins. In order to study cells in-vitro, cell attachment is essential. Microfabricated cell culture chambers can be developed by precise patterning of areas, where cell attachment is required. This chapter discusses electrochemical deposition of conductive polymers doped with ECM proteins. Laminar flows can be used to precisely pattern the flows in the channel and control the deposition on the electrodes. Laminar flow patterned ECD of polypyrrole doped with collagen is achieved.
CHAPTER 8

Conclusions

This research work explains the development of plastic microfabrication techniques specifically epoxy based casting to fabrication of microfluidic platforms that can then be used for different applications in molecular and cellular biology. The second chapter is an extensive review of polymers: different types of polymers, their structures and functions are discussed. Polymer fabrication techniques particularly techniques which can and have been modified for microfabrication of polymers are also reviewed. The third chapter specifically deals with epoxies and techniques to cast them to fabricate useful microfluidic platforms and make high quality structural elements that can then be used for fabricating functional devices for various applications. Miniaturization offers the possibility to integrate multiple functions onto a single platform however integration of control elements for fluidic manipulations, detection and sensing in plastic based systems is difficult due to the material properties. Chapters 4 and 5 discuss two separate techniques for integration of control elements onto plastic based systems. The first one involves embedding active silicon micromachined devices in a plastic microsystem and the other involves surface micromachining to build from the bottom up devices that can be integrated within the system. Chapter 6 discusses the two most important biomolecules nucleic acids and proteins and also techniques for different nucleic acid and protein assays. Demonstration devices for molecular assays were designed and fabricated to perform Polymerase Chain Reaction (PCR) and Capillary electrophoresis (CE). This chapter also explains the testing and results obtained. Chapter 7 details how microfluidics and microfabrication can be used to engineer cellular interactions with surfaces and surroundings. Cell attachment is critical to the normal functioning of the cell and requires Extra Cellular Matrix (ECM) proteins for proper attachment. An electrochemical deposition technique for patterning conductive biomolecules and proteins is explained. Laminar flows in channels are used to precisely control the dimensions of the deposited protein.
Bibliography

Chapter 1:


1.2 Manz, A., Graber, M., Widmer, H. M., Sensors and Actuators. 1990, B 1, 244-248


158

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Chapter 2:


2.6 Elders, J., Jansen, H. V., Elwenspoek, M., Ehrfeld, W., Proc. MEMS’95, 238-244.


Chapter 3:


Chapter 4:


162

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


4.29 A. Meckes, J. Behrens, M. Hausner, M. Gebhard, W. Benecke, Enhancement of air monitoring by combined microsensors and actuators, Concept and design considerations for a miniaturised gas analyser, Proc. µTAS ’96, Basel, Switzerland, pp. 126-128.


Chapter 5:


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Chapter 6:


Chapter 7:

7.2 G. Fuhr and S.G. Shirley. Cell handling and characterisation using micron and

7.3 N.B. Standen, P.T.A. Gray and M.J. Whitaker Editors, Microelectrode

7.4 D.J. Harrison, P. Li, T. Tang and W. Lee. Manipulation of biological cells and of

7.5 P.E. Andersson, P.C.H. Li, R. Smith, R.J. Szarka and D.J. Harrison, Biological

7.6 S. Hediger, A. Sayah and M.A.M. Gijs, Fabrication of a novel microsystem for
the electrical characterisation of cell arrays. Sensors and Actuators. B 56 (1999),
pp. 175-180.

7.7 D.S. Misfeldt, S.T. Hammamoto and D.R. Pitelka, Transepithelial transport in cell

7.8 L.C. Milks, M.J. Brontoli and E.B. Cramer. Epithelial permeability and the
1241-1247.


7.11 Harrison RG. 1912. The cultivation of tissues in extraneous media as a method of

7.12 Weiss P. 1945. Experiments on cell and axon orientation in vitro: the role of
colloidal exudates in tissue organization. J. Exp. Zool. 100:353-86.


7.15 Furshpan EJ. MacLeish PR. O’Lague PH. Potter DD. 1976. Chemical
transmission between rat sympathetic neurons and cardiac myocytes developing
in microcul-trues: evidence for cholinergic, adrenergic, and dual-function

7.16 Grumbacher-Reinert S. 1989. Local influence of substrate molecules in
determining distinctive growth patterns of identified neurons in culture. Proc.


172

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