Polymer Pen Printing: A Tool for Studying 2D Enzymatic Lithography and Printing 3D Carbon Features

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Polymer Pen Printing: A Tool for Studying 2D Enzymatic Lithography and Printing 3D Carbon Features

by

Zhantong Mao

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The City University of New York.

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This manuscript has been read and accepted for the Graduate Faculty in Chemistry to satisfy the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

Polymer Pen Printing: A Tool for Studying 2D Enzymatic Lithography and Printing 3D Carbon Features

By

Zhantong Mao

Advisor: Professor Alan M. Lyons

Polymer Pen Lithography (PPL) is a promising molecular printing approach which combines the advantages of both microcontact printing (low cost, high-throughput) and the dip pen lithography (DPN) (arbitrary writing, high-resolution) into one cohesive lithography method to create 2 dimensional (2-D) patterns with micro/nano-features on different substrates. The goal of this dissertation is to design and develop a new tool based upon PPL, which is not limited to forming 2D parallel patterns, but can also create 3D complex microstructures, finding applications in both biotechnology and Micro-Electro-Mechanical systems (MEMS) technology. This novel approach is named Polymer Pen Printing. Different from PPL using traditional dry-ink printing methods, an inking step is added to each printing repetition in the polymer pen printing process. Thus a wide range of ink materials with diverse viscosities can be transferred to substrates to create functional 2D and 3D microstructures.

The polymer pen printing apparatus used in this thesis has been accomplished and introduced in Chapter 2. As a preliminary attempt, the single polymer pen printing approach was developed by simply attaching a solid polydimethylsiloxane (PDMS) pen tip to a multi-axis robot for small microarray fabrication. Compared to the single pen printing method, multi-pen printing
can create large arrays of features. Therefore, an improved apparatus for polymer pen printing with high-throughput was discussed and built. Silicon molds, which consist of hundreds of uniform pyramidal openings, were photolithographically defined and etched using hydrofluoric acid (HF) followed by potassium hydroxide solution; after surface-modification with fluorosilane, these silicon molds were used to cast arrays of PDMS pyramidal pen tip. The cast PDMS pen array was mounted to a hollow holder with a 45° mirror inside. Therefore, each PDMS pen can be observed and monitored from the microscope on the side. To achieve prints less than 1 micron across, a Z axis stage with nanometer resolution was incorporated; and to control the compression of PDMS pen tips, a force gauge was also incorporated to detect 1 mg of applied force from the tips. The printing process for the multi-pen system is almost the same as single pen system. PDMS pens are coated with ink solution before each printing cycle by dipping into an inkwell and then brought into contact with the substrate surface. Thus multiple patterns, one from each tip, are created in parallel simultaneously. Furthermore, with control of the printing force, feature sizes could be controlled over the range submicron to tens of microns.

Three ink candidates have been printed by polymer pen printing approach to fabricate 2D&3D microstructures. The first ink material is Barium Strontium Titanate (BST) nanocrystallites dispersed in a furfuryl alcohol (FA), which was printed by the single PDMS pen with 100 µm tip diameter (Chapter 3). After printing, samples were heated to crosslink FA monomers, forming a stable polymeric matrix with embedded BST nanocrystallites. Without shear-thinning properties, BST/FA ink cannot be used to build 3D posts, but it has the capability to create circular patterns with different thickness by the single or multi-tier deposition method. It was found that the thickness of film increased linearly with the number of deposits without changing the diameter significantly. This encouraging result could enable the formation of
microcapacitors with multi-tiered structure. Moreover, the study of printing parameters, including printing height and ink pick-up position, shows that changes to the pen positions in the ink reservoir or substrate have essentially no impact on deposit thickness or diameter. Beyond that, the effect of surface chemistry of PDMS pen and silicon wafer have also been studied. The plasma treated hydrophilic PDMS pen can pen transfer more BST/FA than untreated one; and the larger diameters with smaller thickness were obtained on a hydrophilic silicon wafer.

The second ink candidate is a dilute aqueous solution of enzyme Candia antartica lipase B (CALB), which is known to catalyze the decomposition of poly (ε-caprolactone) (PCL) films. By bringing enzymes into contact with pre-defined regions of a surface, a polymer film can be selectively degraded to form patterned features that are requisite for applications in biotechnology and electronics. This so-called “enzymatic lithography” is an environmentally friendly process as it does not require any actinic radiation or synthetic chemicals to develop required features. But the need to restrict the mobility of the enzyme in order to maintain control of feature sizes poses a significant challenge. In Chapter 4, after writing 2D enzyme patterns onto a spin-cast PCL film by single pen printing, samples with CALB were incubated at 37 °C and 95% relative humidity (RH) for up to 7 days to develop features. The CALB selectively degraded the PCL film during incubation, forming openings through the film. The size of these features (10 to 50 µm diameter) is well suited for use as biocompatible micro-reactors.

Previous study of patterning CALB by single polymer pen printing technique resulted in slow etch rates, low throughput and poor image quality. In Chapter 5, I present an improved enzymatic lithography approach, still based on enzyme CALB and PCL system, which can resolve fine-scale features (< 1 µm across) in thick (0.1 – 2.0 µm) polymer films after 5 minutes to 2 hours of incubation at 37 °C and 87% RH. Immobilization of the enzyme on the polymer surface was
monitored using fluorescence microscopy by labeling CALB with FITC. The crystallite size in the PCL films was systematically varied; small crystallites resulted in significantly faster etch rates (20 nm/min) and the ability to resolve smaller features (as fine as 1 µm). The effect of printing conditions and RH during incubation is also presented. Patterns formed in the PCL film were transferred to an underlying copper foil demonstrating a “Green” approach to the fabrication of printed circuit boards.

In parallel, the third ink material is a mixture of 25 wt% graphite dispersed in a high viscosity phenolic resin n-methyl-2-pyrrolidone (NMP) solution, which can be converted into carbon/carbon composites after a pyrolysis process. The 3D polymeric posts were created by depositing multilayers of thixotropic phenolic ink on a silicon substrate by single polymer pen printing method with a 10 µm radius PDMS pen tip (Chapter 6). After pyrolysis at 1000 °C in a nitrogen (N₂) atmosphere, the polymeric features were converted to the glassy carbon/graphite features with a high aspect ratio (>2). These features may be used as microelectrodes.

Last, arrays of needle-shaped glassy carbon have been developed by a drawing approach using multi-pen printing technique followed by simple pyrolysis process (Chapter 7). To build polymeric needles with ultra-high aspect ratio, the polymeric ink was prepared by dissolving phenolic resin in the high boiling point (204 °C) solvent NMP without fillers to achieve good printability and suitable viscosity. By slowly lifting up the print head from substrate, liquid needle structures were formed and then solidified on silicon substrates or gold electrodes due to the solvent evaporation. In addition, suspended resin fibers connected to two electrodes have also been fabricated by precisely controlling the movement of the PDMS pen. After pyrolysis, these resin features were converted to glassy carbon and the 3D structures remained. The electrical characterization results showed that glassy carbon made by this method had relatively low
resistivity \((2.5 \times 10^{-5} \, \Omega \text{m})\). Therefore the glassy carbon based microneedles are well-suited to be electrodes for electrochemical sensors for biological applications.
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Chapter 1. Introduction

Today, microfabrication has emerged as one of the most promising techniques in modern manufacturing\(^1\)\(^-\)\(^4\). This miniaturization technology not only reduces the amount of industrial consumables used in the process, improves production efficiency, and lowers production cost as compared to traditional manufacturing techniques, it is also a breakthrough technology, which has transformed our world in the past decades\(^5\),\(^6\). The resulting micro-systems or micro-electronics from miniaturization technology has brought to us a more convenient, healthy and entertaining life.

Giving smartphones as an example, the device incorporates many micro-devices, such as a processor, silicon microphone, radio frequency microelectromechanical system (RF-MEMS) and microfuel cell. Smartphone has become one of the most important parts in our modern life soon after it was designed and mass produced. Nowadays, the chemical and biochips are also integrated to smart phones to track and monitor personal health which increased their value in the healthcare market. With relatively small size, multi-function, more than one billion smart phones have been sold throughout the world since 2007 and to one third of the population in United States. It is one commercial application for micro-devices. With the driving force from the fast-increasing market, miniaturization science and technology continues to be developed and applied to various fields.

1.1 Microelectromechanical systems (MEMS)

1.1.1 MEMS and microstructures

Mostly, the microsystems developed by microfabrication techniques are referred as microelectromechanical systems (MEMS) in the US, which generally have electrical and mechanical two elements in the past, and have at least one characteristic dimension less than one millimeter\(^7\),\(^8\). Of the present time, the term MEMS is not limited to letter-for-letter interpretation,
but has a broader meaning in science and engineering as all structures or devices in the microscale range with mechanical, electrical, optics, chemical and bio-parts. In fact, it may be more appropriate to call it “micro-system technology” as it is called in Europe or “micro-machined devices” as it is referred to in Japan\textsuperscript{9}. Due to the small scale effects, many physical phenomena are favored in these miniaturized structures, such as the higher heat transfer efficiency, better sensitivity and more prominent electrostatic forces. Therefore, MEMS have a wide range of applications in various fields such as sensing, microelectronic engineering, chemical and bioengineering.

1.1.2 Material used in MEMS

Silicon is the most widely used and favorable material for MEMS manufacturing for several reasons\textsuperscript{10}: 1). It is an ideal structural material with high Young’s modulus, and mechanically stable in different size ranges; 2). It can transduce signal by its electrostatic properties; 3. It has a very high melting point of 1400 °C and a relatively low thermal expansion coefficient; 4. Its microfabrication approaches used in the semiconductor and integrated circuits (IC) industry are well established, which is also an advantage for integrating MEMS with other electronics or IC on the same substrate. In the early stage of MEMS development, MEMS products were all fabricated from silicon. For example, the earliest MEMS product, the pressure sensor, commercially available in 1970s, was made of silicon. Indeed, its theoretical concept or say “discovery stage” can be dated back to 1954, when Charles Smith\textsuperscript{11} at Bell Laboratories published the paper “the piezo-resistance effect in germanium and silicon”, which was recognized as the origin of MEMS by some researchers. Another important MEMS application is electrostatic inkjet printer invented by IBM also in the 1970s\textsuperscript{12}. The inkjet head, which was made of silicon, is one application of silicon’s piezoelectric property. When voltage is
applied, the chamber made by silicon changes shape and squeezes ink droplet out of nozzle to print patterns. From then on, silicon based MEMS developed very fast due to their small size and high cost performance ratio, more and more silicon sensors, actuators, electronics were invented and commercialized.

With the development of MEMS technology, many kinds of materials have been found to be useful for the fabrication process such as ceramic, metal, polymers. Among them, polymer is the least expensive material. Though most polymers are dielectric material, which limits the application as sensor and actuator, they are favorable in microfluidics or other bio-MEMS manufacturing. Instead of silicon and glass, a large number of papers have reported microfluidics devices made by polymers. For example, Poly(dimethylsiloxane) (PDMS) material is one polymer widely used in microfluidics fabrication, due to the simple process. By casting with a pre-designed mold, micro-containers and channels in PDMS can be easily fabricated and packed for medical analysis, such as blood testing cartridges. Another example is the plastic microneedle array for transdermal drug delivery. The sharp-tipped and high aspect ratio, solid or hollow microneedles can pierce through the outer layer of the skin with minimal invasiveness and pain, to deliver precise dosage of drugs. Due to the low cost and environmental friendly property of polymer, polymer based bio-MEMS is disposable, which can prevent cross infection and contamination. Fabrication of 2D and 3D microstructure of polymers is one important part in this thesis, which will be discussed in Chapter 4 and 5.

Recently, carbon has been recognized as a promising candidate material in MEMS that has great potential in various fields such as energy storage, micro-battery, heat transfer and sensors. Under harsh environmental conditions, such as high temperature, high humidity, or exposure to functional liquids (electrolyte solution, biological molecules solution), carbon may be a good
As a matter of fact, carbon has many advantages over silicon. First, carbon is a conductor, which has good electrical and thermal conductivity. Carbon is also an inert material, and has excellent chemical and thermal stability. In addition, carbon is biocompatible and can be easily surface modified with a variety of functional groups and biomolecules, such as enzyme and DNA, which makes carbon be favorable in bio-MEMS. And last, carbon is much less expensive than silicon. Although carbon based MEMS is still in discovery stage, and there is no commercial carbon-MEMS product on market, many carbon microstructures have been fabricated and studied in the past decades. One of the challenges for developing Carbon-based MEMS is that there is no well-developed microfabrication approach for carbon. As we know, micromachining carbon directly is difficult, high-cost and time-consuming. Most of carbon microstructures reported were created by molding or patterning carbon polymeric precursor, followed with pyrolysis in inert or reducing environment. Due to the high electric conductivity, carbon electrode product in macro-range, including carbon paste electrode and carbon fiber electrode, was on market for very long time. Therefore, many researchers focus on minimizing carbon electrode to micron size and creating electrode high density array in a small area for fuel cell and micro-battery application. For example, Madou’s group has developed an array of high aspect ratio carbon posts and more complex 3D carbon structures using SU-8 photoresist, and studied the performance as electrodes. They believed that these carbon structures can be used in Li-batteries and chemical/bio sensors. In this thesis, 3D carbon microstructure was fabricated and more details will be discussed in Chapter 6 and 7.
1.1.3 MEMS applications

Today, MEMS based devices can be found everywhere around us, in our automobile, smartphone, printer, and healthcare devices. Some commercialized products are listed in Table 1.1. The MEMS market was expected to reach 20 billion in the next few years.

**Table 1.1. Application of MEMS**

<table>
<thead>
<tr>
<th>Automotive</th>
<th>Electronics</th>
<th>Communications</th>
<th>Medical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal navigation sensors</td>
<td>Disk drive heads</td>
<td>Fiber-optic network components</td>
<td>Blood pressure sensor</td>
</tr>
<tr>
<td>Air conditioning compressor sensor</td>
<td>Inkjet printer heads</td>
<td>RF Relays, switches and filters</td>
<td>Muscle stimulators &amp; drug delivery systems</td>
</tr>
<tr>
<td>Brake force sensors &amp; suspension control accelerometers</td>
<td>Projection screen televisions</td>
<td>Projection displays in portable communications devices and instrumentation</td>
<td>Implant pressure sensors</td>
</tr>
<tr>
<td>Fuel level and vapor pressure sensors</td>
<td>Earthquake sensors</td>
<td>Voltage controlled oscillators(VCOs)</td>
<td>Prosthetics</td>
</tr>
<tr>
<td>Airbag sensors</td>
<td>Avionics pressure sensors</td>
<td>Splitters and couplers</td>
<td>Miniature analytical instruments</td>
</tr>
<tr>
<td>“Intelligent” tyres</td>
<td>Mass data storage systems</td>
<td>Tunable lasers</td>
<td>Pacemakers</td>
</tr>
</tbody>
</table>

Besides these established devices, there are many other MEMS in development, and find their applications in more areas. To date, the MEMS varies from single part with simple two-dimensional (2D) surface microstructure, such as DNA array, to multiple-component system with complex three-dimensional (3D) microstructures, which may have “intelligence” to process digital signal after integrating with circuits, wireless communication modules and even micro/nano
processor on chip\textsuperscript{27–29}. Following “Moore’s Law”\textsuperscript{30,31}, the prediction of the exponential rates for microprocessor improvement, MEMS with ever smaller feature size, which is down to nanometer, is in demand. As a result, researchers and engineers were motivated to develop microfabrication methods, inventing new tools and processes.

\section*{1.2 Lithography methods}

Microfabrication methods, which include wide range of different approaches, are fundamental in the “micro-world”, and in rapid development with many new approaches being established in the past decades. But the mature and most widely used techniques were developed in the last century in microelectronic and semiconductor industries, which basically consists of subtractive (lithography and wet/dry etching) or additive (material deposition) manufacturing routes.

Lithography is a group of methods that can transfer or create patterns onto a substrate, which is more commonly used in manufacturing of MEMS. Based on differences in the process, lithography can be broadly divided into two categories, namely, subtractive and additive lithography.\textsuperscript{32} For example, photolithography is one of subtractive method; soft lithography and dip-pen lithography are additive lithography methods.

In this thesis, conventional lithography followed by wet etching approaches have been used to develop a new polymer pen printing lithography method. Thus, in the next section, lithography will be addressed in detail.
1.2.1 Photolithography

Photolithography is a very mature technique currently used in the industrial production of microelectronic, semiconductor devices and MEMS, which includes three principal parts: light source, optical system, photomask and photoresist. Light source, such as UV generator, provides photons and energy for “polymerizing” or “depolymerizing” photoresist. The photomask typically is a quartz plate with a pre-patterned chromium coating, which is called “hard mask” and located between light source and photoresist layer during explosion step. Photoresist can be classified into two categories based on their solubility in developing solution after exposure: the positive tone and negative tone. The positive photoresist became developable after exposure, which replicate the pattern from hard mask; and the negative photoresist is just the reverse, the unexposed area is developable, which replicates the inverse of the mask.

Positive photoresist is a mixture with three major components: a non-photosensitive polymer, which is the matrix material and plays main role in etch resistance; a photoactive compound (PAC), which can change its solubility after exposure; and a coating solvent for dissolving polymer and PAC. The most popular positive photoresists are based on Novolak – diazonaphthoquinone (DNQ) system, such as AZ9260 and AZ1518 photoresist used in my project. The chemistry is described as follows: the quinone-diazide photo-reaction occurs in the UV exposure step, causing a dramatic change in solubility of PAC from almost non-soluble to very soluble in basic developing solution. As shown in Fig 1.1, DNQ molecules first release nitrogen and absorb water molecules to form indene carboxylic acid (ICA) under UV exposure. Then in the developing step, these carboxylic acid groups can react with hydroxyl group in the alkaline solution, forming ionized compound, which has high solubility in water. Furthermore, DNQ is recognized as a dissolution inhibitor, which has strong effect on the water solubility of Novolak.
rein. The pure Novolak, the phenol formaldehyde resin, has a hydroxyl group on benzene ring in
each segment, which can be slightly dissolved in alkaline solution. But due to the strong interaction
between DNQ and Novolak resin, the mixture (mostly 1:5 weight ratio for DNQ and Novolak resin)
without UV exposure is insoluble. Under UV exposure, the solubility of resist increases more than
three orders of magnitude due to the conversion of DNQ to ICA. With the help of ICA, basic
developing solution penetrates into resist film easily and “etches” away the material in the exposed
area. Therefore, the positive photoresist replicates the pattern of photomask.
Figure 1.1. The chemistry of Novolak-DNQ positive photoresist. a) DNQ photoreaction process. b) Novolak dissociation reaction in developing solution.
Figure 1.2. The chemistry of SU-8 negative photoresist. a) photoinitiation process. b) epoxy crosslinking reaction with photoacid catalyst in the post-exposure-bake step.\(^{33}\)

The negative photoresists also have three main components: matrix material, photoactive cross-linking agent or catalyst, and the last, coating solvent. The concept for negative photoresist is simpler than positive photoresist. With exposure, the polymer or monomer is crosslinked to form a 3D network, which is undissolvable in developing solution. On the other hand, the unexposed part can be removed by dissolving in organic solvents. Here, the SU-8 negative epoxy-based photoresist is considered as an example (Fig. 1.2), which was also broadly used in carbon microstructure fabrication. In SU-8 system, the monomer of epoxy resin (matrix material) has
eight functional groups, which is dissolved in Gamma Butyrolactone (coating solvent) with photoinitiator, the mixed triarylsulfonium/ hexafluoroantimonate salt. The photoinitiator absorbs UV and generate photoacids in the exposure step. Next, in the post-exposure-bake step, with catalyst photoacids, epoxy groups are crosslinked as thermoset resist, which is undissolvable in the developing solvent. As a result, the pattern on negative photoresist is the inverse of the photomask.

![Photolithographic Process](image)

**Figure 1.3** Schematic representation of the photolithographic principal process with positive or negative photoresist.\(^{34}\)

Photolithography is a multi-step process. In IC and semiconductor industry, it may be done 20 to 30 times to fabricate one circuit with a complex structure. As shown in Fig. 1.3, the standard process normally consists of 5 principal steps: coating of photoresist, UV exposure, aqueous
development, pattern transfer and resist stripping. But for a complete photolithography process, more steps should be added to it.

Before coating the photoresist, the silicon wafer is required to be cleaned carefully by organic solvents followed with deionized (DI) water. Sometime, piranha solution is used to remove the organic contaminators on the surface of silicon wafer. After blowing dry with nitrogen gas, the wafers mostly are spin-coated with wafer primer first. For example, the 1,1,1,3,3,3-hexamethyldisilazane (HMDS), which can improve the adhesion between photoresist and substrate, which is commonly used before coating positive photoresist. Next, the photoresist is spin-coated onto the silicon substrate. The thickness of photoresist layer can be controlled by adjusting the spinner rotational speed. Typically 1~2 µm thickness of photoresist is required for silicon process, with spin speed ranging from 3000 to 6000 rpm. Then the coated sample was pre-baked to remove the solvent left in the photoresist layer. After the UV exposure, the sample was developed in aqueous developing solution to remove the unnecessary part and form desired pattern in photoresist layer. The post-bake step is used to remove the solvent residue and harden the developed photoresist. The silicon wafer used in photolithography has a micron thick protective layer. In fig 1.3, it is silicon oxide (SiO$_2$). So next, the developed sample was immersed in buffered hydrofluoric acid solution, which etches away the exposed SiO$_2$, to transfer pattern from photoresist to SiO$_2$ layer. The last step is the photoresist removal, by dissolving in organic solvent or O$_2$ plasma treatment. These wafers will be further etched by wet or dry methods to form 3D silicon microstructures.

In addition to these conventional photolithography techniques, convenient “maskless” technologies were developed in the last few decades, which is referred as direct writing exposure systems$^{35}$. With different energetic beam source, such as laser, electron beam and ion beam, the
resolution is improved to submicron range. For example, the laser writer Heidelberg DWL 66fs used in my project, can create high resolution patterns with a 0.8 µm feature size.

However, photolithography is a relatively high-cost microfabrication method. The expensive facilities and clean-room environment are not accessible to all chemists. Furthermore, it is only applicable to photosensitive material and non-curved surface. Due to these limitations, researchers developed some alternative lithography methods, such as soft lithography and dip-pen lithography. These methods have attracted more interest in the past decade, which provided a simple and cost-effective way to construct micro-scale or even nano-scale patterns.

1.2.2 Soft lithography

Soft lithography$^{36,37}$ is a collective term for a series of microfabrication methods based on soft polymeric stamp. It is a convenient and inexpensive technique that can create patterns on curved surface or complex 3D microstructures. It consists of two parts: stamp fabrication and patterning process. Basically, the stamp is created by casting prepolymer in a hard master, which is fabricated by conventional photolithography process, shown in Fig. 1.4. The most notable stamp material is polydimethylsiloxane (PDMS), or referred as silicone commonly. PDMS has many advantages, such as transparency, easy process, low Young’s modulus and thermal expansion. In addition, the stamp made by PDMS higher resolution than conventional photolithography, and is reusable for at least several times. The most popular formulation of PDMS is Sylgard 184 from Dow Corning, Midland, MI, which can be cured by thermally induced platinum-catalyzed hydrosilation reaction. After heating, liquid PDMS prepolymer becomes a stable solid elastomer with strong 3D network, shown in Fig. 1.5.
**Figure 1.4** Schematic representation of PDMS stamp or mold fabrication.

**Figure 1.5.** Primary platinum-catalyzed PDMS crosslinking reaction.
Though PDMS is an inert hydrophobic material, its surface can be easily modified to hydrophilic with silanol terminations by an oxygen plasma treatment. Moreover, fluorosilane can be grafted onto silanols to convert surface chemistry to superhydrophobic, shown in Fig 1.6. Therefore, both hydrophobic and hydrophilic materials can be transferred to substrate by PDMS stamps.

**Figure 1.6.** PDMS surface modification: step 1, plasma oxidation; step 2, fluorosilane treatment.

Soft lithography is a family of non-photolithographic methods with different patterning processes, including microcontact printing (µCP), replica molding (REM), microtransfer molding (µTM), micromolding in capillaries (MIMIC), solvent-assisted micromolding (SAMIM), 3D molding and phase-shifting photolithography. The earliest soft lithographic technique is microcontact printing, which was invented by Whitesides’ group in 1993\(^3\)\(^8\). In their paper, hexadecanethiol solution was coated onto a pre-patterned elastomer stamp and then printed onto the gold surface. The self-assembled monolayer (SAM) of hexadecanethiol on gold surface acted as a resist, which protected the gold underneath from etching, results in conductive gold microstructures with 1 µm feature size. The basic process of µCP is shown in Fig. 1.7. Recently, due to the biocompatibility of µCP process, this method have been widely applied in bio-field for
patterning biomolecules, such as enzyme, protein and DNA. In my thesis, the µCP is used in enzyme writing project, which is discussed in chapter 2.

![Diagram of µCP process]

**Figure 1.7.** Schematic representation of the basic µCP process.

However, µCP has an obvious drawback: the prepared stamps are only able to copy existing patterns but not to create new ones. Thus, another method, called Dip Pen Nanolithography (DPN), as a direct writing technique, has been invented.

### 1.2.3 Dip Pen Nanolithography (DPN)

DPN, as a low-cost scanning probe lithography method, has gained popularity in recent years. Similar to µCP, it is developed from the surface characterization technique of atomic force microscope (AFM). For example, both inking and printing are necessary steps in their patterning process. Another common feature is that a wide range of chemicals can be used as inks, including small organic molecules, polymers, inorganic nanoparticles and biomolecules. However, the main difference between two methods is that the hard tip of an atomic force microscope in DPN is used as a carrier to transfer inks onto the substrate instead of PDMS stamp. Typically, the
tip is inked by immersing it in an ink solution. After drying, the ink-coated tip is attached to an AFM station, which is controlled by software to move tip to draw the designed patterns on the substrate. As the tip and the substrate surface come into contact, the existence of meniscus of condensed water vapor (Fig. 1.8 a) would enable inks to be transferred from the tip to the contact surface area, eventually creating patterns on the substrate.

NanoInk Inc.\textsuperscript{41} has designed a new DPN system with multi-tips (multiple printing heads), which is integrated with microfluidic chips as shown in Fig. 1.8b. This system not only increased the throughput, but also has ability to create more complex and multicomponent patterns, which is very attractive to bio-scientists. To print several different materials at the same time without cross contamination, each tip has its own inkwell. Fig.1.8c is an example of deposition of four different antibodies simultaneously using the NLP 2000 System. However, due to the hard solid nature of the tip, DPN has certain limitations. First, AFM tips are made of fragile and costly silicon or silicon nitride. Second, this method is incapable of creating micro scale features. Third, DPN still cannot be applied to non-flat surface. Therefore, researchers continues working on the improvement of lithography technique based on scanning probes.
1.2.4 Polymer pen lithography (PPL)

In 2008, Huo et al. developed a new lithography method, named polymer pen lithography (PPL), which is based on the existing DPN and microcontact printing methods. The key difference between PPL and DPN is that the former uses PDMS soft tips instead of AFM hard tips, and thus avoiding the limitations imposed by the tip material. PDMS tips are cheap, durable, simple to manufacture and can create features with various sizes from 90 nm to hundreds of μm by controlling the force and dwell time of contacting the substrate. In addition, large array of uniform PDMS tips can be easily casted by molding, which can created thousands or even millions of
patterns simultaneously. On the other hand, unlike the µCP which allows only the copying of existing patterns, PPL is able to write different patterns onto the substrate. In summary, PPL combines advantages of both DPN and µCP, therefore, has a wide range of promising applications.

Except photolithography, the categories of lithography as discussed above all employ dry-ink printing strategy, in which the stamp or tip surface is either dipped in or sprayed upon with an ink solution, with drying as the final step of inking. It not only ensures inks to be transferred in small amounts and uniformly attached to the stamp or tip surface, but can also control feature size by varying the humidity. However, this strategy limits the choice of ink materials, and does not allow changing inks during the patterning process. To solve this problem, we attempted to develop a wet-ink printing method to replace the traditional dry-ink printing method, which is named polymer pen printing.

1.3 Objective of the thesis and the scope of work

The main objective of my thesis is to design and develop the novel polymer pen printing technique for fabricating 2D & 3D functional microscale structures by using materials with different rheology properties. More specific objectives include:

(1) To set up a polymer pen printing system based on GIX Microplotter II platform from Sonoplot INC, and develop the print head from a single pen to multiple pens.

(2) To fabricate PCL wells by enzymatic lithography method, and study the factors that may influence the lithography process.

(3) To fabricate 3-dimensional structure carbon features by the polymer pen printing method, including posts, needle arrays and air bridges.
Chapter 2 presents the development of polymer pen printing system with single PDMS pen or photolithographically-defined pen array. For single pen printing, two single PDMS pens have been made with 10 µm or 100 µm radius tip respectively. More work have been done to set up multi-pen printing apparatus, including creating mask files for focused laser writing; developing a process to anisotropically etch silicon to form square pyramidal openings for casting; creating PDMS pen arrays with different height and hardness; designing a hollow holder with a 45° mirror inside to observe tip status; modifying a robot to become a 3D printer with 5 axes of control: x, y, z, and pitch & roll; incorporating a force gauge to control the compression of PDMS pen tips; incorporating a Z axis stage with nanometer resolution to achieve prints less than 1 micron across.

In chapter 3, printing barium strontium titanate (BST) nanocrystals / furfural alcohol (FA) ink by the single polymer pen printing approach has been demonstrated for creating high-dielectric circular patterns, aiming for the microcapacitor array production. Multi-tier deposition method has been investigated to create thicker film. In addition, the effect of substrate wetting property on deposit thickness and diameter is also studied.

Chapter 4 and 5 introduce the enzymatic polymer pen lithography methods. In this part, the enzyme Candida antartica Lipase B (CALB) aqueous solution was deposited onto the biodegradable poly (ε-caprolactone) (PCL) films using the polymer pen printing technique. During incubation in a high humidity environment, the enzyme selectively catalyzes the degradation of the PCL film forming micro-well structures. Moreover, the effect of printing parameters and incubation conditions on lithographic resolution is discussed in detail.

In chapter 6 and 7, the fabrication of carbon 3D microstructures is detailed. We utilized the polymer pen printing technique to create high aspect ratio posts, ultra-high aspect ratio needle
arrays and air-bridge structures, then the pyrolysis process converts these polymeric features to glassy carbon or carbon/graphite composites. In addition, the shrink percentage and electrical resistivity of glassy carbon are also discussed, which shows the potential applications in MEMS.

At The last, chapter 8 is the conclusion of my thesis work. Some future works are also mentioned.
Chapter 2. Design and Development of Polymer Pen Printing System

2.1 Introduction

2D arrays of discrete micro-elements on a solid support, which are referred as microarrays, are useful tools for analyzing highly parallel samples\textsuperscript{1,2}. This technology is useful for genomic research: the DNA chip, a miniaturized platform containing isolated regions of DNA, is essential for effectively discovering and identifying large scale gene expressions\textsuperscript{3-5}. Nowadays, most microarrays are prepared by two distinct printing methods: contact printing and non-contact printing\textsuperscript{6,7}. The former, including robotic pin printing\textsuperscript{8,9} and stamp printing\textsuperscript{10}, is the most widely used, convenient and low cost approach, which transfer molecules by bringing probes into physical contact with a solid substrate. The print head of the non-contact approach is relatively complex\textsuperscript{11-13}, but enables depositing materials without touching the substrate to avoid contamination. Inkjet printers\textsuperscript{6}, which have thermal or piezoelectric actuators in the printer head, are well-known instruments for non-contact printing. After moving the print head be to a certain height over the substrate, the functional materials stored in the print head are forced out to be deposited onto the substrate.

2.2 Sonoplot GIX Microplotter II platform\textsuperscript{14}

Many commercial microarray printing system have been developed in recent years, and most of them deposit low viscosity solutions, and the printed feature size is over 50 µm. The GIX Microplotter II (Sonoplot, Inc, WI) is a benchtop picoliter fluid dispensing system (Fig 2.1a), which is designed for precisely depositing polymer electronics solutions and biomolecules in a cleanroom. The dispensing system consists of two important parts: the robot with working envelope of $35 \times 30 \times 7$ cm and with 5 µm resolution in XYZ axes, and a glass hollow probe.
attached to a piezoelectric actuator as the print probe. 2D patterns such as arrays and continuous features (lines, arcs and bends) are designed in Sonodraw CAD software. In addition, the whole printing process can be observed and recorded from an integrated microscope camera. When dipping the probe into the material well, ink is loaded into the glass hollow probe by capillary forces; during printing, the ultrasonic-assisted technology (Fig 2.1b) used in this system allows the glass plotter to deposit droplets as small as 5 µm diameter and dispense relatively highly viscous fluid (up to 450 cP), which may clog most the print heads of a conventional inkjet printer. Moreover, the volume of the ink that is dispensed by the probe can be well-controlled by adjusting the applied voltage and frequency of the piezoelectric element, with a variability of feature size of less than 10%. The Microplotter II system has been used to fabricate microstructured features for various applications. For example, Zang et al\textsuperscript{15} developed low-cost epoxy microlenses with a diameter of 20 µm and a height of 8.8 µm on a glass substrate by simple and rapid microplotter printing. Galatsis et al\textsuperscript{16} used Microplotter II to create thin-film based transistors with multi-layer structures by depositing conductive silver nanoparticles, semiconductive carbon nanotubes and dielectric polyethylenimine (PEI)/LiClO\textsubscript{4}.

However, the major challenge of using the Microplotter II system is for dispensing high viscosity fluids and inks. These fluids, such as FA monomers, 60 wt% phenolic resin NMP solution, and the graphite flake filled thixotropic polymeric materials used in my thesis, neither can be loaded into the glass hollow probe by capillary suction nor dispensed onto the substrate by ultrasonic vibration. Therefore, in this chapter, the Microplotter II platform was modified from non-contact to contact approach by using solid PDMS pens instead of the glass microplotter.
Figure 2.1. Sonoplot Microplotter II system. a) Photograph of the microplotter system with its controller. b) Schematic of ultrasonic-assisted dispenser inking and printing process. The ultrasonic-assisted dispenser consists of a hollow glass probe and the piezoelectric element.
2.3 Single polymer pen printing system

The single polymer pen printing apparatus was built by attaching a solid PDMS pen onto the high-precision robot to convert the Sonoplot GIX Microplotter II to a pin printer. The other parts of Sonopolt GIX Microplotter II, such as robot control and optical system, are used without modification in the new tool to retain all other functions of the Microplotter II. At the expense of printing speed, the modified printer has the ability to deposit viscous ink materials to fabricate 2D&3D microstructures. Furthermore, the durable PDMS pen can be used for a long time, which decreases the printing cost relative to the expensive and fragile glass microplottors. In this thesis, the single polymer pen printing approach was used to deposit three different inks ranging from a low viscosity aqueous enzyme solution to medium viscosity ceramic/monomer solution to a highly viscous resin mixture (Chapter 3, 4 and 6).

2.3.1 Fabrication of single PDMS pens

PDMS pens with two different sizes were fabricated by using DAP silicone adhesive (DAP Products Inc.). As shown in Fig. 2.2a, the 10 µm diameter pen tip was made by simple draw and cut method. This fabrication process is described more specifically as follows: first, a drop of DAP silicone is applied on a glass slide, then the glass probe is immersed into the material. Next, the glass probe is quickly pulled out, forming a long silicone string. After 5 minutes the DAP is fully cured, the silicone string is cut to form a PDMS pen with about 1 mm length and 10 µm tip diameter. With this pen, phenolic resin and enzyme solution was used as printing inks to create microstructures as small as 10~50 µm diameter. The other single PDMS pen was made using a dispensing approach with a syringe dispenser. By choosing a plastic dispensing syringe needle
with a 200 µm opening, a PDMS pen tip with 100 µm diameter was created (see Fig. 2.2b). This pen was mainly used to create micron thick BST/FA arrays with a diameter of ~ 100 µm.

![Image](image-url)

**Figure 2.2.** Microscope images of PDMS pens. a) 10 µm diameter PDMS pen tip fabricated by draw and cut method. b) A PDMS pen with a tip diameter of 200 µm fabricated by dispensing.

### 2.3.2 Printing process for single polymer pen printing

The printing cycle for single polymer pen printing is illustrated in Fig. 2.3, which begins with the inking step. First, the PDMS tip is dipped into the material well to a pre-set depth. Then the pen tip is lifted up by the robot with small amount of ink sticking on the tip. In the third step, the PDMS pen is brought into contact with substrate to transfer material from tip to substrate. The last step is to lift up the tip leaving ink material on the substrate. By programming the robot to move the single PDMS pen, 2D or even 3D patterns were successfully created with different ink materials.
2.4 Polymer pen printing system with pen array

Single polymer pen printing approach is an effective way to transfer and deposit high viscosity ink onto the solid substrate, but the printing speed is relatively slow, due to the repeated pen movement between sample pattern and inkwell during the printing process. Therefore, the single polymer pen technique can only be used to create small microarrays, limiting its utility. To break through this limit, the single polymer pen printing system was upgraded by integrating a PDMS pen array which is commonly used in PPL system. Thus, large microarrays can be created in a short time.

2.4.1 Literature review of PPL

Polymer pen lithography (PPL) was first reported by Mirkin’s group in 2008 by attaching a cantilever-free PDMS pen array to an AFM platform as the “writing” tool\(^{17}\). Compared to DPN,
which employs AFM tips as print probes, the PPL technique has higher probe density and numbers, allowing larger scale patterning. Besides that, the controllable deformation of PDMS pen tips enables the printer to create features ranging from nm to µm scale by simply applying different pressures. In their paper, a typical PDMS pen array with thousands of uniform tips was cast from a silicon mold fabricated using a conventional photolithography method and wet etching. With 20µm pitch, the pen density can be as high as 250,000/cm²; each pen in the array has an identical pyramidal shape with a tip radius about 70 nm, which has enabled patterning sub-100 nm features.

Most applications for PPL are focused on small molecule deposition. In a typical experiment\textsuperscript{18}, the PDMS pen array is first plasma treated to form a hydrophilic surface. Then a drop of dilute aqueous or ethanol based ink is placed onto the PDMS pen array. After solvent evaporation, a thin layer of functional molecules is formed on the surface of the PDMS pens. Next, the inked PDMS pen array is mounted to an AFM system and leveled. During printing, the relative humidity must be controlled in the range from 40% to 80%, so that the molecules on the PDMS pen can be transferred through the aqueous meniscus between pen tip and substrate. The first application of PPL was demonstrated by patterning 16-Mercaptohexadecanoic acid (MHA) on a gold surface as a protective mask, which was also used to study the relationship between feature size and applied force. Functional nanoparticles, such as Au, Fe₂O₃ and C₆₀, can also be deposited directly by PPL\textsuperscript{19,20,21}. In these experiments, poly(ethylene glycol) (PEG) was added to form an ink vehicle to deliver the nanoparticles. This technique is called matrix-assisted, and enables good control of the size and uniformity of features on different substrates.

PPL can be used in conjunction with click chemistry, as reported by Braunschweig’s group\textsuperscript{21,22}. In their study, one reactant was formed on the substrate and the other reactant with or without catalyst was coated onto the PDMS tip as the ink. Then the reaction can be induced at the
contact area between tip and substrate and the molecules of final product are covalently bonded to the substrate. Several solid-state reactions at ambient temperature have been used to form functional amide bond microarrays, such as Cu-catalyzed azide alkyne click reaction (CuAAC) and Staudinger Ligation, which then can be used to immobilize biological molecules at specific site, forming active probe arrays. Force-accelerated reaction can also be applied in click chemistry by PPL. Due to the soft nature of PDMS material, pen tips become compressed when brought into contact with the solid substrate, resulting in a controllable force in the reaction area. For example, cyclopentadienes (CPs) molecules were patterned by PPL and then covalently bonded to the a single layer graphene substrate via Diels–Alder (DA) cycloadditions for 15 to 30 minutes induced by the printing force from PPL.20

To extend the application, some modifications have been applied to the conventional PPL system. The first one is made to pen arrays. As discussed earlier, one of the advantages of soft PDMS pens is to create features with force-dependent size, ranging from nanometer to microns. But the utility of PDMS pen array results in a great challenge in patterning uniform-sized features over a large area. To improve the feature uniformity and shorten the pen array leveling time, Zheng et al.23 fabricated arrays of pens with a dual-elastomer structure. The hard apex of each pen minimized tip compression, decreasing the size variance of features; and the soft base is very helpful for leveling, which enables all pens in the array to touch the substrate simultaneously. Liu et al.24 inserted a deformable membrane layer between the PDMS pen array and a glass support. The tip displacement can be individually controlled by applying a pneumatic pressure on the backside membrane of each pen. Moreover, with computer control of pneumatic pressure, each pen can create different patterns with same motion.
The inking strategy of PPL can also be modified to generate a multiplexed pattern array\textsuperscript{25}. Instead of using a single inkwell, ink solutions were dispensed by inkjet printer into the recesses of the silicon mold, which was used to make the PDMS pen array. Then each PDMS pen can be coated with a different ink by dipping in its own inversed pyramid-shaped inkwell. Multiplexed protein arrays were fabricated using this inking approach\textsuperscript{26}.

Taking advantage of the transparency of PDMS, PPL has been modified for beam pen lithography (BPL)\textsuperscript{27}. The set-up of PPL and BPL are almost the same except for the PDMS pen array. In BPL, the PDMS pen array is fully coated with an opaque gold layer with hundreds of nanometer-sized openings at the apex, which allows light to pass through to create patterns on photoresist. Compared to conventional photolithography, arbitrary patterns with high through-put can be readily achieved by BPL.

Overall, PPL is a powerful nano/micro-patterning tool. However, as discussed above, PPL is limited depositing dilute ink solutions in 2D patterns. In this section, the novel polymer pen printing (PPP) system with multi-pen was designed and built. The key difference between PPP and PPL is that the former attaches PDMS pen array to a relatively course-scale robot instead of AFM piezoelectric stage, thus avoiding the limitation of short-range movement imposed by AFM system. In addition, the conventional PPL employs a “dry-ink” printing strategy, in which the pen surface is dried before printing. However, this strategy limits the amount of ink material that can be transferred and can only create 2D patterns. Similar to single polymer pen printing, an inking step is integrated to each printing repetition, allowing the ink material to be transferred along with a small amount of solvent, which is referred to as “wet-ink” strategy. In this case, a wide range of materials could be transferred and printed, even if the material shows very poor interaction with PDMS pen.
2.4.2 Apparatus design

The multi-pen printing system has five important parts: a PDMS pen array; a pressure sensor integrated with a special holder for mounting the pen array; the optical microscope observing system; the long range robot (Sonoplot GIX Microplotter II) platform, and the piezo-based stage system with 5 axis tilting and z axis nanomovement. The last two are ready made. Therefore, in this section, silicon mold fabrication, PDMS pen array fabrication, the holder and observing system design will be addressed in detail with some background information.

2.4.2.1 Fabrication of silicon mold

Fabrication of silicon molds for casting PDMS pen arrays can be found in many papers\textsuperscript{17,18,23}. In order to create pyramid openings with larger size and better uniformity, the procedure has been modified. Here, the mold fabrication process consists three major stages: 1. design patterns; 2. generate patterns in the photoresist layer using a Heidelberg DWL66 Laser Writer; 3. create array of pyramidal openings by wet chemical etching.

A 4-inch (100 mm) <100> silicon wafer with 1 µm thick silicon oxide layer was used in this project. Due to the size limitation of the Heidelberg system, the wafer was cleaved into four similar pieces. Thus, the layout was designed on a quarter wafer. As shown in Fig. 2.4, three different 2D array sizes were designed. Each opening corresponds to a PDMS pen, thus three different PDMS pen arrays were fabricated after molding. In one quarter wafer, there are 4 arrays of 400 µm openings (red), 5 arrays of 200 µm openings and 7 arrays of 300 µm openings. The pitch between two openings is fixed at 500 µm; and with dimensions of 6 mm × 6 mm, each array consists $12 \times 12$ openings. The street between each array is 4 mm wide, which is designed for cutting wafers into 1cm × 1 cm pieces after lithography, with arrays in the center. The whole
pattern on the quarter wafer is using CAD software, which can be directly loaded into Heidelberg DWL66 Laser Writer to generate patterns in the photoresist layer on the silicon wafer.

**Figure 2.4.** Graphical representation of quarter wafer design. The yellow squares are the arrays of 300 µm openings, red squares are 400 µm openings and blue squares are 200 µm openings.

The second stage (Fig 2.5 a to b) was done by focused laser lithography in the Micro/Nano Fabrication Lab of Princeton University. The only difference in the microfabrication process
between the conventional lithography and focused laser lithography is that in the latter, one “writes” patterns on photoresist directly without a photomask. In one experiment, the quarter wafer was first spin-coated with a thin layer of photoresist AZ1518, then put into the Heidelberg DWL66 Laser Writer to selectively expose the photoresist. After post-baking and developing in AZ400K solution, the pattern of square openings is created in the photoresist layer as a soft conforming etch mask. Before etching, the quarter wafer was cut into 1 cm × 1 cm pieces using a glass knife to fit in the wet-etching chamber.

Wet chemical etching consists of two parts: isotropic HF etching for transferring patterns to the silicon oxide (SiO₂) layer which then serves as a hard mask (Fig.2.5 c) and the anisotropic KOH etch for making pyramidal openings (Fig.2.5 d to e). To insure good quality and controllable isotropic etching, buffered HF solution (BHF) (6 parts of 40 wt% NH₄F and 1 part 49 wt % HF solution) was chosen to remove the exposed SiO₂ regions. The etching rate for the BHF is about 700 Å/min at room temperature. Therefore, it took about 14 minutes to etch away the 1 µm thick SiO₂ layer to transfer patterns from the soft mask to the hard mask.

After washing away the photoresist using acetone, the sample was etched in 30 wt% KOH solution at 80 °C. The KOH preferentially etches the <100> plane in the silicon crystal, but etching is “stopped” at the <111> plane, producing anisotropically etched etching structures in the <100> silicon wafer. With square opening pattern in the SiO₂ layer well aligned to the crystal plane, silicon square pyramidal openings were formed as the mold for creating PDMS pens.
Figure 2.5. The process flow for silicon mold fabrication.
Figure 2.6. Anisotropic etching in KOH solution. a) Schematic representation of KOH etching setup. b) Over etched silicon pyramidal opening with 300 rpm stirring speed (topview). c) Good silicon pyramidal opening with 50 rpm stirring speed (topview).

As shown in Fig. 2.6 a, during etching, the silicon mold is placed on a stage in the container filled with KOH solution. A magnetic bar is also put underneath the holder to insure good mixing of the reacting KOH solution. Two stirring speeds have been tested to study their effect on silicon etching. As expect, the higher stirring speed 300 rpm results in faster etching. But the silicon is over etched as shown in Fig 2.6b, the protective layer SiO$_2$ is fully removed by KOH etching before forming a nice opening. With 50 rpm stirring, a good opening 400 µm across was etched in 4.5 hours (Fig. 2.6c).

The silicon sample with 400 µm opening is also used to study the etching rate to find the best time for creating pyramidal opening without over etching. Fig 2.7a shows the microscope images of silicon openings after different etch times. The brighter part with reflected light is the silicon <111> side wall; the flat center is the <100> plane. The etching processes until the square-shaped bottom disappeared and a sharp point is achieved. As shown in Fig 2.7b, the bottom width decreased linearly during the first 4 hours and then the change became much slower. The depth of
the silicon opening increases with the difference between top width and bottom width by a factor $1/\sqrt{2}$ (this factor will be discussed in section 2.4.2.2). The silicon etching rate is then calculated as the increase in depth per hour. It is more clearly shown in fig 2.7c that the etching rate during the first two hours is slightly faster than during the following hours, but all of them are in the range from 60 to 80 µm/hour. After 4 hours etching, the speeds slows by a factor of ~5, which may be caused by the small reaction area, or depletion effects arising from the lack of mixing.

**Figure 2.7.** Si <100> plane etching rate in 30 wt% KOH at 80 °C, 400 µm opening sample shown here. a) Microscope pictures at different etching time: 1. 1h; 2. 2h; 3. 3h; 4. 3.5h; 5. 4h; 6. 4.5h. b) Plot of opening bottom width vs etching time. c) Plot of etching rate vs etching time.

Based on the study above, etching time for the different patterns was determined to be: 2.5 hours for the 200 µm opening sample, 3.5 hours for the 300 µm opening sample, and 4.5 hours for the 400 µm opening sample. After KOH etching, the silicon molds are dipped into dilute HF
solution to remove the remaining SiO$_2$ mask and the resulting opening arrays are shown in Fig. 2.8 (2×2 array in the image). Before pen array fabrication, the silicon mold must be treated with fluorosilane. A thin layer of 1H,1H,2H,2H-perfluorodecyltrichlorosilane was formed on the silicon surface after vapor deposition, which prevents PDMS adhesion to the mold. Thus the PDMS pen array can be easily peeled off without breaking the brittle silicon masters.

![Microscope images](image)

**Figure 2.8.** Microscope images of a) 200 µm opening, b) 300 µm opening, c) 400 µm opening.

### 2.4.2.2 Fabrication of PDMS pen array

Sylgard 184 silicone elastomer was chosen to fabricate the PDMS pen array, due to its low cost, non-toxicity, chemical inertness and controllable modulus. It has two components, the polymer base and the curing agent. By changing the ratio between polymer base and the curing agent, the stiffness of PDMS can be controlled. In this project, most of the PDMS pen arrays were made with the standard PDMS with 10 to 1 mixing ratio (polymer base to curing agent), resulting in soft pens for microfabrication. Some harder pens were also made with mixing of ratio 3:1, which was used to create submicron features.

The fabrication process (Fig 2.9) starts from mixing the polymer base with curing agent, and then the mixture was degassed in a vacuum chamber for about 10 minutes to remove air
bubbles. Next, one drop of the degassed mixture was poured onto the silicon mold, then the mold with prepolymer is put back into the vacuum chamber to be degassed for another 10 minutes. After that, a plasma cleaned glass slide (about 20 mm × 20 mm) is placed on top of the silicon mold. By applying some pressure, the remaining air is squeezed out, forming a thin and continuous PDMS layer between the pen array and the glass support. After thermal curing (100 °C for 60 minutes), the PDMS pen array was easily released from the mold. Without further cleaning, the mold can be reused for more than 10 times to fabricate new PDMS pen arrays. Fig 2.10 is the microscope images of different PDMS pen arrays (topview). Bottom width of a single PDMS pen is used to describe the pen size, which is the same as the opening size in the silicon mold. According to the silicon crystalline structure, the angle between the sidewalls (<111> plane) and horizontal surface (<100>) is fixed at 54.7° (Fig. 2.11a). Thus, the height of PDMS pen, h, is correlated with the base width, w, with equation $h = \frac{1}{\sqrt{2}} w$. For example, the height of a 200 µm pen is about 140 µm, which is shown in Fig. 2.11b).
Figure 2.9. Schematic representation of the PDMS pen fabrication.

Figure 2.10. Microscope images of PDMS pen array: a) 200 µm pens. b) 300 µm pens. c) 400 µm pens.
In the inking step, large pens are preferred, as the height prevents wicking ink between pens. But the apex radius of the 400 µm pens is 3~5 µm, and the height of these pens in the same array is not as uniform as smaller pens. The 200 µm and 300 µm pen arrays have submicron tip apex (Fig. 2.11c) and a better pen size uniformity, and so mainly chosen as the main printer probes.

![Diagram](image.png)

**Figure 2.11.** a) A sideview schematic of one PDMS pen. b) Microscopic sideview of a 200 µm PDMS pen. c) SEM image of the apex of a PDMS pen.

Due to the softness of PDMS, pen tips are deformed when the pen array is pressed against the substrate. The size of the tip-substrate contact area varies as the printing pressure changes. As shown in Fig. 2.12, the width of the contact area increases linearly from 6.5 µm to about 35 µm with increasing pressure. Though it is called “force dependent”, in most of cases, the feature size is actually controlled by adjusting the z-displacement of the pen array using the Sonoplot platform but the pressure change is measured. In our newly designed polymer pen printing apparatus, a force-feedback system has been integrated, which enables the function of size control by directly adjusting pressure (discussed in section 2.4.2.4).
2.4.2.3 Design of mechanical parts

In order to mount the PDMS pen array to the robot, four mechanical parts were designed using Rhinoceros software (Fig. 2.13), which were subsequently fabricated at a machine shop from aluminum. Parts a) and b) were designed for course leveling the PDMS pen arrays. Part c is the holder for attaching the PDMS pen array, which is mounted to a pressure sensor. Furthermore, the groove on the bottom is designed for holding a 45° mirror as part of optical observing system, enabling the simultaneous monitoring of tip displacement and pressure. Part d) is a mount connecting robot and part b). As the load capacity of the robot is limited, several large openings were drilled into the two sidewalls of part d to reduce the weight, and insure precise movements.

Figure 2.12. Force dependent plot of a 9 × 9 array with 200 µm pens.
2.4.2.4 Apparatus for multi-pen printing

The polymer pen printing apparatus is shown in Fig. 2.14. Four mechanical parts were assembled and mounted to the xyz robot. Part b) is attached to part a) by three shoulder screws with springs, which is fixed in part d) connecting to the robot. In part a), three micrometer heads are inserted into and fixed in the three large holes with an equilateral triangle arrangement. By adjusting the length of micrometer heads, PDMS pen array can be coarsely leveled. Two piezo stages are incorporated to achieve higher spatial resolution: one is a xyzθxθyθz motorized six-axis aligner (New Focus Model: 8095) for fine leveling the substrate to the pen array; and the other one is the nanopositioner z stage (PI Model: P-611.Z) for submicron fabrication. Between part b) and part c), the red piece is a tension and compression load cell (Futek Model: LRF400), which is used to monitor the pressure applied to the pen tips.
**Figure 2.14.** Polymer pen printing apparatus. Left: photograph of printer; right: schematic image of printer.

The optical system is an indispensable part for leveling the pen array which can only be observed from the backside (through the glass support). In the newly designed polymer pen printing apparatus, due to the holder modification, the microscope from Sonoplot Microplotter II must be moved, which results in a significant challenge of pen observing. Adding a 45° mirror in the holder was the simplest solution to this challenge. Hence, the holder has been designed to integrate a 45° mirror with a groove in the bottom part and a rod at the center. As shown in Fig 2.15, the light turns a 90° angle by reflection of 45° mirror, enabling the pen array to be observed by the microscope on the side. The bottom left is a microscope image of a PDMS pen array through backside.
2.4.2.5 Force-trigger system

As mentioned earlier, the feature size is mostly controlled by adjusting z-extension and the pressure sensor integrated in conventional PPL is only used to monitor the force as tips are deformed. In our polymer pen printing system, the pressure sensor and z-axis nanopositioner were integrated such that the movement of the nanostage was controlled in real-time by the measured force from the pressure sensor. The whole process is described as follows: after inking, the pen array is moved to the position 20 µm above the substrate. When the robot is stopped, a pulse is sent by the computer to trigger the z movement of the nanostage on which the substrate is mounted. When the substrate touches the pen tips, the pressure sensor records the force and compares it to the preset pressure. The nanostage stops when the recorded force reaches the preset force value. After the preset dwell time, the nanostage moves back to its original position with substrate, and the pen array is brought either back to the inkwell or moves to the second position in the pattern.

Figure 2.15. Light path of optical microscope system with a 2×2 pen array image (back view).
2.4.3 Pen array leveling

Polymer pen printing system with a PDMS pen array is designed for high-throughput microfabrication. The pen array creates parallel patterns and each pen print should be identical. Due to the force-dependent nature, it is a great challenge to create uniform features over a large area by different PDMS pens. As shown in Fig. 2.16, with a small angle between pen array plane and substrate surface, the size of resulting features varies. To create features with uniform size, the pen array and substrate must be coplanar, and the process of achieving coplanarity between pen array and substrate is called “leveling”. In conventional PPL, leveling is achieved by tilting the stage via optical or force-feedback approaches, which may take up to 1 hour\textsuperscript{18}. In our polymer pen printing system, two leveling process have been designed: tilting the pen array with micrometer heads and tilting the 6-axis stage.
Figure 2.16. Schematic leveling procedure: a) Process of adjusting micrometers and tilting the stage to level pen array and substrate. b) 2D sectional image of leveling. c) Schematic representation of deformed pens. Black box is the deformation area. d) Microscope images of deformed PDMS pens. Left, unleveled pens with different sized black areas; right, leveled pens with same sized black area.

As PPL, the xyzθxθyθz motorized six-axis aligner is used to tilt the substrate. Three piezomotors in the stage are arranged at vertices of an equilateral triangle to adjust $\theta_x$, $\theta_y$, $\theta_z$ angles of top plane. With open loop control, high angular resolution ($\leq 0.2$ µrad) can be achieved. But due to the slow movement, this stage is only used when precise alignment is required, such as submicron fabrication. Three micrometer heads have been incorporated in the polymer pen.
printing system as a manual leveling system, which enables pen array tilting. In most of cases, micrometer leveling system is adequate to ensure the coplanarity of pen array and substrate, and the size variability of resulting features is as low as 10%.

2.4.4 Challenges for the inking process

The inking process is relatively simple in conventional PPL; ink molecules physically attach to PDMS pens when the whole array is immersed in a dilute ink solution. The inked pen array is dried before mounting to the piezostage and the functional molecules are transferred via a water meniscus in a humid environment. This process is termed “dry-ink”, which is very powerful in printing nanosized features with a single layer of molecules. But the “dry-ink” strategy cannot be applied to viscous ink solution, and the small amount of material transferred with the dry-ink strategy is not enough for 3D additive microfabrication. Thus a “wet-ink” printing strategy was chosen with multi-pen printing so that more material can be transferred to the substrate in a single print, which can be stacked up to create 3D microstructures.

The “wet-ink” printing strategy is very opportune for single polymer pen printing. The millimeter long PDMS pen can be dipped into the inkwell by the robot and moved to the substrate to deposit material without any issue. But in multi-pen printing, this strategy gives rise to a great challenge of inking the pen array. In a typical PDMS pen array, the pitch between pens is only 500 µm and height of individual pen is less than 200 µm. As show in Fig. 2.17a, after dipping the pen array into a regular inkwell, the solution is sucked up and fills the gap between pens, leading to an ill-defined pattern after printing.

Three different inking methods have been proposed to solve this problem. The first one is to create a micro-inkwell for each PDMS pen. According to this method, the silicon mold used for
casting the PDMS pen array can also be used as the inkwell. After alignment, each pen is dipped into one pyramid opening (Fig. 2.17b), hence, preventing excess ink solution from bridging cross adjacent pens. But without a microliter inkjet printer, the ink solution is difficult to be dispensed uniformly into each micro-inkwell. Moreover, with an extra 30 minutes preparation time for alignment, polymer pen printing becomes less convenient.

The third method is to simply bring pens into contact with a wick saturated with dilute ink solution (Fig. 2.17c), which was used in enzyme printing project. The wick is necessary as it not only prevents the adsorption of excess ink, but it also reduces the rate of water evaporation to maintain the concentration and printability of the enzyme aqueous solution.

The last method is called a “doctor blade” approach (Fig. 2.17d). Kapton tape is attached to a glass slide, and then a 1 cm × 1 cm square opening is cut by a blade to make a 60 µm depth inkwell. Ink material in filled into the square opening, and the excess material was removed by blade to form a relatively smooth, uniform and stable ink layer. This method is suited to viscous material with low flowability, such as the graphite filled phenolic resin ink.
Figure 2.17. Different inking strategies. a) Dipping in regular inkwell. The left is the microscope image of inked pen array, where ink solution fills in the gap between pens with tips sticking out. b) Dipping in the silicon mold, each pen has individual inkwell. c) Filter paper soaked with ink as inkwell. d) “Doctor blade” inking method with a 60 µm-deep inkwell made using kapton tape.
2.4.5 Pattern printing

The new multi-pen printing system was first tested to print BST/FA nano-particle ink with controlled pen compression. Droplets of ink were transferred onto an oxidized silicon wafer which was cleaned by piranha solution. The nano-particle dots with square shape were formed as shown in Fig. 2.18. Two dots on the left side were printed by one pen and the other two dots on the right side were printed by the adjacent pen. Changing the pen compression from 20 µm to 50 µm, caused the dot width to increase from 17 µm to 40 µm.

![Figure 2.18](image)

**Figure 2.18.** A single layer nanoparticle BST dot printed by the PDMS pen array: a) tip compressed 20 µm. b) tip compressed 50 µm.

Another application is called high-throughput enzymatic lithography. After printing enzyme patterns on the PCL film using a pen array, samples were incubated to develop the micro-well patterns. As shown in Fig. 2.19a, a 9 × 9 array of PCL micro-well patterns have been successfully fabricated. The opening of the holes has a square shape, which replicates the shape of the deformed polymer pen tips. The 9 different-sized features in one pattern were created by varying the printing force, and the force-dependent relationship is shown in Fig. 2.19b. As a direct
writing lithography method, any arbitrary pattern with square dots can be created by polymer pen printing. One example is shown in Fig. 2.19c, 24 uniform openings make up a “CUNY” pattern. More work have been done and discussed in Chapter 5.

**Figure 2.19.** a) A microscopic image of $9 \times 9$ array of PCL well pattern, inset is an enlarged image of one pattern. b) A plot of printing force vs. the hole-width of a). c) An AFM 3D image of CUNY pattern. d) SEM image of one submicron feature.
The third application for polymer pen printing with a pen array is to create a phenolic needle array using the “doctor blade” inking method. One sample is shown in Fig. 2.20, which was made by 2.5D drawing lithography: continuous lifting of the polymer pen array until the phenolic string breaks in the center. After pyrolysis, the phenolic needles are converted to carbon needles, which is discussed in detail in Chapter 7.

![Figure 2.20. SEM images of a 5 × 5 array of phenolic needle posts.](image)

2.5 Conclusion

Two polymer pen printing systems have been successfully developed, and the “wet ink” printing strategy was chosen for both of them. By attaching a single PDMS pen to Sonoplot Microplotter II, the single polymer pen printing is a convenient patterning tool to create samples with small array.

To increase the through-put, the polymer pen printing system has been fully redesigned and modified to attach a PDMS pen array. The silicon molds with large opening pyramid pits have been successfully fabricated by focus laser lithography and wet etching. The best conditions for
KOH etching, at 80 °C and low stirring speed 50 rpm, have also been found. The casted PDMS array with 200 µm and 300 µm pens are chosen as the printing head, which have good uniformity of pen height and submicron radius of pen apex. The optical observation system and force-feedback system have also been introduced. Some initial results of application of polymer pen printing are listed in the last part. After circumventing the challenges of pen array leveling and inking, the multi-polymer pen printing can be regarded as a powerful tool for 2D and 3D microfabrication.
Chapter 3. Fabrication of 3D Electrodes for Energy Storage Applications

3.1 Background

High dielectric constant ceramics, such as Barium Strontium Titanate (BST), have attracted much attention for fabricating thin-film capacitors\textsuperscript{1,2}. BST has a high dielectric constant as well as good ferroelectric and pyroelectric properties\textsuperscript{3}. To increase the effective dielectric constant of thin-films of BST ceramics, it is necessary to prepare nanocrystallites, which show higher performance reported by many researchers\textsuperscript{4,5}. O’Brien’s group\textsuperscript{6} have synthesized BST nanocrystallites with uniform size distributions, and the tunable 7 to 30 nm size results in controllable dielectric constant of BST ceramics. Fig. 3.1a is a TEM image of BST nanocrystallites with 7 nm diameter. The packing density of functional crystals and the interparticle void space play important roles in determining the dielectric properties of the ceramic film. BST films prepared from nanocrystallites have a 25–35 vol\% empty space filled with air, which significantly decrease the dielectric properties. Therefore, O’Brien’s group developed a novel deposition–polymerization technique to prepare ceramic films, shown in Fig. 3.1b. By infiltrating polymers into the interparticle void, dielectric properties of the films were improved.

The BST nanocrystalline dispersion in a FA binder is a printable ink material developed by O’ Brien’s lab. In this project, single polymer pen printing was used to deposit either a single layer or multilayers of BST/FA to prepare micro-scale capacitors which have potential applications in reconfigurable solid-state power supplies and, with appropriate matrix development, actuators and touch sensors.
3.2 Experimental

3.2.1 Material

The 7 nm BST/FA ink with 30mg/ml Concentration was obtained from Dr. O’Brien’s lab at the City College of New York, City University of New York.

3.2.2 Surface modification

The PDMS single pen tip was treated in an oxygen plasma for 10 seconds to change the wettability from hydrophobic to hydrophilic.

Silicon substrates were treated in three different ways: the detergent-cleaned silicon was prepared by treating the wafer in an Alconox detergent solution for 5 minutes in an ultrasonic bath, and then rinsing with distilled water several times. Next, the wafer was blown with clean air and dried in an oven at 130 °C for 10 minutes. The HF treated silicon was prepared by immersing the detergent-cleaned silicon in a diluted HF solution for 10 minutes, and then rinsing and drying as described in the previous step. The hydrophilic silicon wafer was treated with oxygen plasma for 30 seconds.

Figure 3.1. a) TEM image of 7 nm BST crystalline. b) SEM image of BST/FA film.
3.2.3 Printing Procedure

The single PDMS pen is dipped into the inkwell to the preset depth of 50 µm for 0.5s, coating with BSA/FA ink. Then the pen is brought into contact with the silicon substrate to deposit BSA/FA material onto the surface with a speed and acceleration of 50,000 µm/s and 50,000 µm/s². By repeating the printing cycle, such that the inked pen tip is brought into contact with the silicon substrate at a different location each time, an array of dots is formed. To create a thicker film, a multi-printing approach has been chosen here. Using the 100 µm diameter pen, dot arrays with 1 to 4 tiers were prepared by depositing ink at same position without changing the printing height.

After printing, samples were dried at 60 °C for 30 min to initialize the FA polymerization, and then heated to 120 °C to fully cure the poly (furfuryl alcohol) to form a stable film.

3.2.4 Surface profilometry

The thickness of cured patterns were measured by surface profilometry. A line profile of 9000 µm was acquired with a Tencor Alpha-Step 200 Profilometer with 30 µm/s scan speed. The probe-substrate contact force was set at 1.00 mg to avoid sample damage.

3.3 Results and discussion

3.3.1 Effect of pen tip dimensions on delivering ink

Two PDMS pens with different tip dimensions were used to deposit the BST/FA ink. The smaller pen has a tip with 10 µm diameter, and the larger one has 100 µm diameter tip as shown in section 3.1.2. After inking, BST/FA forms a liquid ball on the larger pen, which is then transferred to the silicon surface to create a circular area with 1~2 µm thickness. On the other hand, there is only a thin layer of BST/FA ink coated onto the 10 µm diameter pen, this results in low
efficiency ink delivery ink and non-uniform dots. The results shown below were printed using a pen with a 100 µm diameter tip.

3.3.2 Effect of tip surface on dot diameter

PDMS is a hydrophobic material, but FA is a hydrophilic organic liquid. Thus, in order to pick and print FA based ink, an oxygen plasma process was applied to modify the wetting characteristics of the printing pen surfaces. The contact angle of water on the as-formed PDMS surface is 114° whereas the contact angle is reduced to less than 1° after oxygen plasma treatment as shown in Fig. 3.2. As expected, the hydrophobic pen tip limits ink quantity and results in smaller diameter deposits whereas the hydrophilic tip can print larger dots and form a complete pattern.

Figure 3.2. Contact angle of water on a PDMS surface. a) As prepared and b) after oxygen plasma treatment.
Figure 3.3. Microscopic images of single tier deposits on silicon substrates with treatment of a) oxygen plasma (CA <1°) and b) HF (CA = 63°); Thickness profiles of single tier deposits on silicon substrates with treatment of c) oxygen plasma and d) HF.

3.3.3 Effect of substrate surface properties on the print size and thickness

The hypothesis for ink diffusion across the substrate is that low energy promotes spreading of the ink on the surface. Thus, the surface chemistry of the substrate is an important factor that may determine the dot size and thickness. In this project, the silicon substrate was treated using different approaches to modify the energy and wettability of the surface. These treatments included oxygen plasma, detergent washing and HF etching creating surfaces on which water formed contact angles ranging from 1° to 63°. The results on a high energy surface (oxygen plasma treated, contact angle < 1°) and a lower energy surface (HF treated, contact angle 63°) are shown in Fig.
3.3. Both surfaces result in well-formed dots with relatively uniform thickness. As expected, on the plasma treated substrate, single dot deposits have a slightly larger diameter than the dots on HF treated substrate (230 μm vs 204 μm). As a result, the thickness values of single dot deposits on the plasma treated substrate are lower (760 nm) than for the HF treated substrate (1310 nm).

3.3.4 Effect of multi-deposit printing on layer thickness

For many applications, we seek to minimize deposit thickness in order to maximize capacitance. However for other applications, a thicker capacitor layer would be desired. Thus, multi-tiered structures were formed by printing ink at the same location. The thickness was found to increase linearly with the number of deposits for all three types of substrates. In addition, the diameter was found to increase very gradually with increasing print cycles. This is an encouraging result as it will enable the formation of relatively high aspect ratio features that are desired with controlled thickness and diameter. Results for a plasma treated silicon substrate are shown in Fig. 3.4.

![Figure 3.4](image)

Figure 3.4. Effect of multi-tier printing on the a) thickness and b) diameter of the deposit.
3.3.5 Effect of tip printing and ink pick-up position on dot size and thickness

Tip printing position during ink transfer from tip to substrate was studied to determine the sensitivity of the process to tip location and compression. The zero position is defined as where the ink on the tip barely touched the substrate and could transfer across. Negative values indicate a lowering process of the tip after touching the substrate, such that tip becomes progressively more compressed against the substrate surface with increasingly negative values. For example, the -50 μm height means that the print position is set at 50 μm below the substrate surface, resulting 50 μm compression of the tip. As shown in Fig 3.5a and 3.5b, decreasing the print height leads to an enlargement of the deposit diameter and a concomitant decrease in deposit thickness. This is consistent with expectations as the tip is compressed against the substrate, occupying volume causing the ink to spread. However this effect is relatively small and not strongly dependent on print height.

The sensitivity of the pick-up position in the inkwell on the deposit thickness was monitored using an oxygen plasma treated silicon substrate. As shown in Fig 3.5c and 3.5d, the deposit thickness is independent of ink pick-up position in the inkwell from -100 to 0 μm. Here, a tip position of zero is defined as the position where the tip first touches the ink. Negative values indicate pen immersed in the ink. The smaller (i.e. more negative) values, the greater the immersion depth. In this experiment, no significant effect was found.
Figure 3.5. The effect of tip print height on a) deposit diameter and b) deposit thickness; the effect of tip ink pick-up position on c) deposit diameter and d) deposit thickness.

3.4 Conclusion

BST/FA ink was successfully patterned using a single polymer pen printing method. Due to the hydrophilicity of FA solvent, the wetting behavior of PDMS plays an important role in determining feature size and thickness. The oxygen plasma treated PDMS pen can transfer more material than the untreated one, forming uniform dots. Analogously, the oxygen plasma treated silicon wafer promotes the expansion of BST/FA ink, results in larger diameter dots but smaller thickness. Multi-tiered structures were also fabricated by printing ink at the same location to obtain a thicker capacitor layer. The thickness increases linearly with the number of deposits without significantly changing the diameter. The last printing parameters, including printing height and ink pick-up position, have also been studied. The results show that changes to the tip position in
the ink reservoir have essentially no impact on deposit thickness or diameter; the diameter is only sensitive to the first touch of printing, once the tip comes into contact with the substrate, further pressure does not have a significant effect.
Chapter 4. “Writing Enzyme” by Single Polymer Pen Printing

4.1 Background

Researchers have great interest in fabricating various types of micro-biodevices for drug delivery\(^1\), cell culture\(^2\) and microfluidics\(^3\). MEMS fabrication methods, including photolithography and soft lithography, have been primarily chosen to fabricate microstructures. However, these techniques are limited to traditional MEMS materials, such as silicon, glass and PDMS\(^4\). In order to extend the application of micro biodevices, researchers have been trying to find new materials and develop “green” techniques.

Enzymatic lithography is a novel approach that can fabricate 3D microstructures in biodegradable and bioresorbable materials\(^5,6\). By “writing enzyme” on the polymer surface, the enzyme catalyzes polymer degradation during incubation to create micro or nano wells and trenches in the polymer films. But due to the biological specificity of enzymes, most examples are based on enzyme-biological molecules, such as DNA\(^7\), lipid\(^8\) and peptide systems\(^9\), which do not have good mechanical properties. Furthermore, these films to be etched are only several nanometers thick, which narrows their applications.

In this work, a new system for enzymatic lithography has been developed\(^10\). Commercial poly(\(\varepsilon\)-caprolactone) (PCL) is chosen as the film material as this polymer is used in many medical applications. Under humid condition and at 37 °C, PCL can be rapidly hydrolyzed by Candida antartica Lipase B (CALB)\(^11\). Fig. 4.1 is the mechanism of PCL degradation, showing the final products as hydroxyhexanoic acid and its oligomers\(^12\). These molecules are water soluble and can diffuse and volatilize readily due to their small molecular weight. Thus, once the CALB is placed onto a PCL film, it can decompose the surrounding PCL to form wells with several hundred
nanometers in depth, which may have the ability to contain and release biologically active molecules in a biocompatible environment. Here, the single polymer pen printing is demonstrated as the fabrication approach, where the PDMS pen can “write” any arbitrary patterns with CALB ink. Water is required for the CALB to rapidly degrade PCL. Therefore, after enzyme writing, PCL samples are incubated at 37 °C and 95% relative humidity environment to develop 3D hole microstructures, which are characterized by atomic force microscopy (AFM).

**Figure 4.1.** CALB-catalyzed degradation of PCL.

### 4.2 Experimental

#### 4.2.1 Material

PCL (Tone Grade 787, Mw=80,000 g/mol) was obtained from DowChemical. Enzyme (Candida antarctica lipase B, CALB) was obtained from Dr. Gross’s lab in NYU Polytechnic Institute (he is in Rensselaer Polytechnic Institute now). 99.8% anhydrous toluene was purchased from Sigma-Aldrich and used as received.

#### 4.2.2 PCL film preparation

Silicon wafers were cleaned by immersing in piranha solution for 20 minutes and then rinsed thoroughly with DI water. To increase the affinity between PCL and wafer, 10 wt% hexamethyldisilazane (HDMS) in xylene were spin-coated on the silicon surface at 3000 rpm for 30 s and then dried for 10 min at 100 °C. PCL pellets was dissolved in toluene with 2.5 wt%
concentration, then the PCL solution was spin-coated onto a HDMS treated silicon wafer at 3000 rpm for 30 s. The resulting PCL films have average thickness 300 nm.

4.2.3 Enzyme printing process

Enzyme patterns were printed on PCL films by transferring small quantities of the enzyme aqueous solution from a reservoir to the film surface using single polymer pen printing method. The PDMS pen with 10 µm-diameter tip was used in this experiment and 5 wt% CALB aqueous solution was prepared as the ink. The Microplotter II system was programmed to dip the PDMS pen into the CALB solution for 0.5 s and then translate the pen to the appropriate location on the substrate (with a speed and acceleration of 5 cm/s and 5 cm/s²). Next the PDMS pen was lowered to contact with the PCL surface to deposit the enzyme onto it. This process was repeated to create any arbitrary pattern. After printing, patterns were incubated at 37 °C and 95% humidity for the desired time.

4.2.4 Atomic Force Microscopy (AFM)

AC (tapping) mode was used to characterize samples during incubation. An image area of 90 µm × 90 µm was acquired with an Asylum MFP 3D-Bio AFM (Asylum Research, Santa Barbara, CA) using silicon AFM probes with 48 N/m spring constant and 190kHz resonant frequency (Nanoscience Instruments, Inc. Phoenix, AZ).

4.3 Results and discussion

4.3.1 A “Enzyme writing” process with the single polymer pen printing

In the “enzyme writing” project, our collaborators used the micro-contact printing (µCP) method to deliver enzyme, where a specific pattern is defined into a stamp28. In my part, the single
polymer pen printing technique was used to “write” any arbitrary pattern into the PCL film by depositing enzyme using a PDMS tip. The polymer pen printing and µCP approaches are similar, such as stamp and pen, are both made from polydimethysiloxane (PDMS). However, polymer pen printing has an advantage over µCP that it enables the formation of any arbitrary image simply by appropriate programming of the robot.

Basically, as shown in Fig. 4.2, the PDMS pen tip is coated with an aqueous solution of CALB during the inking step that is then brought into contact with the PCL film to transfer the enzyme onto the surface. By repeating the printing cycle, such that the inked pen tip is brought into contact with the PCL film at a different location each time, a pattern of dots is formed. After printing the entire pattern, the film was developed at 37 °C and at a high relative humidity (95% RH). The deposited enzyme locally degraded the film surface creating the equivalent of a one-step lithographic process.

![Figure 4.2](image.png)

**Figure 4.2.** A schematic presentation of the enzymatic single polymer pen printing process.

### 4.3.2 Large PCL features

The optical microscope images of a “CSI” pattern covering a 1.2 x 0.6mm area resolved after different incubation times are shown in Fig. 4.3. To apply sufficient pressure to transfer the enzyme from the PDMS pen to the PCL surface, the robot was programmed to extend 20 µm below the surface. This resulted in a pressure that compressed and deformed the low-modulus PDMS
tip, forming enzyme deposits with dimensions as large as 48 µm. Because of the substantial tip deformation used in transferring CALB, the deposits are larger compared to the tip radius of 10 µm. In addition, the CALB ink is deposited, not as a round circle, but in a shape approximating a cardioid that measures 48 µm across the widest point and 20 µm across the narrowest opening. Consequently, this cardioid shape is transferred into the PCL film.

From Fig. 4.3, we also found that during incubation, the dimensions and shape of large features did not significantly change. Lateral growth of the etched holes is not observed to any significant extent. In addition, the etch rates are not affected by the presence of boundaries between spherulite domains. This result indicates that the single polymer pen printing approach benefits from the affinity of CALB for the PCL film.

![Figure 4.3](image)

**Figure 4.3.** Optical microscope images of the enzyme patterned PCL film after different incubation times. a) Before incubation. B) 1 day. C) 3 days and d) 7 days of incubation.
Over a 7-day period, CALB progressively etched the PCL down from the film surface to the silicon wafer on which it was spun (350 nm). Fig. 4.4a shows a 2D view of an individual hole topography. Fig. 4.4b displays the sectional profile of a PCL hole. The PCL wall is straight, and the bottom of the holes are relatively smooth and level, indicating that CALB completely degraded the PCL film in areas to which it was applied. The 95% RH applied in this experiment resulted in a faster etch rate and complete PCL degradation compared to the 30% RH used in the µCP experiment\textsuperscript{10}.

**Figure 4.4.** AFM images of the enzyme patterned PCL film after incubating for 7 days at 37 °C and 95% RH. a) 2D topographic AFM images of one feature. B) Sectional analysis of a.

### 4.3.3 Small PCL features with low printing pressure

At low tip deformations, where the pen was programmed to extend 10 µm below the surface, the round tip diameter and shape are preserved resulting in smaller diameter round holes in the PCL film. However, the sizes and shapes showed substantial variability, shown in Fig. 4.5a and 4.6a. From numerous repeat experiments, the hole size printed at minimal contact pressure is 10 µm ± 50%. Beside this, the lateral diffusion is obviously observed in small features from AFM
images. Fig. 4.5b and 4.5c are the 3D and 2D views of holes arrayed to form the letter “N”. Fig. 4.5d is a sectional profile of a row of 4 holes. Over a 3-day period, the enzyme progressively etched 250 nm thickness into the PCL film while the diameter of the holes increased from 10 to 12 µm with a tapered wall.

**Figure 4.5.** Optical microscope and Tapping mode AFM images of the PCL film after incubating the deposited enzyme for 3 days. a) Optical microscope view. b) 3D topographic view. c) 2D topographic view and d) sectional analysis.
Figure 4.6. Optical microscope and Tapping mode AFM images of the PCL film after incubating the deposited enzyme for 6 days. a) Optical microscope view. b) 3D topographic view. c) 2D topographic view and d) sectional analysis.

With additional incubation time (6 days total), the enzyme etched through the 350 nm film thickness to reveal the silicon substrate (Fig. 4.6) with a concomitant increase in hole diameter to 14 μm. This etch process appears to be isotropic, which lowers pattern resolution. Therefore, further investigation has been done in CALB-PCL system to seek optimal conditions for enzymatic lithography approach. The effect of tip shape, printing pressure, RH, PCL morphology will be discussed in detail in Chapter 5.
4.4 Conclusions

This work shows that enzymes can be spatially arranged rapidly on PCL surfaces by applying the single polymer pen printing method. During incubation at 37 °C and 95% RH, enzymes at PCL surfaces use water in the atmosphere to catalyze polyester hydrolysis, creating hole patterns with defined dimensions (10 to 50 µm) by controlling tip compression. The size of these features is well suited for use as biocompatible micro-reactors. The next part of the enzymatic lithography work, discussed in chapter 5, demonstrates that well-defined features with better resolution can be created more rapidly by multi-pen polymer pen printing, by controlling PCL crystalline morphology, RH, and pen shape.
Chapter 5. A High Throughput, High Resolution Enzymatic Lithography

Process: Effect of Crystallite Size, Moisture and Enzyme Concentration

5.1 Introduction

The use of enzymes to selectively degrade polymers has attracted significant interest as both three dimensional scaffolds \(^1\)-\(^4\) as well as two dimensional patterns \(^5\)-\(^{10}\) can be formed using biocompatible materials with relatively straightforward printing technologies. The need for photosensitizers and solvents, required for traditional lithography, is obviated as patterned enzymes on a surface can decompose specific polymeric materials into small molecules during incubation to create micro- or nano-scale features in the film. For example, DNase I line patterns were deposited on a DNA self-assembled layer using dip pen nanolithography (DPN).\(^{11}\) Patterns 438 \pm 32\,\text{nm} wide and 3.0 \pm 0.1 \,\text{nm} deep were formed after incubation. One problem manifested by this system is the lateral diffusion of enzyme. The width of the resulting features was 2-3 times greater than that of the DNase I printed line. In order to prevent loss of image quality resulting from enzyme mobility across the surface, researchers have attempted to chemically bond the enzyme onto the transfer tools.\(^{12}\) By contacting an enzyme-immobilized polydimethylsiloxane (PDMS) stamp, features in the peptide film were defined without enzyme lateral diffusion. Since the enzyme must be held in contact with the material surface to catalyze the degradation, high resolution patterns can only be achieved in thin (~0.5 nm thick) films using long tip-substrate residence times (300 s).

To create etched patterns rapidly in the thick (100 \,\text{nm} – 10 \,\mu\text{m}) films required for many biological and electronic applications, \(^{13}\)-\(^{16}\) it is not practical to bind the enzyme to the printing tip; for high throughput printing, the tip must rapidly transfer the enzyme onto the biodegradable
polymer surface so that image development (i.e. incubation) can occur outside of the printing tool. Such enzyme transfer followed by image development is comparable to traditional lithography processes where throughput in the exposure tool is the rate limiting step, and image development occurs as a subsequent operation.  

Polymer pen lithography (PPL) is a lithography method that enables a wide range of promising applications including use as a tool to create nano/micro-arrays of biomolecules. PPL is especially well-suited for enzymatic lithography because, compared to conventional techniques such as photolithography and vapor deposition, PPL can pattern enzymes directly without denaturation, thereby maintaining enzyme activity. As opposed to DPN that uses microfabricated silicon pen tips, PDMS pens are used in PPL, which are easier to fabricate into large arrays, thereby lowering costs. The relatively low elastic moduli of the pens enhance durability and provide compliance to non-planar surfaces. By controlling the force applied to the pen array, features with dimensions ranging from nanometers to microns can be created. As compared to micro-contact printing (μCP), PPL can create any arbitrary enzyme pattern by programming the printing sequence. The main challenge with using PPL to deposit enzymes in a specific pattern is that the enzyme becomes mobile on the polymer surface. To maximize throughput in the printer, a system that exhibits strong enzyme-polymer interactions is necessary, such that enzyme mobility on the surface is limited. In this way, the slow incubation process can be shifted away from the printing tool to an inexpensive incubation oven, increasing throughput and decreasing costs.

Previously, we have shown that the enzyme *Candida antartica* Lipase B (CALB) can be selectively bound to poly(ε-caprolactone) (PCL), and single pen printing, has been used to fabricate relatively large features in 300 nm thick PCL. In this paper, we describe how a high
throughput polymer pen printing methodology as well as control of the PCL crystal morphology and incubation conditions enables the formation of micron sized features in thick (up to 2 µm) PCL films. CALB is transferred onto the surface using an array of pyramidal PDMS posts and binds to, and then hydrolyzes, PCL to form hydroxyhexanoic acid; \(^{28,29}\) selective deposition of CALB leads to the formation of patterns in the PCL film. The strong interaction between CALB and PCL limits enzyme mobility; features etched into the PCL film faithfully replicate the original printed enzyme pattern to within the resolution of the fluorescence microscope (~0.5 µm). To gain insights into enzyme mobility on the PCL surface, a high resolution enzyme transfer system was developed and used to characterize the relationship between enzyme transfer area and etched feature size. We show that humidity and PCL crystallization play key roles in controlling polymer etch rates and preventing enzyme mobility, thereby insuring pattern fidelity. This approach was used for the lithographic definition of copper films on circuit board substrates. Patterned PCL films were used as acid etch resists and micron-scale features were transferred into copper foil circuit board laminates thus providing a “Green” route to electronic circuit fabrication.

5.2 Experimental

5.2.1 PCL film preparation

Poly(ɛ-caprolactone) (Tone Grade 787, \(M_w=80,000\), PDI = 2.0) was used as received from Dow Corning and dissolved in the appropriate solvent for spin coating. Silicon substrates were cleaned, dried, spin-coated with 10 wt% hexamethyldisilazane (HDMS) in xylene at 3000 rpm for 30 s and dried for 10 min at 100 °C. PCL was spin-coated at 3000 rpm for 30 s. Resulting film thickness ranged from 0.1 to 6 µm, depending upon the PCL solution concentration as shown in Table 5.1.
Table 5.1. PCL film thickness depends on solvent and PCL solution concentration.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration (wt%)</th>
<th>Film thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>5</td>
<td>0.3</td>
</tr>
<tr>
<td>Chloroform</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

To control the crystallite grain size in PCL, spun films were melted (120 °C for 30 min) and then cooled under three different conditions: (1) PCL with large spherulites was obtained by cooling from 120 °C to room temperature over 10 h; (2) PCL with medium spherulites was obtained by cooling rapidly to 25 °C; (3) PCL with small crystalline domains was obtained by immersing the molten sample in liquid nitrogen for 30 s. All samples were dried in a desiccator for approximately 24 h before use to minimize the amount of water absorbed in the film.

5.2.2 Enzymatic polymer pen lithography

The *Candida antarctica* lipase B in liquid media was obtained from Novozyme, purified by ultra-filtration, lyophilized and frozen until use. Enzyme ink solution was prepared by directly dissolving CALB in deionized water (0.2 mg/mL). Pen arrays were formed on a glass slide (15 × 15 × 1 mm) by casting PDMS (Sylgard 184 Dow Corning) into a photo-etched silicon mold. A typical array was comprised of 81 square pyramidal pens (9 × 9 array) with base length of 300 μm and height of 212 μm (Fig. 5.1a). The pen array was held by a specially designed arm attached to a modified GIX™ Microplotter™ II (SonoPlot, Inc.). The Microplotter provides programmable
motion in three orthogonal axes with 5 µm resolution. The pen array arm is connected to a load cell (FUTEK model LRF400) with 10 g range and 0.001 g resolution. To achieve features <6 µm across, a nano-positioning Z-stage (P-611.Z, Physik Instrumente) with 0.2 nm resolution was used to control the vertical displacement of the substrate. A force-feedback computer controller limited the vertical displacement of the nanopositioner until a preset force was achieved.

The pen array was re-inked before each printing step to insure a consistent concentration of CALB on the pen surface. To re-ink, the pens were brought into contact with a filter paper saturated with CALB solution (Fig. 5.1b and 5.1c). After printing was completed, the film was removed from the printer and incubated at 37 °C at a controlled humidity (74%, 87% and 100%) for a predetermined time. Humidity was maintained by using standard saturated salt solutions, sodium chloride, potassium nitrate and pure water, respectively.
Figure 5.1. Optical microscope images of the PDMS tip array, individual PDSM tip profile, scheme illustrating the inking process and views of the tip array before and after inking. a) Optical microscope image of a $9 \times 9$ PDMS pen array with side view of a single pen (inset). b) Scheme of the inking process. c) Illustration showing the perspective used to optically observe the PDMS tips (from the backside) during inking, and optical microscope images of a portion of the pen array viewed before and after inking.
5.2.3 Differential Scanning Calorimetry (DSC) Studies

A TA instruments DSC Q100 calibrated with indium was used to analyze the PCL samples with different crystal size. 1.5 mg of each sample was loaded into a normal aluminum pan and sealed. The sealed pans were placed into the DSC instrument and heated to 90 °C at a heating rate 10 °C/min under N₂ gas.

5.2.4 Atomic Force Microscope

An Asylum MFP 3D-Bio AFM was used to characterize the etched PCL samples. Images of different areas (90 µm × 90 µm, 20 µm × 20 µm and 5 µm × 5 µm) were obtained by setting the AFM in the AC (tapping) mode and with the use of a silicon AFM probe (48 N/m spring constant and 190 kHz resonant frequency, purchased from Nanoscience Instruments, Inc.). To observe the structure of PCL spherulites, a slow scan rate (0.3Hz) was used. The line profiles shown are convoluted by the slope of the tip (10°).

5.2.5 FITC labeling

A FluoroTag FITC Conjugation Kit (Sigma FITC-1) was used to conjugate fluorescein isothiocyanate (FITC) to purified and lyophilized CALB. The FITC-CALB conjugate was isolated by elution from a column (Sephadex G-25M, phosphate buffered saline) as the first of the two bands detected (A₂₈₀) during elution. The average molar ratio of FITC to CALB, determined by absorbance of FITC-CALB at 280 and 495 nm, is 1.5:1.0.
5.2.6 Live Cell Microscopy

Real-time observations of fluorescently labeled CALB (FITC-CALB) were performed using a Zeiss Cell Observer Microscope. A 488 nm laser was used for excitation and a 520 nm filter was used to observe the emission of FITC fluorescence before incubation. Once incubation began, fluorescence of FITC was self-quenching. 31 To image the etched features, a 505 nm excitation wavelength was used and all of the reflected light was collected, which is defined as bright field. The environmental chamber was maintained at 37 °C. An interior chamber was fabricated to increase the local RH as shown in Fig. 5.2. Humidity within this interior chamber was elevated to ~80% using a saturated potassium nitrate salt solution.

![Figure 5.2](image)

**Figure 5.2.** a) Zeiss live-cell microscope. b) Schematic image of environmental chamber and salt solution chamber.

Temperature inside the live-cell microscope was maintained at 37 °C. In order to control the RH, a plastic incubation chamber was created and attached to the glass-slide holder. Due to the imperfect sealing of the incubation chamber, incubation of enzyme inked PCL surfaces was at a
RH below 80%. Consequently, the etch rate observed in live-cell microscopy was 5 or 6 times slower than for samples incubated in an oven with humidity controlled by salt solutions.

Once incubation began, fluorescence of FITC was self-quenching. To image the etched sample, the substrate was illuminated with 505 nm excitation and all emitted light was collected as bright field; as a result, the partially etched regions were observed as dark features. Under these observation conditions, the exposed silicon substrate appears bright as it reflects the incident illumination. For films ≥300 nm, the fully etched features appear enlarged as the film thickness is greater than the depth of focus of the 40x live-cell microscope lens.

5.2.7 Copper Etching

Copper foil (5 µm thick) laminated to a polyimide substrate (Sheldahl) was cleaned using ammonium persulfate (20 wt%, 30 s, RT), spin-coated with 10 wt% hexamethyldisilazane (HDMS) in xylene at 3000 rpm for 30 s and dried for 10 min at 100 °C. PCL film (2 µm thick) was applied by spin coating a 5 wt% solution in chloroform at 3000 rpm and drying at 120 °C for 30 min. After incubation at 87% RH and 37 °C for 120 min, the exposed copper was etched using a 30 wt% FeCl₃ aqueous solution at room temperature for 30 s. Then the PCL protective coating was removed by immersing sample in 0.2 wt% CALB solution at 37 °C for 30 min.

5.3 Results and discussion

5.3.1 Process overview

The PPL process used in this paper is shown in Scheme 5.1. An array of PDMS pens, mounted onto a robotic arm (print head), is brought into contact with an inkwell saturated with an aqueous solution of CALB. The inked pens are translated and so brought into contact with the
surface of a PCL film for a predetermined time and specified force. During this contact time, CALB is transferred to the PCL surface. The inked film is then removed from the printer and incubated in a humidity controlled environment at 37 °C for a fixed time. After sufficient incubation, the pattern is fully developed. No chemical development, rinse steps or other approaches to remove either degradation products or enzyme, are required.

Scheme 5.1. Enzymatic patterning of PCL film by polymer pen lithography.

5.3.1.1 Printing System

The pyramidal pen array is made by casting PDMS into a silicon mold against a glass slide. In contrast to conventional PPL processes, where the print head is attached to a modified AFM, we use a relatively coarse-scale (5 µm resolution) three axis robot, to translate the PDMS pen array. The robotic arm provides a significant advantage over an AFM as it allows for the translation of the print head over large distances (31 x 30 x 7 cm). This enables the use of an external inkwell and facilitates the re-inking of the pens after each print step. Thus process steps a and b in Scheme 1 are repeated for each feature in a pattern before incubation (step c). The PDMS pen array typically consists of 81 pens in a 9 x 9 array as shown in Figure 1a and is connected to a 0.001 g resolution force gauge. For printing fine scale features, co-planarity of the print head and the sample as well as a high resolution z-axis motion (<100 nm) is required; this is not possible with the 3-axis robot alone. To achieve these features, a tilt stage was mounted below
the robotic arm to enable pitch and yaw control and a piezoelectric z-stage was mounted on top of the tilt stage to enable fine scale (<10 nm) translation of the substrate upwards to contact the PDMS pens. A force-feedback loop was used to reproducibly control the contact force.

### 5.3.1.2 Inking Process

To transfer a uniform and reproducible amount of CALB onto the PDMS pens, the array is brought into contact with the inkwell with a specific force. Instead of carefully dipping pens into micro-inkwell,\textsuperscript{23,24} the pens are simply brought into contact with a wick saturated with CALB solution to coat with enzyme before each printing cycle (Fig. 5.1b and c). A wick is necessary as it is difficult to ink the pen array directly in an open inkwell without absorbing excess CALB solution that can bridge across adjacent pens, degrading resolution. Similarly, using a saturated wick alone proved insufficient as the solution evaporated over time; a relatively dry wick would not transfer sufficient solution. To maintain a constant level of saturation, the wick was placed over a CALB solution reservoir as shown in Figure 1b. After contact with the wick, the CALB solution could be observed on each PDMS pen as shown in the Fig. 5.1c. The volume of liquid absorbed onto each PDMS tip is small (< 100 nL) and so the solvent (i.e. water) evaporates quickly under the experimental conditions. Thus, by the time the PDMS tips contact the PCL surface, excess solvent has evaporated and the tips transfer “moist” enzyme.

PDMS is a hydrophobic polymer with nonpolar methyl groups on the surface; the resulting limited wettability restricts the ability of the pens to absorb aqueous inks. Therefore, the pen surface must be chemical functionalized before inking. For example, oxygen plasma treatment is frequently used to increase the wettability of PDMS, but this effect is not permanent. We used a simplier procedure to modify the PDMS pen wettability in this experiment. The pen array was
pre-treated with the CALB solution (~ 5 mins) during which time CALB was adsorbed onto the PDMS surface increasing its hydrophilicity. Proteins are known to denature on a hydrophobic surface such that the hydrophobic moieties adhere to the hydrophobic surface, exposing hydrophilic moieties to the aqueous solution.³²⁻³⁴ This thin denatured layer is irreversibly bound to the PDMS and enables the CALB ink to wet the pens. After treatment, the contact angle of the PDMS surface decreased from 106° to 84° as shown in Fig. 5.3. The treated pen array can be used for several months. After inking, the PDMS pens are brought into contact with the PCL surface with a controlled force and contact time to transfer the CALB. By programming the printing sequence, any arbitrary pattern of enzyme ink deposits can be generated, one pattern for each pen in the array.

![Figure 5.3. Enzyme treatment of PDMS surface.](image)

**5.3.1.3 Incubation**

After the enzyme-patterning PPL process was completed, the PCL film with CALB was incubated in an oven at 37 °C at a controlled relative humidity (RH). CALB catalyzed the degradation of the PCL films during incubations, forming features etched through the film stopping at the underlying silicon substrate. Because of the interactions between CALB and PCL, the width of the etched features is comparable to the width of the contact area between PDMS pen
and PCL; no mobility of CALB across the PCL surface was observed. Fig. 5.4 is an optical microscope image of a 9×9 array of “CUNY” patterns resolved after incubation (87% RH). Each “CUNY” pattern (Fig. 5.4b) is composed of 25 square features generated by the same pen within the printhead; all “CUNY” patterns were created simultaneously, each by a separate pen.

**Figure 5.4.** a) Optical microscope image of a 9 × 9 “CUNY” patterns incubated for 1 h at 37 °C. b) One enlarged “CUNY” pattern.

**5.3.2 Effect of applied force on feature size**

By controlling the force with which the PDMS pen array contacts the PCL surface, the size of the features can be varied. As shown in Fig. 5.5a and Fig. 5.6, the width of the features increases from 6.5 to 16.0 µm as the applied force to the array was increased from 0.25 to 2.60 g. To form the 10 µm wide features in Figure 5.4a, a force of 0.8g (0.01g/pen) was applied to the pen array in each print step. Deviations from a perfect square shape are consistent with the deformation of the low-modulus PDMS pens (Fig. 5.5b).35
Feature sizes below 6 µm could not be reproducibly resolved using the SonoPlot robot alone as the 5 µm step size of the robotic arm was too coarse to control the force with adequate precision. Features less than 1 µm were resolved (Fig. 5.5c) that exhibit straight sidewalls (Fig. 5.5d) when a nano-positioning stage, coupled to a force-feedback controller, was used to control the vertical displacement of the substrate. In this way a force of 0.002 g was applied to the PDMS pen array (2.5 x 10^{-5} g/pen) which limits the compression of the pen to < 1µm in width.

**Figure 5.5.** a) Optical microscope image of a 4 x 4 array of individual features; columns of features were printed with 0.25, 0.93, 1.77, and 2.60 g of force. b) optical microscope image of compressed PDMS tip ~0.012 grams/pen. c) AFM image of a <1 µm wide feature in quenched PCL film after incubation using a print force of 2.5 x 10^{-5} g/pen. d) Line profile of AFM image 1c.
Figure 5.6. Plot of printing force vs. feature width after incubation.

5.3.3 Effect of film properties

Image quality is affected by the thickness of the PCL film. Thinner films result in more precise replication of the printed enzyme pattern and edge quality. As shown in Fig.5.7a and 5.7b, films that are 100 and 300 nm thick faithfully reproduce the printed CALB pattern within 0.5 µm difference. However, at 2 µm film thickness, the reproducibility and feature quality diminish. This loss in reproducibility and feature quality is further amplified for 6 µm thick films (Fig. 5.7c and 5.7d). Features vary in size and line edge quality decreases in micron-thick PCL films as the spherulite size becomes smaller than the film thickness. This indicates that lateral etching occurs to a greater extent with increased film thickness. Furthermore vertical etch rates decrease from 20 nm/min to 16 nm/min with an increase in the film thickness from 100 nm to 2 µm. When the film is sufficiently thick, the etching process cannot be completed. Features in the 6 µm thick PCL film were not fully etched at 87% RH after 3 days. This reduction in rate may be due to a decrease in
the pH that results from an increase in the concentration of degradation products as discussed further in CALB mobility section.

**Figure 5.7.** Image quality of features defined in PCL films as a function of their thickness: a) 100 nm. b) 300 nm. c) 2 μm. d) 6 μm.
The quantity of water absorbed into the PCL film before enzyme deposition also affects etch rate and image quality. After the film is spun down onto the silicon substrate and thermally treated, it is fully dried over silica gel in a dessicator for 12 hours to minimize the amount of absorbed water. If the film contains too much moisture, then the CALB mobility will increase laterally resulting in the loss of image fidelity.

### 5.3.4 Effect of CALB concentration on image quality

One strategy to increase the etch rate and shorten incubation time is by transferring more CALB onto the PCL surface; the CALB solution concentration cannot be further increased (above 0.2 mg/mL) without causing precipitation. Increasing the number of prints at the same location increases both the concentration and uniformity of FITC-CALB coverage as shown in Fig. 5.8a. After the first print cycle (left hand column in 5.8a) the fluorescence is relatively weak and not uniform. After three print cycles (right hand column in 5.8a) the fluorescence is brighter and the uniformity is improved obviously. During incubation, features developed faster and with greater uniformity when more enzyme was transferred as seen in Fig. 5.8b and 5.8c (features in these two figures were observed under bright field illumination; under this observation condition, PCL residue is black and the exposed silicon substrate appears white as it reflects the incident light.) The effect of multiple prints is most easily seen in Fig. 4b where the right hand column (3 prints) is almost fully developed, the left hand column (1 print) is developed in only a few small areas, and the central column (2 prints) exhibits intermediate development. The increased CALB concentration on the surface does not, however, affect the width or fidelity of the etched features (Fig. 5.8c). Although the multiple-print process leads to higher etch rates, a single CALB print step results in an overall faster process since printing, a serial process, is the rate-limiting step.
whereas incubation is conducted in parallel. All subsequent results (sections 2.5-2.7) are based on a single CALB print step.

Figure 5.8. Effect of number of FITC-CALB print cycles on fluorescence intensity and etch rate.
FITC-CALB printed 1, 2 and 3 times per location in columns from left to right.  a) Fluorescence image before incubation; higher fluorescence intensity is observed with the increasing number of print steps b) Partially etched sample incubated for 45 min, observed in bright field. c) Fully etched sample using same imaging conditions as in b (white areas result from reflections from the silicon substrate).

5.3.5 CALB mobility on the PCL surface

Water is necessary to increase CALB’s activity for PCL hydrolysis where PCL ester bonds are cleaved to ultimately form hydroxycaproic acid and corresponding dimers. The etch rate of CALB increased with higher RH. To achieve high quality images, the RH during incubation was carefully controlled with the use of saturated salt solutions. At low values of RH (74%), the etch rate was slow and features could not be etched through the full film thickness (300 nm) after 12 h of incubation (Fig. 5.9a). At saturation (~100% RH), water condensed onto the CALB pattern forming micro-droplets on the PCL surface. CALB degraded the PCL film rapidly at this high humidity; a 300nm-thick PCL film could be etched through in 5 min. However, the mobility of
CALB increases under these condensing conditions resulting in uncontrolled feature shapes and dimensions (Fig. 5.9b). The highest humidity that could be controlled without observing water condensation was 87%. At this RH, high etch rates were attained without loss in image quality (Fig. 5.9c).

**Figure 5.9.** PCL film (300 nm thick) incubated at: a) 74% RH for 12 h. b) 100% RH for 30 min. c) 87% RH for 30 min.
Figure 5.10. a) AFM image of a 100 nm thick sample (quenched) incubated at 87% RH for 5 min. b) Line profile of AFM image 5a. c) AFM image of the same sample shown in 5a after incubation at 87% RH for 12 hours. d) Line profile of AFM image 5c.

Fig. 5.10a shows an AFM topographic image of a 4 × 3 array of square features developed in PCL after incubation at 87% RH. CALB etched through this 100 nm thick PCL film in 5 min to form the pattern. Additional incubation time did not affect feature size; incubating the film for an additional 12 h caused no significant change in the feature width as shown in Fig. 5.10 a to c (openings increased from 8.67 ± 0.23 µm after 5 min incubation to 8.77 ± 0.13 µm after 12 h of incubation; values based on 4 width measurements/condition from the AFM line profiles measured at the top PCL surface). The observed 0.10 µm increased width corresponds to an upper limit of
the feature lateral growth rate of 0.1 nm/min. This lateral grow rate is significantly slower than the vertical etch rate of 20 nm/min. This consistency of the feature width over extended incubation times demonstrates the stability of the CALB-PCL interaction. Once CALB etches through the films, the remaining enzyme in the etched region no longer catalyzes further expansion of the etched pattern.

In order to track CALB’s diffusion across the PCL surface during incubations, a fluorescein isothiocyanate labeled CALB conjugate (FITC-CALB) was printed onto the surface and the fluorescent pattern was followed in a live-cell imaging microscope. Fluorescence from FITC-CALB is clearly visible on the surface immediately after printing (Fig. 5.11a). After incubation (4 h, ~80% RH) the PCL film was fully etched, and the FITC-CALB is preferentially observed along the PCL side walls as can be observed by increased fluorescence in Figure 6b. The relatively long etch time was required due to the lower humidity (~80%) in the live cell microscope than in the incubation oven. Comparing Fig. 5.11a and 5.11b, etched features observed in the final stage replicated the printed enzyme pattern and were within 0.5 µm (corresponding to the resolution of the microscope and approximate size of the PCL spherulites) of the printed enzyme features. Lateral diffusion was suppressed even in this environment with relatively poor humidity control. Fig. 5.11c shows the fate of CALB as a function of time using higher magnification of a single feature in a 300 nm thick film. During incubation, CALB etched down through the PCL film stopping at the silicon substrate. Most of the CALB is attached to the sidewall after the pattern develops. The coating of the sidewalls with immobilized FITC-CALB is more evident in a thicker (2 µm) PCL film as shown by a three-dimensional stack of live-cell microscope images in Fig. 5.11d.
**Figure 5.11.** Real-time observations by live-cell microscopy of fluorescently labeled CALB during etching of a 300 nm thick PCL film prepared by quenching. a) Fluorescence image before incubation where the light areas correspond to fluorescence from FITC-CALB printed on the surface. b) Fluorescence image of fully etched features after 4 h incubation at 37 °C, ~80% RH. c) A series of high-magnification fluorescence images of the same feature during etching as a function of time in the microscope. d) Three-dimensional fluorescence image of an etched feature in a 2 μm thick PCL film with FITC-CALB attached to the side walls. Inset shows a model of the etched feature as a guide to the eye.
Fluorescence intensity from FITC-CALB decreases during incubation. This may be due, in part, to photo-bleaching of FITC \(^{37}\) as well as self-quenching when the dye comes in contact with the Si substrate. One possible explanation for the lack of lateral etching is that accumulation of degradation product, acidic 6-hydroxycaproic acid, leads to a lower local pH on the walls that results in reduced CALB activity. \(^{38,39}\) CALB is irreversibly inactivated in the presence of acids with a pKa ≤ 4.8; the pKa of 6-hydroxycaproic acid \(^{40}\) is 4.75.

### 5.3.6 Effect of PCL crystallinity on image quality

Previous work has shown that the crystallinity and spherulite morphology of polyesters, including PCL, influences enzyme binding to surfaces and the rate at which enzyme-catalyzed hydrolysis occurs.\(^{41}\) Enzymes can more easily access and bind to polymer chain segments that reside in amorphous domains that have greater mobility than chain segments in crystalline domains. PCL is a crystalline polymer that forms spherulites upon solidification from the melt. To determine the effect of crystallinity on CALB etch rates, PCL films were prepared at three different cooling rates. Films cooled slowly exhibited the largest spherulites (> 300 µm), the highest melting point (\(T_m = 60.9 ± 0.5 \degree C\)) and the slowest etch rate (0.16 µm\(^3\)/min). Conversely, PCL films quenched from the melt exhibited the smallest spherulites (<1 µm), the lowest melting point (56.0 ± 0.6 \degree C) and fastest etch rate (1.28 µm\(^3\)/min). Cooling at an intermediate rate resulted in spherulite size, \(T_m\) and etch rate between these two cases (Table 5.2). The etch rate for PCL films exhibiting the lowest \(T_m\) is 8 times faster than for the sample with the highest \(T_m\). Higher \(T_m\) results from crystalline domains that are more densely packed due to their greater degree of crystalline perfection. The consequence of a more densely packed PCL crystalline phase is greater resistance to CALB degradation.\(^{42}\)
Table 5.2. Effect of PCL morphology on etch rate and edge quality. Melting points are averages of five different polymer samples.

<table>
<thead>
<tr>
<th>Grain size</th>
<th>Melting point (°C)</th>
<th>Average etch rate (µm³/min)</th>
<th>Time to fully etch 300 µm film</th>
<th>Edge quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Grain (&lt; 1 µm)</td>
<td>56.0±0.6</td>
<td>1.3</td>
<td>15 min</td>
<td>Smooth</td>
</tr>
<tr>
<td>Medium Grain (50~100 µm)</td>
<td>58.0±0.4</td>
<td>0.64</td>
<td>30 min</td>
<td>Rough</td>
</tr>
<tr>
<td>Large Grain (&gt; 300 µm)</td>
<td>60.9±0.5</td>
<td>0.16</td>
<td>120 min</td>
<td>Rough</td>
</tr>
</tbody>
</table>

The spherulite size not only affects the etch rate, but also the feature edge quality. Spherulite size was inferred from the AFM results, which show the surface topography of the film (Fig. 5.12). From these images, boundaries between individual grains can be discerned and variations in the surface height profile reveal striated patterns characteristic of crystalline lamella embedded in amorphous regions of spherulites. Combining the AFM surface profiles with the DSC results, we correlated spherulite size with crystallinity. Films with higher degrees of crystallinity form etched features with rough edges as shown in the corresponding AFM images (Fig. 5.12a and 5.12b). The feature edge follows the directions of molecular alignment within the spherulite, preferentially etching what is presumed to be amorphous regions between lamellae. In these samples, the spherulites are much larger than the features being patterned. However, boundaries between adjacent spherulites do not affect etch rates as shown in Fig. 5.12a. Rapid quenching led to poorly crystallized spherulites that are smaller than the printed feature. Here, the edge of the feature is defined by the boundary between spherulites and has a roughness limited by that size (~0.5 µm). In addition, all of the features have vertical side walls as shown by the AFM line profiles (Fig. 5.12c, d, g and h) and SEM images (Fig.5.13). Thus, the combination of high
etch rate and small grain size of the quenched sample leads to significant improvements in the enzymatic lithography process.

Figure 5.12. Effect of PCL morphology on edge quality of etched features. a) Medium-grain PCL film. b) Large-grain PCL film. c) Line profile of AFM image 5.12a. d) Line profile of AFM image 5.12b. e) Small-grain PCL film. f) Enlarged small-grain PCL film. g) Line profile of AFM image 5.12e. h) Line profile of AFM image 5.12f.
5.3.7 Pattern transfer into copper foil

To demonstrate the usefulness of our enzymatic lithography approach for “Green” electronics, the PCL film was used as an etch resist such that features formed by the enzyme were transferred into an underlying copper film. Copper-based printed circuit boards (PCBs) are used extensively for electronic systems, including fine scale flexible circuits for integrated circuit (IC) packaging. Films for copper etching must be resistant to the acids that dissolve copper, adhere well to the copper substrate, be sufficiently thick to preclude pinholes and be sufficiently thin to resolve the finest feature sizes. Films used for IC packaging are typically 0.1 to 10 µm thick depending upon the resolution required and the method used to apply the film.

The process used for forming patterns in copper foil is shown in Scheme 5.2. A PCL film (300 nm thick) was spin-coated onto a copper foil clad polyimide substrate. We used our polymer pen printing method to transfer CALB onto the surface, which was subsequently incubated to define arrays of features in the PCL layer. Using the PCL layer as an etch mask, enzyme-defined features were transferred into the copper by etching in ferric chloride solution. An SEM image of

**Figure 5.13.** SEM image of 1.9 µm-depth hole. a) Without tilting. b) With 45° tilting.
the etched copper features is shown in Fig. 5.14a An array of copper holes, 10 µm wide and 5 µm deep, revealing the underlying polyimide film (bright yellow part in Fig. 5.14b), was observed after removing the PCL layer by immersing the sample in CALB solution at 37 °C.

**Scheme 5.2.** Process schematic for the fabrication of copper features by enzymatic polymer pen lithography.

**Figure 5.14.** a) SEM image of 10 µm wide, 5 µm deep features etched into copper foil, revealing the polyimide support film. b) Optical microscope image of etched copper feature.

Our demonstration of image transfer from enzymatically defined PCL to form 10 µm wide features in 5 µm thick copper foils would advance the state-of-the-art for the fabrication of copper-clad laminate interconnection circuit boards. These circuit boards form the basis of IC packages...
that provide electrical interconnections between the silicon ICs and a motherboard. The current minimum dimension of copper features on such circuit boards is approximately 50 µm due to limitations associated with optical lithography on non-planar laminate substrates as well as equipment cost constraints. Features smaller than 10 µm formed in PCL could be transferred into thinner copper foils providing a path for further resolution improvements. Enzymatic lithography using polymer pen printing would thus satisfy the need for greater interconnection density, while providing a “Green” approach that would reduce the overall environmental impact of the process.

5.4 Conclusion

A simple but highly-efficient approach to enzymatic lithography is described, capable of resolving features with dimensions ≤1 µm. Once deposited onto the surface, CALB interacts with the PCL film, preventing migration of the enzyme across the surface and, consequently, preventing broadening of the etched feature width in films up to 2 µm thick. Etching of PCL by CALB occurs rapidly external to the printer; patterns with straight sidewalls were developed in 100 nm thick PCL films in less than 5 min when incubated at 37 °C and 87% RH. To achieve high image fidelity, several factors must be controlled including: relative humidity during incubation as well the moisture concentration, crystallinity and thickness of the PCL film. This enzymatic lithography process constitutes a significant advance over typical photolithographic methods for the fabrication of biocompatible devices as well as electronic circuits. Not only does the process require no photosensitizers or chemical development, but both the CALB and PCL are biodegradable. Thus this technique offers an environmentally friendly route to fabricate electronic circuits, reducing the environmental impact of the process while reducing the need for, and cost of, hazardous waste disposal. Introduction of patterns at surfaces of biomaterials is also expected to prove useful in biomedical applications such as bioresorbable scaffolds for tissue engineering.
Chapter 6. Fabrication of High Aspect Ratio Glassy Carbon Posts by Multi-deposition Method

6.1 Background

Carbon has several allotropes, such as graphite, diamond and glassy carbon, according to their different crystal structures. In addition, in the last decades, scientists have discovered and invented more carbon allotropes including C60, carbon nanotubes, graphene, etc\(^1,2\). Various forms of carbon allotropes have different mechanical, thermal and electrical properties, making them useful in a wide range of fields. For example, glassy carbon, which is the easiest one to make among all carbon allotropes, can typically be converted from polymeric precursors by a pyrolysis process above their decomposition temperature in an inert environment\(^3-5\). As a non-graphitized carbon structure, the glassy carbon is built up by a large number of randomly-oriented intertwining carbon microfibrils with sp\(^2\) hybridized orbitals, giving rise to its electric conductivity, thermal conductivity, chemical stability and high-temperature stability\(^6\).

Carbon microfabrication has become very attractive recently, due to potential applications in microelectronic, electromechanical and chemical sensor technologies. Some lithographic methods have been applied to fabricate 3D carbon features, such as focused ion beam (FIB) milling\(^7\) and depositing\(^8\), and reactive ion etching (RIE)\(^9\). As a writing technique, the former one can create carbon patterns on substrate directly, whereas for the latter, the fabricating process is more cumbersome with multiple steps. In the first, protective metallic mask with designed patterns is deposited on the surface of the carbon matrix, and then the sample is treated in a plasma to remove the unprotected carbon, transferring the pattern from the mask to the carbon substrate. The final step is wet etching, in which metallic mask in acidic solution is removed. Both methods are
successful in preparing micro-featured carbon, but they also share some common drawbacks such as their long preparation time and high-costs.

Carbonization of patterned polymeric precursors has been leveraged as an indirect way to develop 3D carbon microstructures. As an example, Whitesides’ group\textsuperscript{10} fabricated glassy carbon microstructures by micro molding. In their study, poly (furfuryl alcohol) precursor was first molded to form polymeric microstructures, which was followed by carbonization at elevated temperature to convert the polymer features to carbon features. Photoresist is another kind of precursor which can be used in fabricating micro-textured carbon. Taking advantage of patterning by photolithography or focused ion lithography, complex and repeatable carbon topography can be prepared\textsuperscript{5,7,8,11}. For instance, by carefully controlling the multi-step photolithography and pyrolysis process, Lee et al\textsuperscript{12} prepared freestanding 3D carbon microstructures using SU-8 photoresist.

In this work, a simple and low cost two-step approach has been developed. The graphite flake-filled phenolic resin N-methyl pyrrolidone (NMP) mixture was used as the carbon precursor. Then this thixotropic material was deposited by single polymer pen printing to form high aspect ratio polymeric posts. The final 3D carbon features were obtained after carbonization at 1000 °C in N\textsubscript{2} atmosphere.

\textbf{6.2 Experiment}

\textbf{6.2.1 Material}

Phenolic resin 12114 was received from Plastics Engineering Company (Sheboygan, WI). Graphite 4827 with nominal 2 µm size and 113 m\textsuperscript{2}/g surface area was received from Asbury
Graphite Mills Inc. (Kittanning, PA). N-methyl pyrrolidone (NMP) was purchased from Sigma-Aldrich (Milwaukee, WI).

6.2.2 Printing Procedure

Phenolic resin has been chosen as the carbon precursor in this project due to its high carbon yield and low price\textsuperscript{13–15}. To prepare ink for polymer pen printing, phenolic resin was first dissolved in NMP solvent to form a 55\%–60\% phenolic NMP solution. The resulting mixture was then kept at room temperature for 12 hours to insure complete dissolution such that no more solids were observed in the solution. Next, a suitable amount of graphite was added to the solution and then mixed well to obtain a slurry. The PDMS pen with 10\(\mu\)m-diameter tip was mounted onto the robot of Sonoplot Microplotter II, which was then used to perform polymer pen printing to build up polymeric posts. Typically, a PDMS pen is dipped into the phenolic mixture for 0.5s with 5\(\mu\)m depth in the material, and then moved by a robotic arm to a position 200 \(\mu\)m above the silicon substrate, followed by the PDMS pen moving vertically down and coming into contact with the substrate at a speed of 1 cm/s and an acceleration of 1 cm/s\(^2\), thus transferring the material from the pen surface onto the substrate. High aspect (>2) ratio polymeric posts were fabricated by repeating this process four times but each time increasing the printing height of the pen above the substrate by 15 \(\mu\)m or 20 \(\mu\)m.

6.2.3 Ultraviolet light (UV) treatment

Phenolic posts were treated with UV light before pyrolysis to pre-cure phenolic resin. The UV light source (Dymax BlueWave 200) was set 1 cm above the silicon substrate, and the posts were exposed to UV light with power density 1 W/cm\(^2\) for 1 min.
6.2.4 Pyrolysis process

Carbon posts were obtained from the pyrolysis process. Polymeric posts were first kept in the Lindberg/Blue M™ 1200°C Split-Hinge tube furnace with a N₂ flow of 150 ml/min for 30 min at room temperature, and then heated to 1000 °C with a heating rate of 60 °C/h. Samples were kept at 1000 °C for 0.5 hour before they were allowed to cool down to room temperature at the same rate of 60 °C/h.

6.2.5 Surface treatment of substrate

Silicon wafers were chosen as the substrate in this project. To investigate the effects of the chemical nature of the surface on printing, we performed a series of treatments on the silicon wafer to insure cleanliness and to control hydrophobicity. Similar to the process described in section 3.2.2, first, the silicon wafer was immersed in an alconox solution for 5 minutes in an ultrasonic bath, and then rinsed with distilled water several times. This was followed by a drying process, in which the wafer was blown with clean air and dried in an oven at 130 °C for 10 min, thus turning it into a detergent washed silicon. Next, the washed silicon was immersed in a diluted HF solution for 10 min to remove silicon oxides on the wafer surface, and then dried by a similar process as described in the previous step, producing an HF treated silicon. The HF treated silicon wafer was then treated with a piranha solution (the volume ratio of concentrated H₂SO₄ to 30% H₂O₂ is 7 to 3) for 10 minutes to form a thin layer of silicon oxide on surface. After drying, piranha treated silicon was obtained. The last type of substrate is Hexamethyldisilazane (HMDS) treated silicon, which was made by spin coating a 10% HMDS xylene solution onto a piranha treated silicon at 3000 rpm for 30s and then dried in an oven at 130 °C for 10 minutes before use.
6.3 Results and Discussion

6.3.1 Formulation of phenolic NMP ink

Phenolic resin is one of the most common and inexpensive thermosetting plastics. And it is also a polymeric precursor that can be converted to carbon by pyrolysis in an inert atmosphere with high yield\textsuperscript{16}. But due to its solid state, phenolic resin cannot be deposited directly by single polymer pen printing. Therefore, making phenolic resin into a high concentration solution is an essential step. With good solvency property and a high boiling point (202 ~ 204 °C), NMP was chosen as the solvent for dissolving phenolic resin. Due to the poor solubility of phenolic resin, the final fully dissolved solution with 55 ~ 60 wt% of phenolic resin, can only be obtained in 12 hours after mixing phenolic resin and NMP solvent.

In order to fabricate polymeric posts by printing the ink material must be modified to be thixotropic. Adding an appropriate amount of Cabosil (surface modified silicon oxide nanoparticles) or graphite flakes is the most convenient but yet the most effective way to achieve this. The use of a small amount of Cabosil produces thixotropic material, but may decrease the electric conductivity after pyrolysis; whereas filling with a large amount of graphite not only makes the mixture acquire its thixotropic property, but can also increase the conductive carbon content and reduces shrinkage caused by pyrolysis (discussed in Chapter 7). The printability of phenolic ink decreases as the content of graphite filler increases. After several attempts, phenolic NMP solution with 25wt% graphite flake (compared to solution) was considered to be the most suitable choice for the purpose of this experiment, which shows good thixotropic property for posts fabricated using a repeat print or “stacking-up” process as well as good air-stability for processing more than 24 print cycles without significant changes affecting the viscosity of the ink.
6.3.2 Effect of substrate surface properties on the polymeric feature size

It is hypothesized that the chemical nature of the substrate surface could affect material transfer from the PDMS pen to the substrate as well as the spreading of the ink on the substrate surface. Hence, by varying the hydrophilicity of the silicon wafer, we can study the effect of surface chemistry on printing. Table 6.1 shows the contact angles of silicon wafers after different treatments. It is obvious that the silicon wafer coated with HMDS and the HF treated wafers have the highest contact angles and greatest hydrophobicity. On the other hand, after piranha treatment, the silicon wafer becomes very hydrophilic with a contact angle of nearly 0° due to the presence of a thin layer of silicon oxide.

Table 6.1. Contact angle of silicon wafer after different treatment.

<table>
<thead>
<tr>
<th>Silicon Surface</th>
<th>Water Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent-cleaned</td>
<td>47</td>
</tr>
<tr>
<td>HF treatment</td>
<td>62</td>
</tr>
<tr>
<td>Piranha treatment</td>
<td>~ 0</td>
</tr>
<tr>
<td>HMDS treatment</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 6.2. Material dot size on different substrate surfaces.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter of Phenolic NMP Ink Dot (µm)</th>
<th>Diameter of Graphite filled Phenolic NMP Ink Dot (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent-cleaned</td>
<td>125 ± 15</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>HF treatment</td>
<td>100 ± 10</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>Piranha treatment</td>
<td>&gt; 150</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>HMDS treatment</td>
<td>115 ± 10</td>
<td>48 ± 5</td>
</tr>
</tbody>
</table>
The comparison of the dot diameters printed on silicon substrate with different surface treatments, as shown in Table 6.2, reveals that the chemical nature of the silicon surface plays an important role in depositing the non-filled phenolic NMP ink. Under similar printing conditions, which results in the same amount of material transferred each time, the phenolic NMP solution dots printed on the hydrophobic surface are much smaller than the hydrophilic surface. However, on the other hand, this did not cause significant impacts on the thixotropic ink material, where the size variation is less than 10% on all silicon surfaces. In addition, the graphite filler also limits the ink expansion on the substrate. The dots made using the thixotropic ink are only half the size of the pure phenolic resin dots, which is favorable for creating posts. Therefore, under this experimental framework with printing thixotropic material, the surface properties of the substrate do not have significant influence on the fabrication of polymeric posts.

6.3.3 Multi-deposition of polymeric posts

With the multi-deposition method (Fig.6.1), polymer pen printing technique has been applied in constructing polymeric posts. Thixotropic ink is repeatedly printed at the same position on the substrate, but each time the new material was deposited at an increased height, thus gradually forming high aspect ratio posts. By conducting several experiments, we have also found that the best material pick-up depth is 5 µm in the inkwell to form 20 µm diameter posts. In addition, we also discovered that compressing the tip helps transfer material to the substrate. If the pen tip just touches the surface, only a small amount of material was deposited, making posts impossible to be built up. When the PDMS tip is compressed, the produced pressure helps to transfer all of the material from the pen tip to the substrate surface. Print heights for four-layer deposition are shown schematically in Fig. 6.1a. The silicon surface is set as 0 µm. Thus, the first print position is -20 µm, which means the print position is set at 20 µm below silicon surface, and so the tip is
compressed as a consequence. The second print is targeted to be just on the surface of the silicon substrate. But due to the existence of the first layer, with 8~10 µm height, the pen tip is still compressed and the second layer is deposited onto the first layer. Continue with third and fourth prints, which are shown in Fig. 6.1b, the posts with four layers are formed.

**Figure 6.1.** Schematic representation of the multi-deposition method: a) printing height increasing; b) fabrication schematic of a four-layer post.

**Figure 6.2.** Step by step fabrication of 4-layer posts.
Fig. 6.2 shows the microscopic images of polymeric posts with different layers. The height of the posts increased with the number of layers, but the diameter kept the same with precise positioning. The final phenolic/graphite composite posts, which are prepared by superimposing four layers of material, have 40 µm height and 20 µm diameter.

### 6.3.4 Pyrolysis Process

Phenolic resin is the reaction product of phenol and formaldehyde, which can be cured by heating without catalyst and curing agent. After pyrolysis at high temperature in an inert environment, most of hydrogen atoms and oxygen atoms in the phenolic resin molecules are removed, which converts the phenolic resin to glassy carbon with over 50% yield\(^\text{13}\). Fig. 6.3 is the pyrolysis reaction of phenolic resin, and the final product glassy carbon has complex structures with both hexagonal and pentagonal carbon rings\(^\text{17}\). The electric conductivity of glassy carbon is two orders of magnitude less than graphite in parallel direction, but the isotropic property broadens its application in microelectronics. Fig. 6.4 shows the thermal gravimetric analysis (TGA) plot of 60 wt% phenolic NMP solution. With 40% solvent, the final carbon yield of ink material is about 35 wt%, resulting large shrinkage of printed features.

![Pyrolysis process of phenolic resin](image)

**Figure 6.3.** Pyrolysis process of phenolic resin.
The pyrolysis process is shown in Fig. 6.5. It is clear that from Fig 6.5a to c, the volume of four-layer posts shrink substantially to 21% after pyrolysis due to solvent evaporation and densification of the ink material. UV treatment was applied to samples before pyrolysis to reduce shrinkage. High content of graphite (25 wt%) in polymeric posts could absorb UV energy and release heat, which evaporates part of the solvent and partially cures the phenolic resin. The UV-induced crosslinks not only help posts to maintain their shape during heating process and resist pyrolysis shrinkage, but also, results in higher carbon yield\textsuperscript{18}. Average shrinkage data of UV treated samples is shown in Table 6.3. After the UV treatment, the diameter of the posts did not change; only the height decreased by 15%. Pyrolysis of UV-treated sample caused the diameter and height to shrink by 35% and 45% respectively, which means shrinkage is anisotropic. Different shrinkage can also be found between posts and base. The base of the posts underwent almost no

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6_4.png}
\caption{TGA plot of 60 wt% Phenolic NMP ink.}
\end{figure}
shrinkage at all, due to strong good adhesion between the carbon and the substrate\textsuperscript{11,12}. The final volume of carbon posts converted from UV-treated sample is 26% of the original phenolic posts volume, which is 5% higher than the carbon posts without UV treatment.

\textbf{Figure 6.5}. Fabrication of carbon posts by pyrolysis with or without UV treatment. a) Original phenolic posts. b) UV treated phenolic posts. c) Carbon posts pyrolyzed directly from original phenolic posts. d) Carbon posts pyrolyzed from UV treated phenolic posts.

\textbf{Table 6.3}. Post shrinkage after UV treatment and pyrolysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Origin</th>
<th>UV</th>
<th>Pyrolysis</th>
<th>Carbon Volume (P/O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter (µm)</td>
<td>Height (µm)</td>
<td>Diameter (µm)</td>
<td>Height (µm)</td>
</tr>
<tr>
<td>1 layer</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>2 layers</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>3 layers</td>
<td>17</td>
<td>24</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>4 layers</td>
<td>21</td>
<td>46</td>
<td>21</td>
<td>42</td>
</tr>
</tbody>
</table>

*4 layers sample without UV, after pyrolysis, height shrank to 2/5. Volume change is around 21%.
*Typical standard deviations of the diameter and height are \textasciitilde 2 microns.
6.4 Conclusion

In summary, high aspect ratio micro-carbon posts (height/diameter > 2:1) have been successfully constructed by single polymer pen printing technique followed by pyrolysis for the first time. This method is not only more economic than any other lithography technique, but also simplifies the process of fabricating micro-carbon posts. An ink formulation for building up carbon precursor posts has also been found. With 25 wt% graphite flakes, phenolic NMP ink exhibits good thixotropic property and printability. This work shows great potential to create carbon posts as microelectrode arrays on silicon substrates. One application is shown in chapter 7, where carbon posts were built on flat gold electrodes and connected with suspended carbon fiber bridges. These carbon micro-posts on electrodes is not only used to raise up the carbon fiber bridge from the substrate, but also decreases the contact resistance between gold and carbon fiber.
Chapter 7. Polymer Pen Printing Technique for Carbon Microneedle and Air-bridge Fabrication

7.1 Introduction

With the development of microfabrication techniques, devices made with biocompatible materials are attractive technology for life science and medical device applications\textsuperscript{1,2}. Three dimensional (3D) microneedle arrays is one of the most promising microstructures\textsuperscript{3,4} due to their size range relative to human tissues. The sharp-tipped and high aspect ratio microneedles can pierce through the outer layer of the skin with minimal pain, which enables applications such as transdermal drug delivery\textsuperscript{5,6} and in vivo real-time sensing\textsuperscript{7}. When the size of the needle tip decreases to several microns or even to submicron dimensions, microneedles can be used to measure the extracellular field potentials of single cells in vivo or in vitro, which is the signal generated by live cells\textsuperscript{8}.

Many lithography methods have been applied to fabrication of microneedles with various materials\textsuperscript{9-16}. For example, silicon microneedles with different sizes and geometries were fabricated with established microelectromechanical systems (MEMS) methods, which were reported in many papers\textsuperscript{9-11}. Typically, the silicon substrate is first patterned by photolithography, and then followed by dry\textsuperscript{10} or wet etching\textsuperscript{11} to form solid or hollow silicon needles. Other approaches can be applied to fabricate polymeric microneedle arrays. With a pre-defined master, polymer needles were fabricated by soft lithography\textsuperscript{12}, solvent casting\textsuperscript{13} and injection molding\textsuperscript{14}. Another interesting technique is drawing lithography, which was used to fabricate ultrahigh aspect ratio needles\textsuperscript{15,16}. The process is relatively simple. A positive mold with pillar arrays was brought into contact with melted thermoset plastic and then slowly pulled up to draw liquid bridges.
between substrate and pillars. After cooling, the bridges were solidified and broken at the narrow neck by further drawing.

However, the electrical conductivity of these microneedle materials is limited, which is insufficient to directly meet the demand for electrodes. Therefore, most microneedle-based biosensors have complex design with integrated conductive parts. Yu et al.\(^9\) successfully measured the electrocardiography (ECG) on skin with a hollow micro-electrode system. Besides the hollow silicon microneedles, this system has another three parts: the back reservoir, the sodium chloride electrolytic solution and Pt coated polymer die. In this device, conductive sodium chloride solution was used to transfer signal from the *stratum germinativum* (SG) layer to the Pt film electrode, which was connected to an outward lead wire. Sodium chloride solution, solid carbon fibers\(^17\) and carbon pastes\(^18\) could be filled into the hollow needles as the signal detector and transportation part of the electrochemical sensor devices, but free-standing carbon microneedles were seldom reported.

Due to the good electrical and thermal conductivity as well as chemical and thermal stability\(^1\), glassy carbon was chosen as the microneedle electrode material. Good biocompatibility\(^19\) and easy surface modification properties\(^20\) also makes glassy carbon a promising material for in vivo/vitro biosensing. Moreover, glassy carbon is one of most cost-effective materials, and can be easily produced from different polymeric precursors using a simple pyrolysis process in an inert atmosphere\(^21\).

The approach of fabricating glassy carbon microstructures usually contains two steps: i). Fabricating structures of polymeric precursor and ii). Pyrolysis. Conventional lithographic methods, including soft lithography\(^22\) and photolithography\(^23,24\), have been used to construct 3D
structures of carbon precursors. For example, Whitesides et al.\textsuperscript{22} have successfully fabricated complex glassy carbon microstructures, including free-standing grids, curved grids, diffraction gratings and high aspect ratio structures by soft lithography (micro-molding in capillaries or micro-transfer molding). In their study, the furfuryl alcohol-modified phenolic resin was first molded using a poly(dimethylsiloxane) (PDMS) master, followed by pyrolysis at an elevated temperature to convert the phenolic precursor to carbon features. But soft lithography has some limitations: the pattern is fixed for each mold and it is difficult to be applied for high-throughput manufacture. Taking advantage of easy-patterning by photolithography, complex and repeatable carbon topography can be fabricated conveniently with photoresist polymeric materials. By carefully controlling the multi-step photolithography and pyrolysis process, Madou’s group\textsuperscript{25} developed a large array of high aspect ratio carbon posts using SU-8 photoresist, which has potential for Lithium battery application. Fabrication of suspended carbon micro/nano structures is another research area of significant interest to the carbon-MEMS community, because of their potential as electrical interconnections between microelectrodes in electronic devices. Moreover, the suspended carbon wires are great platforms for gas sensor applications\textsuperscript{26}. E-beam\textsuperscript{27} exposure techniques have been used to cure the top layer of SU-8 after the initial photolithography to create suspended microstructures. Electro-mechanical spinning\textsuperscript{28} was also studied to form individual polymeric fibers directly between SU-8 posts, which can be converted to carbon nanowires with higher electrical conductivity than regular glassy carbon\textsuperscript{29,30}. After pyrolysis, suspended carbon wires were formed between carbon posts with good ohmic contact. Chung et al.\textsuperscript{31} found another low cost way to create “carbon bridge” structures by using decomposable paraffin wax as a support with SU2.5 epoxy on the top. After pyrolysis, the wax was totally decomposed and the epoxy was converted to carbon forming suspended “bridges” between posts. These suspended structures were
thermally and electronically isolated from substrate to avoid the deleterious interactions between carbon and substrate.

Herein, we present a simple and novel approach, named “polymer pen printing” (PPP) to build 3D microneedle arrays from viscous phenolic resin N-Methyl pyrrolidone (NMP) solutions. The high aspect ratio microneedles with submicron-sized tips were formed by drawing force, which is similar to the drawing lithography discussed previously. After printing, the precursors with 3D microstructures were pyrolyzed at 1000 °C in a N₂ atmosphere to create carbon needles. In order to determine the resistivity of pyrolyzed carbon and carbon-carbon composites, carbon bridges were created across two electrodes on a chip by single pen PPP followed by pyrolysis. Moreover, phenolic/graphite pillars were built on the gold electrodes by multi-deposition method, then the microwires were created to connect pillars, forming air-bridges high above the substrate. Using the two-probe method, the resistance of carbon bridges was measured and the resistivity of pyrolyzed glassy carbon was calculated using SEM to measure the wire diameter.

7.2 Experimental

7.2.1 Materials

Phenol formaldehyde resin used in our experiment was a resol resin from Plastics Engineering Company (Sheboygan, WI). Graphite flake 4827 was from Asbury Graphite Mills Inc. (Kittanning, PA). N-methyl pyrrolidone (NMP) 99+% was from Sigma-Aldrich (Milwaukee, WI).
7.2.2 Substrates

HF treated silicon wafers were used as the substrate for carbon needle array fabrication. The silicon wafer was first cleaned with oxygen plasma treatment, followed by immersing in the 5% HF solution for 5 min to remove silicon oxide. After rinsing with deionized water, the silicon wafer was blow-dried with clean air and then cleaved in to 2 cm × 2 cm pieces.

Two types of dielectric substrates with gold electrodes were used for carbon air-bridge and pillar fabrication. One type of chip was made of silicon with photolithographically defined gold electrodes on the surface. The other chip type was a ceramic substrate with thick-film gold patterns fused to the surface.

7.2.3 Fabrication of phenolic resin microstructures

7.2.3.1 Preparation of phenolic resin NMP inks and inkwells

Phenolic resin (6 g) was added into NMP solvent (4 g) and mixed well to form a 60 wt% Phenolic NMP solution. The resulting mixture was then kept at room temperature for at least 12 hours until no more solids and air bubbles were observed in the solution. Next, a suitable amount of graphite flake (0, 4, 8, or 16 wt% relative to the phenolic NMP solution) was added to the solution and then mixed well to obtain slurry as the ink material.

To make a 60 µm-depth inkwell, kapton tape was applied to a glass slide, and then the tape was cut by a blade to make a 1 × 1 cm square opening. Before printing, about 0.05 ml of phenolic ink was applied into the square opening, then the excess material was removed using a razor blade to form a relatively smooth, uniform and stable layer of phenolic NMP ink.
7.2.3.2 Fabrication of PDMS pen array

For silicon mold fabrication, a <100>-orientated silicon wafer with 1 μm of thermally grown silicon dioxide layer was spin-coated with photoresist first, and then the pattern of 300 μm × 300 μm square array on 500 μm pitch was generated by direct laser lithography using Heidelberg DWL66 Laser Writer. After developing the photoresist, the exposed oxide surface was removed by dipping the wafer in buffered HF solution for 14 minutes, and the recessed pyramidal openings were formed by anisotropic etching in 30% KOH solution at 80 °C for 3.5 h. The final step is to coat a layer of 1H,1H,2H,2H-perfluorodecyltrichlorosilane on the etched mold surface by vapor deposition.

The PDMS pen array can be cast into the prepared mold. A silicone pre-polymer 184 (liquid phase) was mixed and poured into the silicon mold and degassed for 10 minutes. A piece of O₂ plasma-cleaned glass slide was placed on top of the filled mold. After curing at 100 °C for 60 minutes, the PDMS pen array was bonded onto the glass slide which could be peeled off from the silicon mold. The glass slide was cleaved into smaller pieces (about 2 cm × 2 cm) with the PDMS pen array in the center. Typically, a PDMS pen array has 100 (10 × 10) pen tips, and each pen is 212 μm tall.

7.2.3.3 Printing system

The backside of the glass slide, without the PDMS pen array, was attached to an aluminum holder. The holder is a hollow pillar with a 45° mirror inside. The pen status can be monitored using a side-mounted microscope. The holder is connected to a force gauge, which was mounted to the robot arm of a GIX™ MICROPLOTTER™ II (SONOPLOT, INC. Middleton, WI) with
5μm spatial resolution along x, y, z axes. With this precise control of movement, different polymeric microstructures can be made.

### 7.2.3.4 Fabrication of phenolic resin needles

The process for fabricating phenolic needles is shown schematically in Scheme 7.1. Inking the pen array is the first step of the printing cycle. The PDMS pens are dipped into the ink reservoir for 1 s to a depth of 30 μm into the ink, and then moved by the robot to a position 100 μm above the silicon substrate. To create polymeric needles, the inked PDMS pen array was first moved vertically down to come into contact with the substrate at a speed of 30 μm/s and an acceleration of 30 μm/s². After 3 seconds, the PDMS pen array was then lifted up with same speed and acceleration to a height of 500 μm above the substrate, forming needle-shape phenolic posts. Any excess material remaining on the pen tips after printing was removed using a foam tipped swab.

![Diagram of phenolic needle fabrication](image)

**Scheme 7.1.** Fabrication of carbon needle electrodes by multi-pen printing.


7.2.3.5 Fabrication of phenolic resin air-bridges

A single PDMS pen was cut from the pen array and attached to the robot to make air-bridges, which is called a single polymer pen printing method. By controlling the pen’s movement, described below, bridges with different arc heights can be created (Scheme 7.2). Typically, after inking with phenolic NMP solution, the PDMS pen was moved above a gold electrode on a ceramic or silicon substrate and lowered to contact surface with a 10 µm compression. With a speed of 30 µm/s and an acceleration of 30 µm/s\(^2\), the pen was lifted up to 200 µm and then moved 100, 200 or 300 µm distance in x or y direction to form a polymeric wire. Next, the pen was moved vertically down and came into contact with another gold electrode. The last step is to lift up the pen quickly with 10,000 µm/s velocity and an acceleration of 10,000 µm/s\(^2\) so that the end of polymeric wire can be transferred to the electrode.

Scheme 7.2. Fabrication of carbon air-bridges by single polymer pen printing.
7.2.3.6 Fabrication of phenolic resin pillars

The single polymer pen printing method can also be used to fabricate phenolic resin pillars as described in Chapter 6. Different from needle and bridge structures, to build a phenolic pillar, only the 16 wt% graphite filled phenolic resin material was used. Also, during the inking process, the pen was dipped 20 µm into the inkwell to pick up smaller amount of ink. In the printing process, the PDMS pen with ink material was moved vertically down 20 µm below the surface of the substrate so that the tip was compressed, then was lifted up quickly with a velocity of 10,000 µm/s and an acceleration of 10,000 µm/s², thus all of the ink material from the pen surface can be transferred to the substrate. The pen was inked again and printed onto the substrate with 10 µm compression. This process was repeated 8 times such that the contact height was increased 10 µm above the previous print height. In this way, pillars 80 µm high and 30 µm diameter were fabricated.

7.2.4 Pyrolysis process

The as-printed samples were first kept in an N₂ atmosphere in a tube furnace for 30 min at room temperature, and then heated to 150 °C with a ramp rate of 10 °C/min. Next, samples were kept at 150 °C for 60 min to remove the NMP solvent and cure the phenolic resin, following by heating up to 1000 °C with same ramp rate. After 60 min at 1000 °C, samples were allowed to cool down to room temperature by shutting down the furnace power.

7.2.5 Characterization

A simple two-point electrical measurement was used to estimate the resistivity of fabricated glassy carbon. Two sharp tungsten needle probes with a tip diameter of 10 µm were used to make electrical contact with the gold electrodes and the current – voltage (I-V) plots were recorded using a Keithley model 4200-SMU semiconductor characterization system by sweeping
the voltage from 0 to 1 volt. An AMRAY 1910 Field Emission SEM was used to image the microstructures of phenolic resin and carbon (before and after pyrolysis). Samples were not coated with conductive metal.

7.3 Results and discussion

7.3.1 Polymer pen printing system

A polymer pen printing (PPP) system built by modifying a Sonoplot GIX Microplotter II. In our PPP system, a PDMS pen array is attached to a relatively course-scale robot instead of an AFM piezoelectric stage used for polymer pen lithography (PPL) aimed to nanofabrication. The PPP system avoids the limitation of short-range movement imposed by AFM system. Another difference is that conventional PPL employs a “dry-ink” printing strategy and the pen array is dried before printing. Using “dry ink” limits the creation of patterns to 2D as particle-filled inks cannot be used. With a long-range movement robot, we developed a “wet-ink” printing method to integrate an inking step into each printing repetition. As a result, large volume of ink can be transferred with good precision using PPP.

7.3.2 Fabrication of microstructures

By using our PPP tool, arrays of microscale phenolic resin needles have been successfully fabricated, as shown in Scheme 7.1. During the inking process, the PDMS pen array is only dipped into the ink to a depth of 30 μm to obtain equal volumes (~ 0.01 nl) of resin material on each pen tip. After inking, PDMS pens with resin material were brought into contact with the silicon substrate. With a 10 μm tip compression, ink from the lower part of the tips was transferred to the silicon surface but ink on the upper part remained on the PDMS pen. The ink-substrate contact area was about 30 μm diameter. By slowly lifting up the PDMS pens, the phenolic resin was
stretched to form micro-wires. Due to the low relative humidity (~ 25 % RH), the solvent in the ink evaporated rapidly and resulted in a dry micro-wire surface. The needle-shaped phenolic posts with 30 µm diameter base were formed after the center of the phenolic ink wire dried and broke as the pen was lifted above the substrate.

Figure 7.1. SEM images of phenolic resin needles printed on a silicon wafer without coating. a) Array of needles made by phenolic resin without graphite. b) One needle from the array in a). The inset is a high magnification image of the needle apex. c) Array of needles using 8 wt% graphite filled phenolic ink. d) One needle from the array in c). The phenolic ink samples were not metallized before SEM imaging, as a result charging occurs which results in artifacts on the surfaces.
To obtain the good uniformity of the needle height, the RH of the printing system should be controlled between 25~30%. If the RH is lower than 20%, the ink on the tip becomes partially dried during the movement from inkwell to substrate, resulting in fully-transferring of ink from tip to substrate without forming phenolic resin wires between them. When the RH is too high, the formed needles are much taller (over 300 µm), which cause them to curve substantially. Some wires would not break until the pen was lifted 1000 µm above the substrate.

Fig. 7.1a and 7.1c shows two 3×3 arrays of needles. The needles in each array have almost the same height and are perpendicular to silicon substrate. In Fig. 7.1a, the needles with ultra-high aspect ratio (> 50 : 1) were made using a phenolic resin ink without graphite, which are about 100 µm high and have a apex with 1 µm diameter (Fig. 7.1b). In order to improve the conductivity of these carbon needle electrodes, graphite flakes were added into the phenolic ink. The resulting glassy carbon/graphite composite material after pyrolysis will have higher electrical conductivity. Fig. 7.1d shows the needles made by phenolic resin with 8 wt% graphite. These needles are only 38 µm high, with a diameter of 4 µm at half height. Thus they have a much smaller aspect ratio than the pure resin needles. The graphite filler decreases the elongation property of phenolic resin ink which results in shorter and thicker needles (Fig. 7.2).

![Figure 7.2](image.png)

**Figure 7.2.** The effect of graphite flakes content on needle height.
Figure 7.3. Shrinkage of phenolic needle posts. a) Conversion of phenolic needle to carbon needle by pyrolysis (no graphite fillers). b) Height shrinkage of phenolic needle posts plotted as a function of graphite content.

7.3.3 Shrinkage during pyrolysis

The phenolic resin powder has over 55 wt% of carbon yield\(^3^4\), however, the ink contains 40 wt% NMP solvent. As a result, the final carbon yield is about 35 wt%. Due to the high aspect ratio structure and strong adhesion to the substrate, anisotropic shrinkage was inevitable during the pyrolysis process\(^3^1\). Fig. 7.3a shows the conversion from a phenolic needle (no graphite) to a
glassy carbon needle. The needle shape was retained after pyrolysis with a shrinkage in height of 58%. As shown in Fig. 7.3b, with the increase of graphite fillers content, the height shrinkage decreases, rate of change is small, decreasing from 58% ± 7% to 50% ± 9% as the graphite content increases from 0 to 16%. Due to the needle shape of the posts, the shrinkage is highly anisotropic. Different from height shrinkage, there was almost no shrinkage at the base. The good adhesion of phenolic resin to silicon restricts lateral shrinkage along the substrate.

### 7.3.4 Glassy carbon air-bridges on chip

Measurement of the electrical conductivity of micro-carbon needles is quite challenging because it is difficult to contact the submicron apex of the carbon needles with the conventional micro-sized probe. By bonding glassy carbon nanowires on microelectrodes using focused-ion beam-based platinum deposition, Lentz’s group successfully measured and studied the electrical conductivity of glassy carbon nanowires. In our research, we found another way to measure the resistance of glassy carbon wires. Carbon air-bridges between two gold electrodes were easily built. Two-point electrical measurement method can be used to record the I-V curve of carbon features, and the resistivity of carbon can be calculate from the plot. The resistivity of pyrolyzed carbon can be estimated.

Fig 7.4a shows a phenolic resin air-bridge on two electrodes separated by 300 µm and was converted to a carbon bridge after pyrolysis (see Fig. 7.4b). Following the path of the pen, the phenolic resin wire has curved structure and is ~300 µm above the silicon surface. After pyrolysis, as shown in Fig 7.3b, the wire shrunk to form a straight line between the two electrodes. From a 90° view in the SEM (Fig. 7.4c and d), the carbon wire remains above the surface, but only has a
height of several microns. The good adhesion between the carbon base and the gold electrode can be seen in Fig. 7.4d, and results in low contact resistance.

Figure 7.4. Bridges between electrodes. a) 45° view of phenolic resin air-bridge. b) 45° view of carbon air-bridge. c) 90° view of the carbon air-bridge. d) High magnification view of c), shows the arc of the air-bridge.

In order to increase the height of carbon bridges, carbon pillars were built first on the electrodes by a multi-printing method. With further compression of the PDMS pen and speeding up of the pen movements, phenolic resin on the PDMS pen tip can be fully transferred to the substrate and form a small cylinder with 30 µm diameter and 10 µm height. These cylinders were stacked up to form ~80 µm high pillars (Fig.7.5a and 7.5b). Then phenolic resin wires can then be drawn from one pillar to another to form bridges.
Figure 7.5. SEM images of the fabrication of carbon air-bridges on carbon pillars. a) Phenolic resin pillars. b) High magnification image of one phenolic resin pillar. c) Carbon bridges between two carbon pillars on different electrodes. d) Submicron carbon wire between carbon pillars.

Compared to the carbon needles, after pyrolysis (< 40% vs) vertical shrinkage of posts (<40%) was less than for needles (> 50%). As shown in Fig. 7.5c, the carbon pillar (50 µm tall) was converted from an 80 µm high phenolic resin pillar. The average diameter of “carbon wire bridge” between these two pillars is about 1 µm (Fig. 7.5d).
Figure 7.6. A typical I–V curve of carbon bridges.

7.3.5 Electrical resistance of carbon bridges and resistivity of glassy carbon.

Many carbon air-bridges have been created before, but the electrical resistance of these bridges has seldom been measured\textsuperscript{35,36}. The common electrical test methods are very difficult to be applied to micron or nano-sized carbon structures. By fabricating bridges on large gold electrodes on silicon substrates, the two-point electrical resistance measurement could be used to estimate the electrical properties of micro-textured carbon. Fig. 7.6 is a typical I-V curve of one carbon bridge between two carbon pillars, and the electrical resistance is 37,000 Ω, which is calculated from the reciprocal of the slope. Because the resistance of gold electrodes and carbon pillars were three or four orders of magnitude lower than carbon wires, the resistance of carbon wire is approximated to 37,000 Ω. Using the dimension of carbon wires, the resistivity of carbon can be calculated as follows:

\[
\rho = R \times \frac{A}{l} = 37000 \times \pi (0.50 \times 10^{-6})^2/(2.0 \times 10^{-4}) = 1.4 \times 10^{-4} \Omega m
\]
Glassy carbon has a wide resistivity range which mainly depends on the precursors and pyrolysis temperatures\textsuperscript{23,37}. In order to get the lowest resistivity of glassy carbon converted from phenolic resin, we also studied the effect of pyrolysis process. As discussed in many research papers, the higher the pyrolysis temperature, the lower the resistivity of glassy carbon. In our experiment, the temperature increased from 800 °C to 1000 °C, reducing the resistivity by almost an order of magnitude (Fig. 7.6a) from $2.0 \times 10^{-4}$ to $2.7 \times 10^{-5}$ Ωm. The curing time at 150 °C of phenolic resin is another important factor. A higher degree of cure will result in a higher cross-link density and thus greater carbon yield and higher final density. As shown in Fig. 7.6b, as the curing time increases, the resistivity decreases. The resistivity decreased from $6.3 \times 10^{-5}$ Ωm to $2.7 \times 10^{-5}$ Ωm by increasing the curing time at 150 °C from 0 to 1 h, after pyrolysis at 1000 °C. Finally, using a cure time of 1 hour and a pyrolysis temperature 1000 °C, the lowest resistance of glassy carbon $2.0 \times 10^{-5}$ Ωm was achieved, which implies fully formed glassy carbon.

**Figure 7.7.** Factors that affect the resistivity of glassy carbon. a) The effect of pyrolysis temperatures. b) The effect of curing times.
Table 7.1. The resistance of 100 µm-long carbon bridges with different graphite content and the estimated resistivity of carbon/graphite composites.

<table>
<thead>
<tr>
<th>Graphite content (wt %)</th>
<th>0</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance (Ω)</td>
<td>721</td>
<td>177</td>
<td>177</td>
</tr>
<tr>
<td>Estimated Resistivity (×10⁻⁵ Ωm)</td>
<td>3.1</td>
<td>0.9</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Graphite flakes were added to the phenolic ink to decrease shrinkage as well as increase the electrical conductivity after pyrolysis. Because the elongation of phenolic resin ink wires decreased as graphite flakes were added, the challenge of making long carbon bridges using the thixotropic graphite/phenolic ink, was challenging. However, 100 µm-long carbon bridges were fabricated by keeping graphite concentration of the filled ink below 10%. As shown in Table 7.1, graphite fillers significantly improved the electrical conductivity of glassy carbon; the resistance for carbon/graphite bridges decreased from >700 Ω to 177 Ω with, and the estimated resistivity of carbon bridge material decreasing from 3.1×10⁻⁵ Ωm to 0.5×10⁻⁵ Ωm.

7.4 Conclusion

In summary, we formulated a polymeric precursor ink by dissolving 60 wt% phenolic resin in high boiling point NMP solvent and obtained good printability, air-stability and controlled rheology for 3D printing. Three different phenolic resin microstructures, including microneedle arrays, air-bridges and pillars, were successfully fabricated using the polymer pen printing approach. These polymer ink features were crosslinked and subsequently converted to glassy carbon by pyrolysis while retaining their overall shape. With 35 µm height and submicron apex (as small as 0.5 µm), carbon needles converted from pure phenolic resin needles have ultra-high
aspect ratio. The suspended carbon wires with 50 to 300 µm length air-bridge structures show good ohmic contact with gold electrodes, facilitating the resistance measurement for estimating the resistivity of pyrolyzed carbon. The lowest resistivity of glassy carbon, $2.0 \times 10^{-5} \, \Omega \text{m}$, was achieved by using a curing time of 1 hour and a pyrolysis temperature of 1000 °C, which is comparable to the best glassy carbon reported ($3.0 \times 10^{-5} \, \Omega \text{m}$)$^{38}$.

The resistivity of pyrolyzed carbon structures could be reduced by adding graphite fillers to phenolic ink, which also decreased the shrinkage during pyrolysis process. On the other hand, the presence of graphite makes the phenolic ink thixotropic, shortening the elongation of the ink wire possible during the needle and bridge fabrication processes. By controlling the printing speed, high aspect ratio (> 2:1) phenolic/graphite pillars were created by multi-depositing 16 wt% graphite filled phenolic ink with increased print height. Phenolic wires were then created directly between pillars. After pyrolysis, carbon air bridges with 50 µm height were formed.

With good electrical conductivity, carbon needles have a great potential to be a low cost alternative to vertical electrode arrays used for neuroscience studies. High carbon air-bridges can be precisely positioned on and integrated into MEMS devices by polymer pen printing, which may find applications as interconnects in high-frequency integrated circuits$^{39}$ or suspended carbon nano/micro-heaters in gas detectors$^{40}$. 


Chapter 8. Conclusions and Outlook

8.1 Conclusions

This research work has aimed at developing a novel bench-top microfabrication apparatus and exploring its feasibility for fabricating 2D & 3D microstructures for MEMS applications. Through modifying the Sonoplot GIX Microplotter II platform with PDMS tip arrays (fabricated using conventional microfabrication methods, such as focused laser lithography, soft lithography and wet etching), a low-cost, convenient and versatile polymer pen printing system has been successfully developed based on a concept derived from polymer pen lithography. In addition to these basic functions, I developed a 2D microarray pen writing lithography system and showed that that 3D microstructures can be fabricated by pen printing techniques.

The single polymer pen printing system was developed first with a solid PDMS tip. The route for upgrading the polymer pen printing system from a single pen to pen array is presented in detail. First, the process of PDMS pen array fabrication, including silicon mold fabrication, was successfully optimized, resulting in a uniform PDMS pen array for printing. Next, mechanical parts were designed that enabled leveling the tips to the substrate while creating a light path for optics. A force gauge and the z-movement stage were integrated, enabling the force-feedback control of feature size. Last, but not least, two simplified inking process were proposed and successfully applied to the enzyme and phenolic-based ink printing studies.

Although single polymer pen printing is limited to patterning small microarrays, by printing dilute enzyme aqueous solution, BST/FA solution and thixotropic phenolic resin solution, we demonstrated that a wide variety of materials with different ink properties can be successfully printed and used to create various 3D microstructures. In addition, the results show that the surface
chemistry of the PDMS pen and silicon substrate have a significant effect on the size of the deposited features. The hydrophilic PDMS pen can pick up more ink material than the hydrophobic pen which increases the efficiency of ink transfer and forms larger features. On the other hand, with a thin layer of silicon oxide created by piranha or oxygen plasma treatment, the hydrophilic silicon wafer promotes the spreading of the ink, resulting in larger features for water based or hydrophilic solvent based ink. Such substrate surface chemistry modifications do not have significant influence on thixotropic inks do to their different rheology compared to low viscosity inks. A summary of the behaviors of different inks as a function of substrate wettability is shown in Table 8.1

**Table 8.1.** Single polymer pen printing results for inks with different viscosities.

<table>
<thead>
<tr>
<th>Ink</th>
<th>Viscosity</th>
<th>Volume of transferred ink</th>
<th>Effect of hydrophilicity of PDMS pen (10 µm tip)</th>
<th>Effect of surface nature of silicon substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALB aqueous solution</td>
<td>Low</td>
<td>small</td>
<td>No significant effect</td>
<td>Hydrophilic: Larger diameter Smaller thickness, Hydrophobic: Smaller diameter Larger thickness</td>
</tr>
<tr>
<td>BST/FA</td>
<td>Medium</td>
<td>small</td>
<td>More ink transferred</td>
<td></td>
</tr>
<tr>
<td>Phenolic NMP solution without filler</td>
<td>High</td>
<td>Large</td>
<td>No significant effect</td>
<td>Larger diameter</td>
</tr>
<tr>
<td>Phenolic NMP solution with filler</td>
<td>Thixotropic</td>
<td>Large, but less than pure phenolic ink</td>
<td>No significant effect</td>
<td></td>
</tr>
</tbody>
</table>
In the applications of polymer pen printing, we found that both additive and subtractive approaches could be used for 3D microstructure fabrication. The former is used in fabricating micro-posts with high aspect ratios, needles and air-bridges by stacking or dragging thixotropic ink material with the polymer pen. Pyrolysis after printing results in 3D carbon microstructures being formed from the phenolic ink features. The electrical resistivity of pyrolyzed glassy carbon with 3D microstructure \((2.0 \times 10^{-5} \, \Omega \text{m})\) is lower than the published glassy carbon data \((3.0 \times 10^{-5} \, \Omega \text{m})\)\(^3\), indicating the potential of polymer pen printing process to fabricate carbon-MEMS. In contrast, high resolution enzymatic lithography is a subtractive process. With optimized conditions, such as 87% RH and small \(<1 \, \mu \text{m}\) PCL crystallites, enzymatic polymer pen lithography addressed in my thesis is more practical than any other enzymatic lithography methods due to the lack of lateral diffusion of the enzyme across the surface as well as its rapid but well-controlled pattern development.

Overall, in this thesis, the developed polymer pen printing system has proven to be a valuable tool for fabricating 2D & 3D microstructures in a low cost way. The 3D microstructured carbon is successfully fabricated, as well as the improving of polymer pen printing based high-throughput, high resolution enzymatic lithography method.

8.2 Outlook

There are three areas in which my thesis can be expanded in the future. First, the resolution of the polymer pen printing system can be upgraded, fabrication of new pen arrays. For example, the Sylgard 184 PDMS used in this project is too soft, leading to difficulties in pen array leveling and nano-feature fabrication. Therefore, the original PDMS pen array in polymer pen printing system could be replaced by a higher modulus pen array\(^1\). In addition, a dual-part design can also
be applied in pen array fabrication\(^2\). The design of pens with hard tips and soft bases could reduce the tip deformation while improving pen planarity and feature uniformity over large areas.

Another approach to improving print quality is to modify the wettability of the tips. The PDMS pens can be surface modified such that the very tip is modified to be hydrophilic while the remainder of the array is superhydrophobic, which may prevent excess solution from being trapped between pens. In this way, the conventional inking process, dipping pen directly in a large inkwell, can be simplified and higher resolution achieved.

Second, explore applications for fabricated 3D microstructures. One approach would be to use the PCL defined micro-wells to study cell-drug interactions; and the other one is to demonstrate that carbon needles can probe electrical properties of individual cells.

The third direction for future research would be to explore the general applicability of enzyme inks. For example, in the enzymatic lithography project, instead of CALB-PCL system, different pairs of enzyme and biodegradable polymer, such as the enzyme trypsin combined with the substrate poly l-lysine (PLL), can be applied to determine if strong enzyme-polymer interactions are a general phenomenon, or a special feature of the CALB-PCL system.
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CHAPTER 6


**CHAPTER 7**


**CHAPTER 8**