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Soft Micro-Channels For Cell Culturing And Migration Studies

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Soft Micro-Channels for Cell Culturing and Migration Studies

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North Carolina A&T State University

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The Graduate School
North Carolina Agricultural and Technical State University
This is to certify that the Master's Thesis of

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has met the thesis requirements of
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Greensboro, North Carolina
2014

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BIOGRAPHICAL SKETCH

Sara Abbasirazgaleh was born on August 1, 1989. She found her passion and enthusiasm for science especially in physics and math when she was a high school student. She finished her high school and pre-university education by receiving a diploma in Math and Physic in 2007. After finishing her high school education, she departed her home country to embark on a new journey, overseas. She began her undergraduate education in electronics engineering majoring in nanotechnology at Multimedia University, Malaysia in 2007. In 2012, right after graduating from her program she continued the pursuit of her passion by starting the Nanoengineering program at North Carolina A&T State University, USA.

DEDICATION

This thesis is dedicated to my parents, my siblings for their love, continuous support and motivation and to whom who believes.

ACKNOWLEDGEMENTS

Words are insufficient to express my gratitude towards those who contributed and supported me along the way to accomplish this research work successfully.

First and foremost, my utmost gratitude to my supervisor and academic advisor Dr. Shyam Aravamudhan for his guidance, assistance, encouragement and his precious advices that helped me to hurdle all the obstacles in the completion of this thesis work. I would also like to extend my appreciation to the rest of my committee members for sharing their knowledge and experience. Furthermore, I am thankful to university and the Joint School of Nanoscience and Nanoengineering and my faculty members for giving me the opportunity and providing me with the necessary resources.

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ABSTRACT

Various techniques and methods have been studied and developed to aid nerve regeneration and repairing nerve injuries. Among all, nerve grafting is the gold standard for bridging the gap between the injured nerve stumps. Despite the advantages of this technique, there are also various drawbacks that have encouraged the exploration of alternative, less invasive methods for promoting nerve regeneration.

In this thesis, we have fabricated soft micro-channels for cell culturing and migration studies which could act as an interface capable of long-term, reliable, and high-resolution stimulation device for nerve regeneration. Micro-channels fabrication is performed using a combination of photolithography technique and physical vapor deposition (PVD) methods. Initially, the surfaces of the micro-channels are treated with oxygen plasma to convert the surface of PDMS from hydrophobic to hydrophilic and to further provide an optimal environment for cells to adhere and grow. Next, *in vitro* studies were performed on the fabricated micro-channels to demonstrate feasibility of the platform to promote adherence and growth of PC12 cells (cell line derived from a pheochromocytomas of the rat adrenal medulla).

CHAPTER 1:

INTRODUCTION

1.1 Introduction

Repairing never injuries dates back to the 17th century [1]. By the 19th century, Huber introduced various surgical methods such as stretching or transposing nerves which utilize nerve grafts to assist in nerve regeneration [2]. However in the recent century, research has shown that nerve grafting is the optimal choice for treatments, since the tension across a repair site can severely inhibit nerve regeneration [1].

One of the methods developed to promote regeneration of peripheral nerves, is bioengineered hollow tube nerve interfaces coated with specific molecular or biological cues, such as, collagen, biodegradable polymers, polyimide, silicon and many other biomaterials. Peripheral nerve interface such as the Utah array, Sieve electrodes, multi-electrode array and penetrating electrodes (either single, needle array, longitudinal intrafascicular electrodes (LIFESs)) are some of the other approaches used and have been intensively studied. These interfaces usually provide great sensitivity as a nerve interface, however they are considered to be very invasive.

Among the clinical approaches, autografting is the gold standard for bridging the ends of nerve stumps of peripheral nerves. Autografting is the most common used technique because it is biocompatible, non-toxic and provides structural supports to promote axonal adhesion and regeneration. There are various challenges to this technique, for instance dependence of autograft on modality of regenerating nerve, limited availability of disposal nerve segments, multiple

surgeries required due to small nerve defects, loss of function at the donor site, multi lengths of nerve graft is required to bridge the gap between the injured nerve stumps and it may contain inhibitory chondroitin sulfate proteoglycans (CSPGs) that impede the nerve regeneration process [3].

Many of these approaches are designed to endure the low impedance of extracellular volume and high ionic diffusion and dispersion. As a result, the extra-cellular potential measured by these techniques is smaller than 10 uV. The extra-cellular recording is measured at the node of Ranvier, which is repeated at over ~2mm segments. It is done at this specific spot on the axons since the extra cellular potential is at its highest level.

Another clinical approach is biomaterial based tabular conduits to bridge the nerve stumps. Regeneration using tabular conduits starts by forming fibrin cables that are used as a substrate for growth of Schwann cells. It has been unsuccessful in promoting regeneration across gaps longer than 10 to 15 mm in rats.

One of the alternative methods is bioengineered nerve interface such as nerve cuffs, penetrating electrodes of various types and regeneration sieves. Among all, nerve cuffs have reached the zenith point in its developmental aspect. It is used for applications that do not demand highly detailed spatial selectivity of simulation or recording with the nerve. This rises from the fact that spatial selectivity is limited by electrical contacts at the periphery of the nerve and also that communication with deeper fascicles are very challenging. It is possible to achieve higher selectivity by dispersing sieves electrodes within the nerves, hence increase the interaction with axons. By utilizing this technique, the measured extra-cellular signal for recording and simulation is extremely small.

1. 2   Motivation

Nerve damage occurs either through a trauma or surgical procedure due to some sort of complication in the process. As we mentioned earlier in the chapter, there are various techniques and methods that have been developed and used clinically to perform *in vitro* investigation of neurite migration and peripheral nerve regeneration. Aside from advantages that each of theses techniques offer, some suffer from major drawbacks that paves the path towards exploring new alternatives that could offer the same benefits while eliminating, if not minimizing, the shortcomings.   Some of the main challenges in nerve regenerations are misrouting of regeneration axons at the lesion site, slow regeneration rate (less than 1mm per day), progressive reduction in the capacity of supporting Schwann cells to aid axon regrowth, accurately identify and guide thousands of the regenerating fibers, high density signal processing and trade off between the selectivity of interfacing and degree of injuries to nerves.

This thesis work is mainly focused on providing a platform to guide regenerating axons from nerve stumps to another or in another words to provide directional regeneration of axon fibers. Moreover, we hope that fabricated micro-channels in this thesis would provide channels with higher density signal processing, meaning that we are able to study one channel (nerve fiber) independently from others, while the stimulation and recording may be performed simultaneous among the channels.

1. 3   Research Objectives

The long-term objective of this research is to design and fabricate an interface capable of long-term, reliable and high-resolution stimulation/recording of peripheral nerves. Towards this goal, the initial goal is to develop high-density 3D micro-channels for stimulation and

regeneration of peripheral nerves. Next, investigate in vitro axonal growth and Schawnn migration through the 3D array of micro channels with embedded electrodes, including study of substrate mechanics (conformability), materials biocompatibility and effect of the biochemical and topographical cues on axonal growth.

The short-term goals of this work are divided into four phases. In the first phase, the goal is to fabricate 2D soft micro--channels in PDMS. Next, the PDMS surface is hydrophilized to provide a suitable environment for cells to grow and adhere. Next, various functionalization schemes including PDL and collagen to aid adhesion of cells to the surface are optimized. Lastly, cells are cultured and monitored on the fabricated soft micro-channels. After phase II, hydrophilization of PDMS surface, cells were cultured on the flat PDMS surface. At this stage, various biomaterial including PDL and collagen are used to aid adhesion of cell to the surface. The final phase consists of culturing PC12 cell on PDMS fabricated micro-channels.

1. 4   Organization of This Thesis

The contents of this thesis are divided into five chapters. Chapter 1, provides an introduction to nerve regeneration, with focus on peripheral nervous system. The current clinical approaches are briefly discussed. This chapter also elaborates on motivation for this research and fabrication of soft micro-channels. Furthermore, the objectives and goals of this thesis work are discussed.

Chapter 2 gives comprehensive literature review on nervous system, its components, peripheral nervous system, clinical approaches and guidance channels. In addition, discussion on challenges and materials for nerve regeneration are provided. Then discussing PDMS based

materials, its hydrophobic properties and functionalization methods. Followed, by an overview on cell adhesion on PDMS via biomaterials such as collagen, PDL and Laminin.

Chapter 3 discusses methods and techniques used in fabricating PDMS based micro-channels, including photolithography and physical vapor deposition (PVD). Also, protocols and techniques used for culturing cells on PDMS surface are discussed.

Results obtained from hydrophilization of PDMS surface, cell adhesion to the surface of plasma treated PDMS and cell adhesion and migration in the channels are presented in Chapter 4. Furthermore, this chapter includes results from characterization methods such as AFM, FTIR, contact angle measurements and optical microscopy.

Lastly, Chapter 5 contains conclusions of this research, future works and perspectives of cell culturing and migration using soft-micro channels.

CHAPTER 2:

LITERATURE REVIEW

2. 1   Nervous System

Nervous system is categorized to central nervous system (CNS) and peripheral nervous system (PNS). Central nervous system (CNS) is consisting of brain, spinal cord, optics, olfactory and auditory - conducts and interprets signals, ends excitatory stimuli to PNS. On the other hand peripheral nervous system (PNS) includes cranial nerves from brain, spinal nerve from spinal cords, sensory nerve cells bodies.

2. 1. 1   Cellular Component

Cellular component of nervous system composed of neurons and neuroglianeurons. Neuroglianeurons consist of cell body called soma and its extensions, axons and dendrites. Dendrites transmit electrical signals to the neuron cell body and axons conduct impulses away. Neuroglia or glia cells are supports cells that aid the function of neurons, includes of Schwann cells in PNS and astrocytes and Oligodendrocytes in CNS. They also can go through mitosis in PNS: Schwann cells surrounds all the axons, outer layer of Schwann cells are called neurilemma.

2. 1. 2   Anatomy of Nervous System (PNS)

Endoneurium surrounds the individual axons and their Schwann cells sheath. Endoneurium is composed of predominantly orientated collagen fibers. Next, the perineurium formed from many layers of flattened cells (i.e., fibroblasts) and collagen, surrounds groups of axons to form fascicles. Finally, epineurium, an outer sheath of loose fibrocollagenous tissue,

binds individual nerve fascicles into a nerve trunk. Epineurium, an outer sheath of loose fibrocollagenous tissue, binds individual nerve fascicles into a nerve trunk.

2. 1. 3   Nerve Injury and Regeneration

When a nerve transected or severed, distal portion start to degenerates as a result of protease activity and separation from metabolic resources of the nerve cell bodies. This occurs in all the axons distal to the injury sites and is so called Wallerian degeneration.   Cytoskeleton begins to break down and the cell membrane to dissolve, Schwann cells at distal ends shed myeline lipids, phagocytotic cells (macrophage, Schwann cell) clear myeline and axonal debris, in addition they also produce cytokines which enhances axon growth following debris clearance the regeneration process starts at proximal end and continues towards the distal stump or end normally new axonal sprouts from the nodes of Ranvier, non- myelinated area of axons located between Schwann cells they grow up till they reach the distal target.

By using a hollow conduit required additional steps to [4] after the injury a fibrin bridge is formed across the defect sites and through the conduit, is consist of macrophages and other cells involved in debris clearing the febrin bridge retract as the Schwann cells and capillaries begin to grow across the gap and the regeneration process proceeds as normal.

2. 1. 4   Current Clinical Approaches

The two most commonly used clinical approaches in peripheral nervous system (PNS) are direct end-to-end surgical reconnection of damages nerve ends and use of autologous nerve graft for large gap in nerves. However, suturing the ends of the two nerves together not desired since the tension induced in the nerve cable prevents nerve regeneration [4]. For larger gap injuries in nervous system the autologous nerve graft is preferred. Though this method has the

disadvantage of loss of function at the donor site and the need for multiple surgeries due to small nerve defects (size of several millimeters).

2. 1. 5   Challenges

The main challenges in PNS are to find alternatives to autologous nerve graft and the clinical recovery rates. The recovery rate for the above mentioned method typically approaches only 80/100 for every injury treated. In addition to the previous mentioned challenges, misrouting of regeneration axons at the lesion site and the slow regeneration rate, approximately less than 1mm per day, are of other concerns. Furthermore, there is always has to be a tradeoff between the selectivity of interfacing and degree of injuries to nerves hence there is a need for a highly sensitive neuroprosthetic interfaces that can record or stimulate the extracellular signals as AP are passing through them, independent of node of Ranvier.

2. 1. 6   Guidance Therapies

In guidance therapies, physical guidance of axons are used based on various materials such as autologous nerve grafts, bone, metal tubes, and fat sheath [4]. This approach requires to accurately aligning the nerve fascicles and nerve graft so that tension on the damage nerves is reduced and the functional recovery is enhanced [4]. Autologous nerve graft is the gold standard for repair of peripheral nerve defects [4]. Nonetheless, nerve guides or nerve guidance channels provide a conduit for growth and diffusion of growth and reduce the infiltration of scar tissue as they are used to direct the axons sprouting from the proximal ends.

It is worthy of mention that still none of the materials, natural or synthetic materials, has exceeded the performance of autograft. New research focuses on new composite from combination of materials and biomolecule.

2. 1. 7   Materials

2.1.7.1   Autologous Tissue Grafts

For autologous tissue grafts materials such as nerve graft, vein graft, muscle grafts, epineurial grafts, Tendon grafts are being used. They offer various advantages such as being more likely to be biocompatible, less toxic and provide support structure to promote cell adhesion and migration. Like any other materials they come with some disadvantages such as potential difficulties with isolation and controlled scale-up as for natural tissues like muscle, vein graft and combination of both. Regardless of the fact that results obtained using autologous tissue grafts material has been good, the major drawback still remains, must be removed from the patient.

2.1.7.2   Nonautologous Tissue and Acellular Grafts

There is more attention towards the nonautologous tissue and extracellular matrix (ECM)- based materials, for instance allegonic and xenogenic tissues. They have the advantage of being removed from animals but not the patient. However, tissues may possess some risk of disease transmission and therefore need to be used in conjunction with the immunosuppressants or need to be processed before to remove the immunogenic components.Various techniques has been used to process intact nonautologous tissue such as thermal techniques, radiation, chemical processes, focused on preservation of ECM structure and removal of the immunogenic cells.

2.1.7.3   Natural Based Materials

Focused on use of purified natural ECM proteins and glycosaminoglycans by modification. It can be used an appropriate scaffolding ECM molecules such as laminin, collagen and fibronectin play role in axonal development.

2.1.7.4 Synthetic materials

Synthetic materials are used since their chemical and physical properties can be specifically optimized for a particular application. Biocompatibility of synthetic material is challenging as the body's inflammatory response can vary considerably from one material to another. Furthermore, some materials tolerated by the body's immune system are unfortunately incompatible with cell adhesion and tissue repair as we make them more cell friendly. Several general properties that the materials used for nerve guidance channels must possess [4] are must be readily formed into conduit with desired dimension, must be sterilizable, must be tear resistance and easy to handle or suture.

In addition must be pliable or flexible, maintain their structure as resist collapse during the implantation and over the time course for regeneration need to be semipermeable [4]. Permanent materials are not used because they might become infectious over time; provoke a chronic inflammatory response, potential to compress the nerve as time passes [4]. In general, prefer nerve guides that degrade as the nerves regenerate.

2.1.8   Biomolecular Therapies

2.1.8.1   Neurotropic Factor to Promote Regeneration

From neurotrophin family, neurothrophic factor, the neurotrophins include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Other factors that are important: ciliaryneurotrophic factor (CNTF), glial cell line-derived growth factor (GDNF), and acidic and basic fibroblast growth factor (aFGF, bFGF).

Nerve growth factor (NGF) expresses at low level in healthy peripheral nerve and is unregulated in distal stump upon injury. Disadvantageous of using NGF is exogenous NGF to spinal cord injury causes sprouting of uninjured sensory axon that has been shown that has serious side effects such as chronic pain and in appropriate neural reflexes [4]. BDNF supports motor neuron survival, promotes the axonal growth factor and sensory neurons [4].

2. 2   Hydrophilization of PDMS

Polydimethylsiloxane (PDMS) is a group of polymeric organosilicon compounds, which is often referred to as silicones. Also, known as dimethicone, one of the several types of silicone oil (polymerized siloxane). It is the most widely used silicon based organic polymer known for its unique properties such as rheological (or flow) properties, optical transparency, chemically inert, nontoxic, non flammable, water repellence, strong resistance, elasticity, flexible surface chemistry, low permeability to water and low electrical conductivity and low density surface properties [5-7]. It has a wide range of applications ranging from contact lenses, medical devices to elastomers used in shampoos since dimethicone makes hair shiny and slippery, food as antifoaming agent, caulking, lubricating oils, heat resistant tiles and etc. Recently, it has found attention for biomedical field such as medical implants and biomedical devices [8, 9].

Polydimethylsiloxane is a soft polymer which chemical formula $CH_3[Si(CH_3)_2O]_nSi(CH_3)_3$, n is the number of repeating monomer $[SiO(CH_3)_2]$ units. It shows viscoelastic properties, meaning that at long flow time or high temperature acts as a viscous liquid similar to honey. However, at short flow times or low temperature it acts like an elastic solid similar to rubber. In other words it is a thin pourable liquid when *n* is very low and a thick rubbery semi-solid when *n* is very high. It has a quiet flexible polymer backbones or chains due

to the siloxane linkages. As a result, it becomes loosely entangled when molecular weight is high which turn gives it a high level of viscoelasticity. Moreover, its shear modulus varies with the preparation condition, typically raining from 100kPa to 3MPa and has very low loss tangent (tan S<<0.0001). After polymerization and cross-linking of the PDMS, it looks like a solid surface as of metal, shinny however clear and hydrophobic.

This material has been on top of the chart for research in various fields especially for biomedical applications such as micro-fluidic devices[9]. This is because its cheap, transparent, flexible material, biocompatible, low toxicity and high oxidative and thermal stability [8] [9]. Additionally, it offers a rapid fabrication of devices using relatively simple and inexpensive instrumentation elastomeric properties, biocompatibility, gas permeability, optical transparency, ease of molding into sub micrometer features, ease of bonding to itself and glass, relatively high chemical inertia and low manufacturing cost [7] [9]. Also, It does not swell or dissolve in a number of solvents and is permeable to most gases including oxygen [7]. Whether oxidized or not the solid surface of PDMS would not allow aqueous solvents to infiltrate and swell the material hence it can be used in combination with water and alcohol solvents without material deformation, although most organic solvents diffuse into the material and making them incompatible with PDMS devices.

Major drawbacks of PDMS are its hydrophobic nature with contact angle about 110° and porosity allowing absorption and adsorption of a wide variety of molecules [10]. Adsorption kinetics influence the biological activity of proteins adsorbed to PDMS, adsorption rate have a significant influence on the conformation and subsequent biological activity of the adsorbed protein layer [7].

Depending on the application there are challenges in regards to its surface properties and reducing the surface hydrophobicity. As mentioned earlier PDMS surface is hydrophobic hence difficult immobilization of biomaterials [8]. Using surface modification PDMS surface reduced in hydrophobic protperties and ecomes hydrophilic. Although, this might not last long asPDMS surface has a quick hydrophobicity recovery time. This can be due to its low glass transition temperature of less than 120 C [9]. Surface modification of PDMS and changing hydrophobicity of the surface without altering the chemical properties of the surface is time consuming and costly [11]. The hydrophobic nature of PDMS is because of the methyl moieties chemical groups at its surface [12]. It is worth it to mention the surface can be further modified to show super hydrophobic properties. For instance, a facile one-step laser etching method was used by M. H. Jin and et al. to fabricate surface at micro, sub-micron and nano regime that shows super hydrophobic character with contact angle greater than 160° and sliding angle lower than 5° i.e. referred to as lotus effect [5].

Various methods has been purposed and employed to reduce the hydrophobicity of the surface and enhance its adhesion properties such as oxidation of the surface by ultraviolet, plasma treatment, CO_2 pulsed laser, polymer grafting on PDMS, etc. Advantages of hydrophilic surface treatment can mention the capability to increase adhesion and capillary effects [11]. In general the surface modification techniques used for PDMS hyrophilization are categorized to these three groups:

1. Gas phase processing including plasma oxidation, ultraviolet (UV) irradiation, chemical vapor deposition (CVD) and sputter coating of metal compounds.
2. Wet chemical methods such as layer-by-layer (LBL) deposition, sol-gel coating,

silanization, dynamic modification with surfactants and protein adsorption.

3. Combination of both, for instance silanization and LBL methods on PDMS pretreated by methods such as plasma oxidation.

Methods such as bonding polymers to polymers of inorganic materials as using thermal welding, solvent welding or chemical adhesive bonding are processed in high temperature or use solvent to soften the material which introduces disadvantages [11]. Better method would be the low-pressure oxygen plasma, indirect corona or simply with an oxygen rich butane gas flame treatment [11]. Furthermore, biological surface modification methods using adhesion proteins can be used however they have disadvantages in clinical uses such as protein might degrade over a long time or when used in a harsh conditions resulting in alteration of surface properties [10].

Hou and et al. presented ccontrollable patterning of cells on PDMS surface by hydrophobin (HFIB) and collagen modification, immobilized on the surface using copper grids as a mask [8]. HFBI self-assembly on the surface of PDMS converted it from hydrophobic to hydrophilic surface that facilitated the immobilization of collagen. Plasma treatment of the surface is one of the most commonly used methods to modify PDMS surface properties. Gases such as oxygen, nitrogen and hydrogen which dissociate and react with the substrate surface creating chemical functional groups [9]. Plasma oxidation changes the surface chemistry by adding Silanol (SiOH) groups to the surface. The oxidized surface resists adsorption of hydrophobic and negatively charged species, can be further oxidized by trichlorosilanes, oxidized surface are stable for 30min in air, after that the hydrophobic recovery of the surface is inevitable independently of the surrounding medium. Both atmospheric air plasma and argon plasma are used for surface modification.

Tan and et al. purposed a novel plasma treatment using scanning radical microjet approach with oxygen microplasma offers advantages such as formation of localized pattern without need for a mask, higher surface treatment rates and lower damage compared to normal plasma treatment [9, 13].

Major challenges with oxidation is the recovery caused by migration of uncured PDMS oligomers from bulk to the surface and the rearrangement of highly mobile polymer chain featuring Si-OH bonds towards the bulk at room temperature [9]. PDMS when exposed to the air forms groups of hydrophilic silicondioxide (SiO_2) and silanol (Si-OH), oxidation process. In contact with air surface rearranges and new hydrophobic groups are created at the surface and with in 2 hours contact angle of a water drop increases with more than 50° [14]. Hydrophilic surfaces are unstable and quickly revert to their original states due to the diffusion of low-molecular-weight (LMW) chains from the bulk PDMS to the oxidize surface [15]. After oxidation can be kept hydrophilic by keeping the surface in contact with water or polar solvents [14]. In addition, chemical composition and geometry of substrate rise the super hydrophibic properties of the surface, most commonly wetting state are described using Cassie-Baxter and Wenzel model [12].

Oxygen RF Plasma process does not require any vacuum or ventilation lines, this process is truly manufacturable and suitable for in-line continuous processing, using plasma time and cost can be reduced [11]. It does not produces chemical waste, provides high efficiency of surface activation by dual action of radicals and charged particles (electrons and ions), does not alter mechanical properties of bulk materials, involve simple operation [11, 15].

2. 3   Photolithography Technique

Photolithography is one of the most common used techniques in IC fabrication. It can be used to fabricated feature smaller than 1um (100nm). A major advantage that photolithography offers is in parallel production of complex circuits and devices in a short period of time. Like any other device it comes with limitation too. The fundamental limitation of the photolithography determined by diffraction limit. This limits the size of the features than can be produced using lithography techniques approximately to the wavelength of light being used. General steps in lithography start by applying a thin uniform layer of photoresist on the surface to be patterned. Photoresist is the polymer-based light sensitive material that comes in two category of positive and negative resist. Depending on which category it belongs to different will react differently upon exposure to light. In positive resist region exposed to the light will becomes soluble to the photoresist developer and the unexposed region stays insoluble while in negative photoresist the region exposed to the light becomes insoluble to the photoresist developer and unexposed region can be removed by the developer. A predesigned mask of desired patterns positioned on top of the wafer or substrate. The mask consists of a glass substrate with a patterned layer of Cr. The reason behind using Cr is because it is not transparent to UV light which makes it is possible to illuminate chosen areas of a substrate through the mask, e.g. common photolithography.

Exposure to UV light alters the chemistry of the photoresist and changes its solubility relative to unexposed resist. There are two types of resist, "positive" and "negative." When a positive resist is exposed to UV light, the energetic photons of the light break certain bonds in the long-chain polymers of the resist, causing them to become shorter and thus more soluble. A developer easily washes away the exposed areas, leaving behind a copy of the pattern on the mask. When a negative resist is exposed to UV light it causes cross-linking between the resist

polymers making the exposed areas less soluble. The developer removes the exposed resist, leaving behind a negative image of the mask. After development, wafer provides a negative image of mask hence it is used a mold to get the PDMS mold with the positive image of the mask. In order to do so, after making the PDMS mold aluminum is deposited all over the substrate using physical vapor deposition (PVD) technique. The reason for depositing the PDMS mold with aluminum is to avoid two PDMS substrates from sticking together.
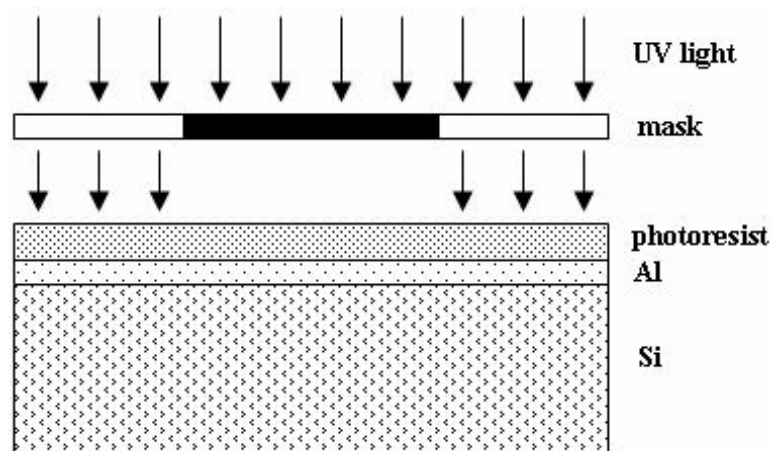

Figure 2.1 [16]: Cross section of photolithography process

2. 4   Physical Vapor Deposition

Physical vapor deposition also known, as PVD is a technique used to deposit a thin film by using the vaporized form of desired material, usually metals such as aluminum and gold. It is called physical since the coating process involves physical processes such as high-temperature

vacuum evaporation with subsequent condensation, or plasma sputter bombardment. There various type of PVD system such as cathodic arc deposition, electron beams PVD, evaporation PVD, pulsed laser deposition and sputter deposition. In electron beam physical vapor deposition, electron bombardment in high vacuum caused depositing material to be heated to a high vapor pressure. The evaporated material is transported by mean of diffusion to condensed on the substrate and hence deposition occurs. Evaporation deposition uses electrically resistive heating mechanism in low vacuum to heat up and alters the phase of material to be deposited to a high vapor pressure. Cathodic arc deposition utilizes a high power electric arc discharge to knock out highly ionized vapor from the source materials while in pulsed laser deposition method a high power laser is used to blast away material. Sputtering deposition uses glow plasma discharge to bombard the surface of sputtering material. Some of the common material used to be deposited using chromium nitride, gold, Titanium aluminum nitride, aluminum, zirconium nitride and etc.

Some f the advantages of this techniques are: film can be deposited at a high rate, low surface damage, deposited film seems to be harder and more resistant towards corrosion, excellent abrasion resistance, little residual gas and impurity intake, no/low substrate heating and high temperature and good impact strength. In addition, physical vapor deposition can deposit any type of organic or inorganic materials. It is also more environmental friendly comparing to other convectional techniques such as electroplating. Some of the limitations are it is difficult to obtain accurately controlled alloy compounds, no in situ substrate cleaning, poor step coverage, variation of deposit thickness for large or multiple substrate and X-ray damages.

2.5   Cell Adhesion

PC12 cell line is used in this study to investigate cell adhesion and growth on PDMS substrate.   PC12 cell line is derived from rat adrenal phaeochromocytoma. PC12 cell is commonly used cell line in studies of neurons and nerve regeneration studies since they have an embryonic origin. Due to their embryonic origin in they can easily be differentiated into neuron like cells. After differentiation, they show similar properties as neurons, meaning that they release neurotransmitters. By adding nerve growth factor also known as NGF to these cells they stop proliferation and start to have similar shapes as neuron. On the other hand, nerve growth factor works reverse on PC12 cells. Besides the above-mentioned reason, PC12 cell line is easy to handle and culture, there a lot of information available on their proliferation ad differentiation.

Study done Tao Zhou and et. al. titled " neurons derived from PC12 cells have the potential to develop synapses with primary neurons from rat cortex" [17], has shown that the PC-12 derived neurons are able to develop into mature neurons by differentiation using NGF and further to form intercellular contacts with host neurons.

CHAPTER 3:

METHODOLOGY

3.1   Introduction

This chapter contains comprehensive details of fabrication process including challenges and obstacles that we had to overcome during research period. Step by step fabrication process and cell culturing aspect of research are discussed in the current chapter. Wafer processing, fabrication of PDMS mold and finally desired micro-channels are discussed in the beginning section of the chapter. Optical Lithography and Physical Vapor Deposition (PVD) techniques were used. Furthermore, this chapter discusses about modification of PDMS surface to alter its hydrophobic surface, preparing substrates for cell culturing by conditioning PDMS surface using biomaterials such as PDL and Collagen and to observe and monitor cell adhesion and growth.

3.2   Fabrication of Soft Micro-Channels

All the process flow steps in mold fabrication, including lithography steps followed by the physical vapor deposition (PVD) process are discussed in this section. Photolithography is one of the most common used techniques in IC fabrication. It can be used to fabricated feature smaller than 1um (100nm). A major advantage that is offered by this technique is in parallel production of complex circuits and devices in a short period of time. The fundamental limitation of the photolithography is determined by diffraction limit that is in turn limited by wavelength of light used. As a result, size of the

features that can be fabricated using photolithography method is approximately equal to the wavelength that device operates on.

In photolithography, surface of substrates is initially cleaned. After preparing the wafer and cleaning its surface from dust and other contaminants, photoresist of choice is spun on the wafer followed by soft baking of phtotoresist. Then, mask aligner is aligned properly and prepared for exposure. After exposure is done, photoresist is developed to remove the undesired regions. Photoresist exposed to the UV light will be cross-linked in the shaped of the UV mask. The unexposed, uncross-linked coating, can then be removed using developer leaving a hardened pattern on the silicon wafer. The last step is known as hard bake step in which the developed resist is heated. This is called a stamp that will be used to mold an elastomer later on in the fabrication process. Figure 3.1 shows the process flow in photolithography steps followed by PDMS fabrication of mold.

3. 2. 1   Photolithography Process

Usually a silicon wafer is used as a substrate for photoresist patterning using photolithography. Although, depending on application the type of substrates used it may vary or may be even doped. Substrates used in this research were 4 inch (100mm) Si wafer and Si doped with boron (B) that were purchased from Wacker Siltronic, PB1194 and D92654W094, respectively. Initially, wafer was cleaned using RCA1 standard also known as SC1 or AMP in the industry. This standard set includes cleaning of the Si wafer with the solution of Ammonia Hydroxide (NH_4OH), Hydrogen Peroxide (H_2O_2) and DI Water (H_2O) in ratio of 1:1:5, respectively. The wafer was submerge in the mixture and then placed for 10min at 80 ^{o}C on the hotplate. During the process Ammonium

Hydroxide (NH$_4$OH) builds a very thin layer of SiO$_2$ on the wafer and the Hydrogen Peroxide (H$_2$O$_2$) etches away the oxide layer along with all the other impurities presents on the surface of the wafer.
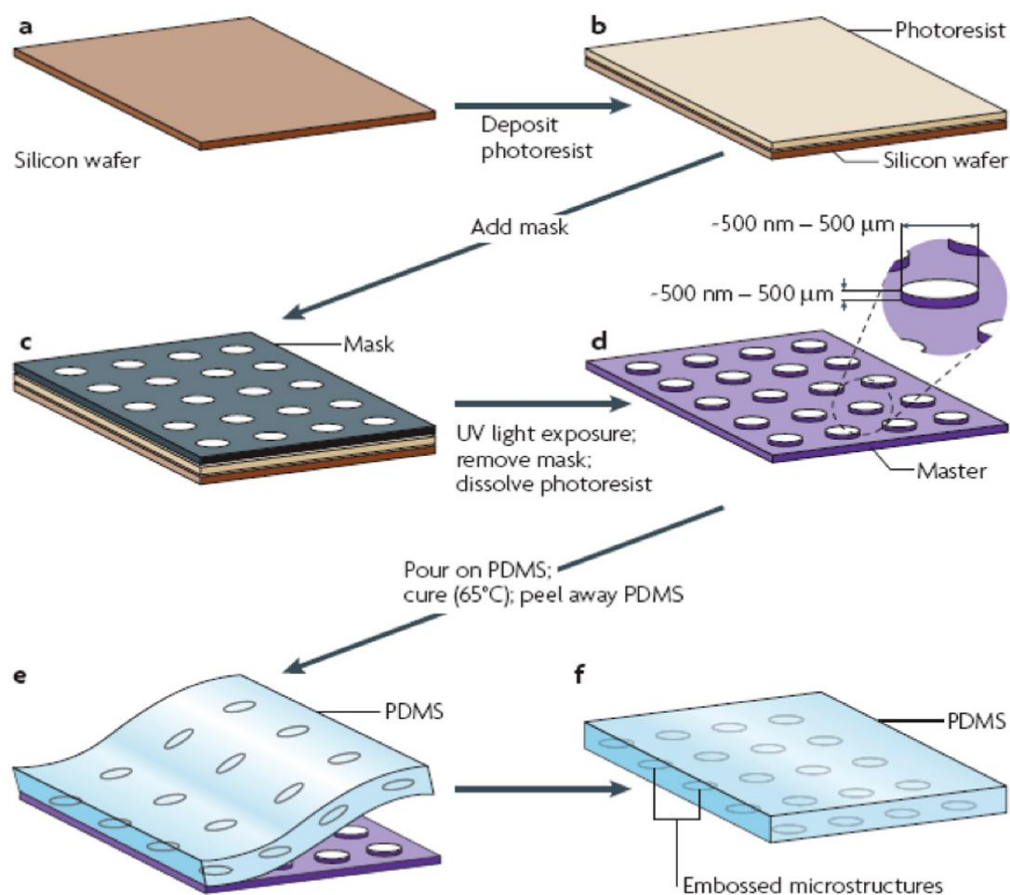

Figure 3.1 [18]: PDMS mold fabrication using SU-8

It is usually used in the semiconductor industry to remove organic contaminants, oxide layer or ionic contamination. After 10 minutes, the solution plate was moved to solvent fume hood in which the wafer was washed with DI water and air-dried using nitrogen pump. Finally, the RCA1 solution was dumped according to the cleanroom protocol by pouring down under the fume hood and then draining with plenty of water to flush (three times). It is worthy of note that RCA cleaning solution cannot be used after 24 hours at room temperature since it loses its effectiveness. However, it can be reused before passing the 24 hours.

Additionally, photo-mask was cleaned by Acetone and Isopropyl alcohol (IPA) followed by drying using nitrogen pump before and after exposing the wafer to UV light. The next step is to coat an even uniform layer of photoresist of choice over the wafer surface. Photoresists that were used in this thesis work were positive resist S1818 and negative SU-8 photoresist.

There are various photoresists that can be used depending on the application, desired thickness and other parameters. To do the patterning, initially we started with positive photoresist known as S1813 and then we changed to a more viscous photoresist from SU8 family called SU-8 3035. Using the positive resist S1818 thickness up to 2um is attainable. On the other hand using the SU-8 resist up to 200 um thickness can be achieved. The primary reason behind using SU-8 resist was to obtain a larger depth for micro-channels. Additionally by using SU-8 resist, processed wafer can be used as a permanent stamp for soft fabrication of micro-channels using PDMS without damaging micro features on the wafer.

After wafer preparation, wafer was placed onto the spin coater and pump was turned on. Before starting the spin coating procedure, wafer was checked to makes sure it was centered on the spin coater by running a test program at 500 rpm's. It is important to note and uneven spin coating results in a non-uniform coating and in turn into a non-uniform image and fabrication. After centering the wafer, in case of positive resist 2mL of HMDS was poured followed by poring about 4mL of S1818 resist over the wafer or in an enough amount to cover the whole wafer. As for negative resist, SU-8, adhesion promoter was no required. Therefore approximately 1 mL of SU-8 per inch of wafer diameter as suggested in the material data sheet was poured on the wafer. Wafers used in this fabrication were 4-inch wafer hence, about 4mL of SU-8 was poured on the surface. Then, the spin coater was sealed and program of choice was started. Once the program was stopped and before removing the wafer from the stage, removed the resist meniscus at the edge of the wafer.

Program of choice was created with the desired speed and time. The first spin cycle was run at 100 revolutions per minute (rpm) for 10 seconds to spread the SU-8 to the edges of the wafer and as for the positive resist it was set for 10s at 500rpm. The second cycle is the user-defined cycle in which the coater then ramps up to the user-defined rpm. This is the cycle that defines thickness of resist on the wafer after spin coating procedure.

Depending on the type of resist that has been used a constant ramp up speed provides different thickness, hence one needs to refer to the thickness vs. rpm chart for the appropriate spin settings for the desired device thickness. This information is

provided in the resist data sheet. Lastly, a ramp down cycle slowly brings the coated wafer to a stop.
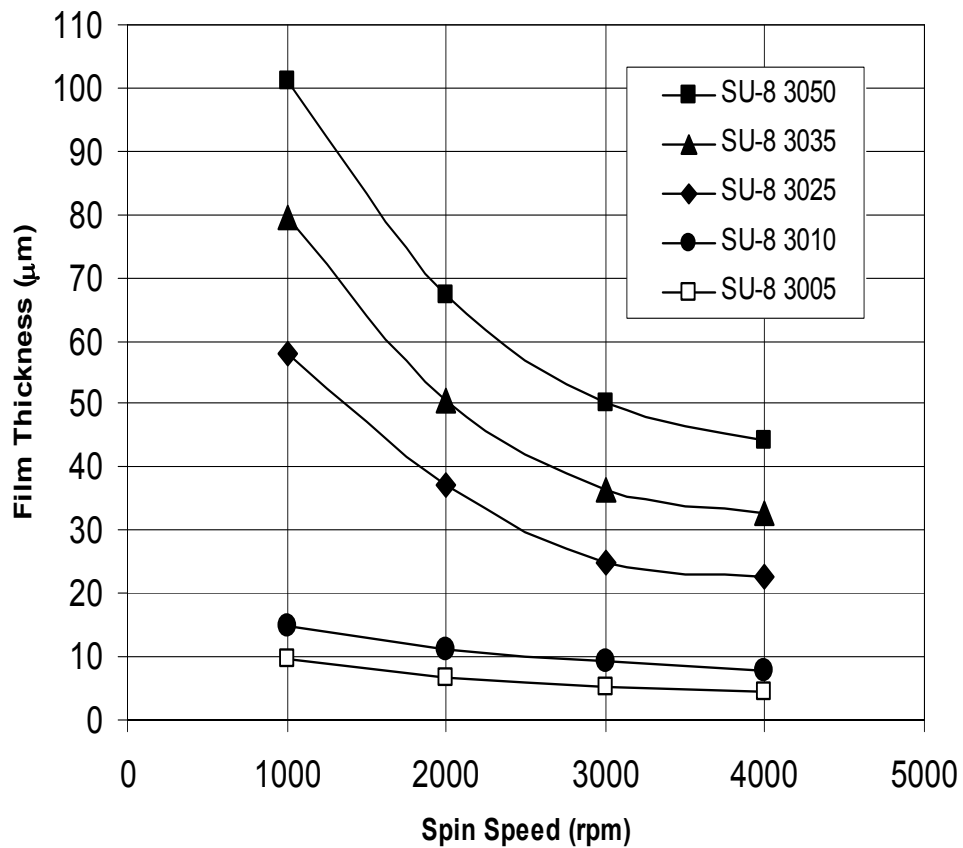

Figure 3.2 [19]: Photoresist thickness versus spin speed

Then, wafer was pre-baked (also known as soft-bake step) by placing the wafer on a hot plate at 65°C before increasing to 95°C. The time at each temperature varies

depending on the thickness of resist. This information was obtained by referring to the pre-bake heating chart for details.

After soft-bake process, mask aligner on the lithography device was aligned. Initially, mask was cleaned by Isopropanol (IPA) and then was placed on the mask holder by turning the mask vacuum on. Afterwards, wafer was placed in the cassette located on the cassette holder in the lithography device. For this research purposes, the positive resist was exposed for 7 sec where as the negative resist was exposed to UV light for 10min.

Exposure time is determined by thickness of resist dividing exposure energy by the mean exposure intensity (recorded on UV power meter), in our case 20 mJ/cm2. The quotient to the division is the seconds required to expose the resist of that thickness to the UV light. After exposure, wafer was hard baked and again depending on the thickness and resist that was used time and temperature for post bake step varies. S1818 resist was hard baked for 1min at 90 ^{o}C. However, negative resist SU-8 was hard backed on hot plate at temperature of 90 ^{o}C for 15min followed by 24hours waiting period. At last, processed wafer was developed in the developing solution. For positive resist S-1818 wafer was merged in the developing solution for 7sec while for negative resist 7min developing time was used with appropriate developer.

Following safety protocols were carried about at all the time during the fabrication steps. In handling with chemicals, chemical gloves are used in addition to be wearing double disposable gloves. Safety goggle and mouth mask are worn at the whole time.

3. 3   PDMS Fabrication

3. 3. 1   Materials

Polymer casts were prepared using PDMS from a Sylgard-184 kit from Dow Corning Corporation USA.

3. 3. 2   Procedures

Initially, weighing dish was placed on the digital balance and tare the balance. After balancing the electronic weighting machine, required amount of PDMS base and curing agent in the desired ratio was poured. It is worthy of note that depending on the research purposes and application this ration may vary. For the purpose of the ongoing research PDMS mold was prepared using 10:1 ratio. Measured weight is 20g and 2g of base agent and curing agent respectively. Using a glass spatula or mixer the mixture was thoroughly whisked for 10min in order to make sure the curing agent is uniformly distributed and mixed in base agent. A uniform distribution of curing agent results in a uniform cross-linked PDMS substrate. During the mixing process air is introduced to the mixture.

These air bubbles need to be removed from the mixture before curing process, hence need to degas the mixture by placing it in the desiccator pump for 1 hour. Before placing the mixture inside the bell-jar desiccator pump, the fabricated Si wafer coated with HMDS was placed in a clean 6-inch glass petri dish. To learn about the fabrication and processing of the Si wafer coated with HMDS refer to previous section. The glass petri dish used was cleaned with ethanol and oven dried. In addition, the petri dish was

customized to provide a support for Si wafer so that we could prevent Si wafer from sticking to the bottom of glass petri dish after curing process. When placing the Si wafer on the support was done. Then, the mixture was poured at the center of the wafer followed by placing it in the desiccator connected to a vacuum pump for an hour.
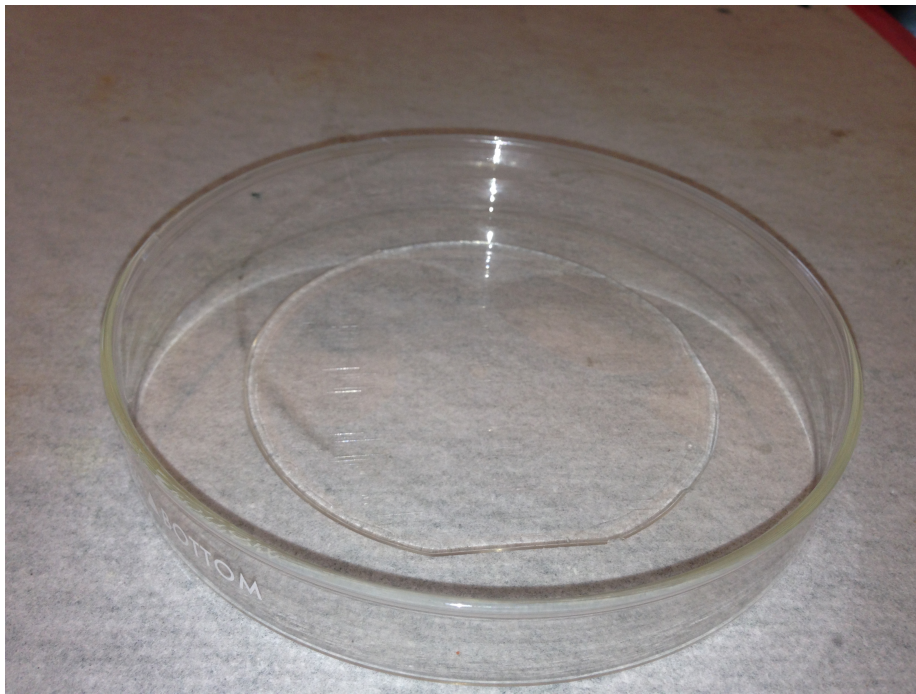

Figure 3.3: Fabricated PDMS Mold

After the degassing process, the PDMS coated mold was placed in the oven at 60 ºC for 1 hour to cure. The Oven was preheated for 1 hour at the same temperature. Finally, the cured PDMS was released from the mold by pulling it lightly from the

corners. Fabricated PDMS was then placed in a cleaned and oven dried 6-inch glass petri dish. Note that if the PDMS was very sticky after giving it enough time to cure, it could be due to either not having enough curing agent or not being mixed thoroughly.

3. 4   Cell Culturing

Prior to the cell culturing on PDMS fabricated substrate, following sterile procedures are performed to make sure that surface of PDMS substrate is clean and sterile enough to provide a viable environments for the cells to grow and adhere. First, all substrate were washed with hot water then followed preparing then in a petri dish for autoclaving procedure. Afterwards, plasma treatment of PDMS is carried out using RF plasma etcher discharging at 13.56 MHz. This plasma reactor is made from a circular of two ways cruciform glass vessel and can be pumped to a base pressure of order of 10^2 Torr using a rotary pump vacuum system. In this research work, a constant flow rate of air gas (-mTorr) and 200 W RF power was used for 30s. All the procedure was carried out while using gloves and following all the labs protocol and material handling safety steps. Immediately after the plasma etch, substrates were coated either with PDL, collagen or PDL plus collagen. After 24 hours of incubation period, substrates were washed three times with PBS and PC12 cell suspension is seeded to the substrate. Prior to the cell seeding cell viability is counted using hemocytometer to make sure cells are viable enough for the experimental purposes.

All the cell culturing procedures were taken place within a laminar flow hood regardless of the type of cell used in this case, Class I (use with animal tissues). The

laminar flow bio hood minimizes the risk of infection entering from the outside environment. In addition, protect lab users from potential pathogens being transmitted from the culturing area into the working environment. Other common sterile techniques followed in the labs are wearing gloves and goggles at all the times, wiping the hood and other working areas with70% ethanol before beginning and after completing an experiments, keeping sterile pipettes in their wrappers prior to use and not using the same pipette/pipette tips to draw media from different bottles.

One main cell lines was mainly used throughout the studies. The cell lines being PC12 neurons were fetched from ATCC (American Type Culture Collection). Cell cultures were maintained according to the standard protocol prescribed by ATCC. RPMI media is used for PC12 cells as seen in Table below. The cells were maintained at 95% 02 and 5% $CO2$ under normal humidified conditions.

Morphological observation of cells were done using inverted microscope and pictures were taken periodically to observe the changes in cell growth and morphological behaviors of cells before and after culturing on PDMS substrate.

CHAPTER 4:

RESULTS AND DISCUSSION

4. 1 Introduction

This chapter presents data obtained at each four phases of research. First section, demonstrate fabricated channels using optical lithography technique and physical vapor deposition. Afterwards, data obtained from hydrophilization of PDMS surface via plasma treatments is elaborated. In last section of the chapter cell adhesion and growth over flat PDMS flat surface and PDMS fabricated channels is discussed.

4. 2 Photolithography

Fabrication of channels was done using optical lithography based on deep UV exposure. In our fabrication steps we used both positive and negative resist. Figure 4.1 shows picture of the Si wafer mold obtained after photolithography process. In case of, positive resist Si wafer was used directly to obtain intended micro-channels. However, in case of the negative resist, PDMS mold was obtained from the Si Wafer and then micro-channels are obtained. Optical microscopy of channels suggest that although we are able to obtain deeper depth for channels, using current mask and lithography instruments we have it is difficult to get the resolution we desired to, specially as it goes to smaller dimension features. This could be due to the wavelength of the light used in our system as we know that SU-8 resist works the best and has maximum absorbance at 365nm

wavelength. In another words, it is not feasible to expose SU-8 resist to g line ultraviolet wavelength of light.
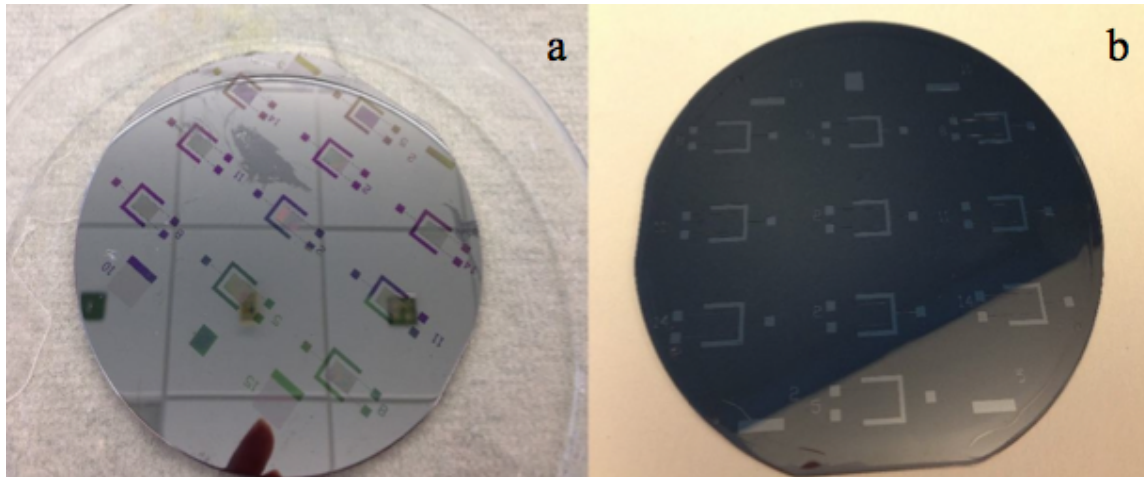

Figure 4.1: a) fabricated Si mold using S-1818 b) SU-8

However, using the S-1818 fabricated micro-channels were matching the specification as required for the research work. The drawback of using S-1818 is mainly due to the depth that can be achieved using this resist. As mentioned earlier, in previous chapter, using positive resist of S1818 up to 2um thickness or in other word depth is achievable. This thickness doesn't provide enough depth to accommodate cell seeding and culturing in the channels. As a result, cell seeding on seeding area becomes very challenging. Same reason is the motivation behinds switching from using positive S-1818 resist to SU-8 negative resist.
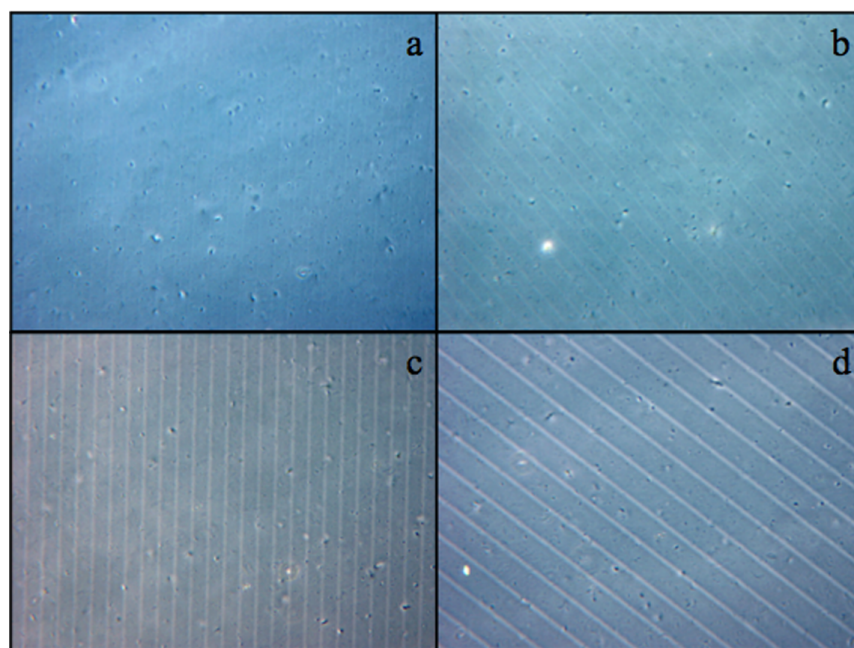
Figure 4.2: Optical microscope image of PDMS micro-channels a) 2um b) 5um c)

10um d) 20um

4. 3 Surface Hydropilization of PDMS Substrate

In this thesis work we used plasma etcher to perform surface hydrophilization of PDMS surface. Here we studied how changes in plasma treatment time changes surface properties of PMDS substrate. To confirm our results and surface hydrophilization, Contact angle measurement, Fourier transforms infrared spectroscopy (FTIR) and Atomic force microscopy (AFM) instruments were used.

4. 3. 1   Contact Angle Measurements
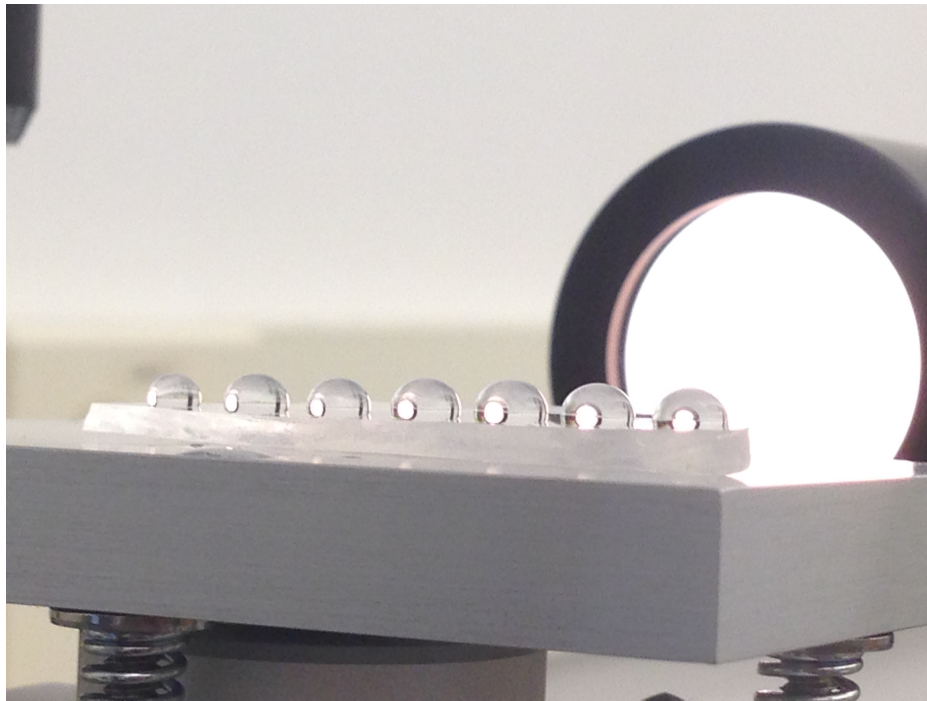
Figure 4.3: Hydrophobic properties of bare PDMS substrate

Three different ratio of curing agent to base agent were studies here and contact angles measured were taken three times. Both left ad right angles were measured and the mean of all measured data was taken as the contact angle measured. All contact angle measurements were performed with a Rame-Hart Automated Dispensing System.

All PDMS substrates were made all under same condition and process. After curing of PDMS was done. They were washed with hot water and immediately afterwards autoclaved to remove all other particles from the surface and also to provide the same conditions and environments as we follow later on to do cell culturing.

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Looking at the results obtained in Table 4.1, we observed that as the plasma treatment time increase from 5min towards 30min, contact angle for a given ratio of base to curing agent increases. Moreover, plasma treatment of PDMS surface reduces contact angle comparing to bare PDMS surface.

Base : Curing Agent Ratio	10:01	5:01	20:01
Bare	106.3167	108.8167	110
5m	8.8667	30.5	36.1333
10m	15.3	39.75	42.1166
20m	28.4	49.1	53.7
30m	50.325	78.8833	82.1

Table 4.1: Contact angles for three ratios of base to curing agent and plasma treatment time
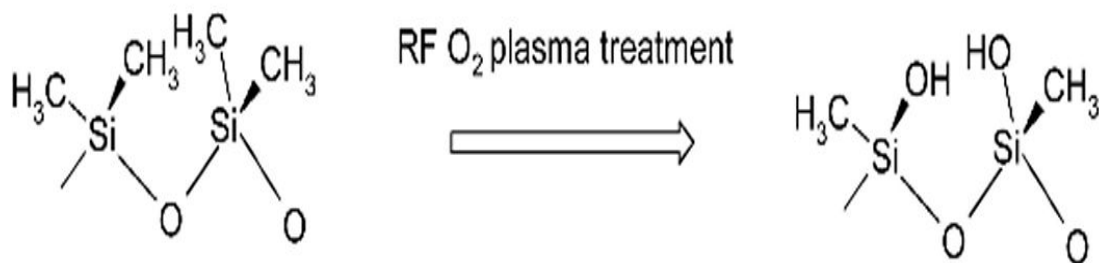
Figure 4.4 [20]: Possible change in surface functional group before and after plasma treatment
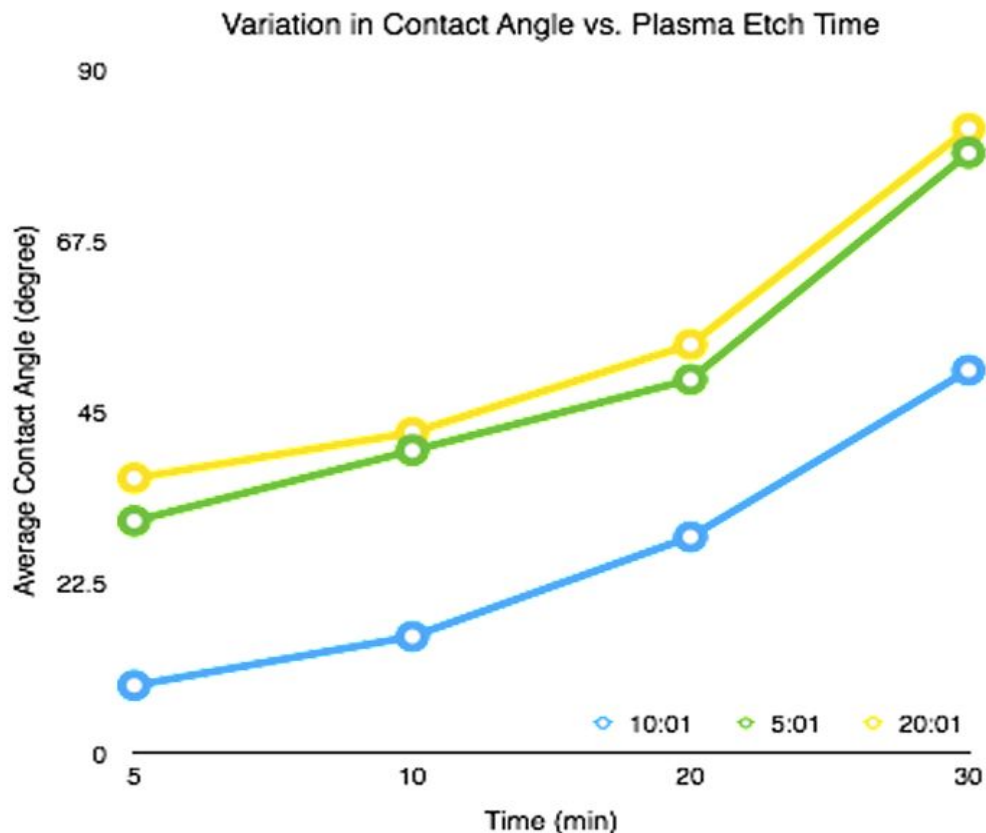
Figure 4.5: Contact angle vs. plasma treatment time

Furthermore, measured data shows that 10:1 ratio of base to curing agent has lower contact angles both in bare PDMS and plasma treated substrate compared to the other two ratios, 5:1 and 20:1. In addition, contact angles measured on 5:1 ratio of PDMS has lower but very close to the ones measured on 20:1 PDMS substrates.

Our analysis suggests that obtained data are in --- with theoretical understanding of changes in surface functional groups of PDMS surface before and after plasma treatment.
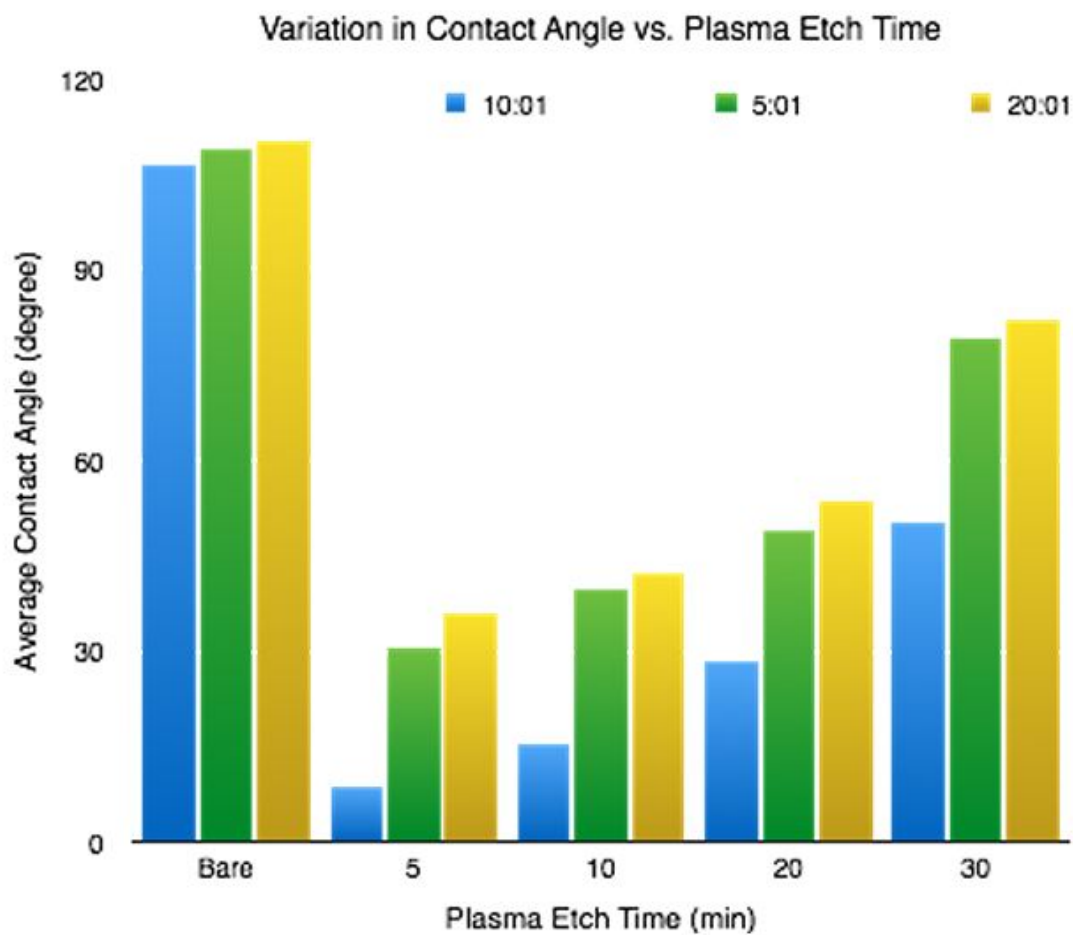
Figure 4.6: Variation of contact angles measured vs. plasma treatment time

3. 2. 2 FTIR Analysis

Fourier transforms infrared spectroscopy (FTIR) of PDMS surface was done to confirm mofication of surface functional groups after plasma treatment. Also, to further

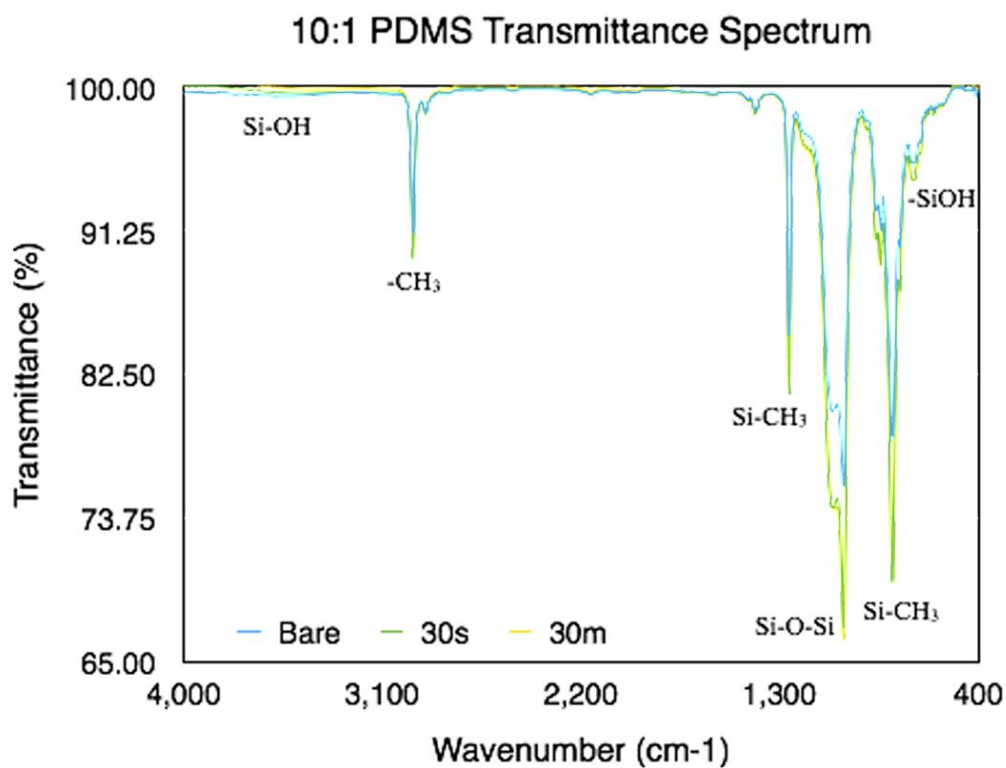study how these changes vary by changing ratio of curing agent.

10:1 PDMS Transmittance Spectrum

Figure 4.7: Transmittance spectrum of 10:1 PDMS

Based on the data that we have obtained we can conclude that bare PDMS has lower peaks comparing to plasma treated substrates. Furthermore, as we increase the plasma etch time we can see that that peaks tends to get higher and absorption increases.
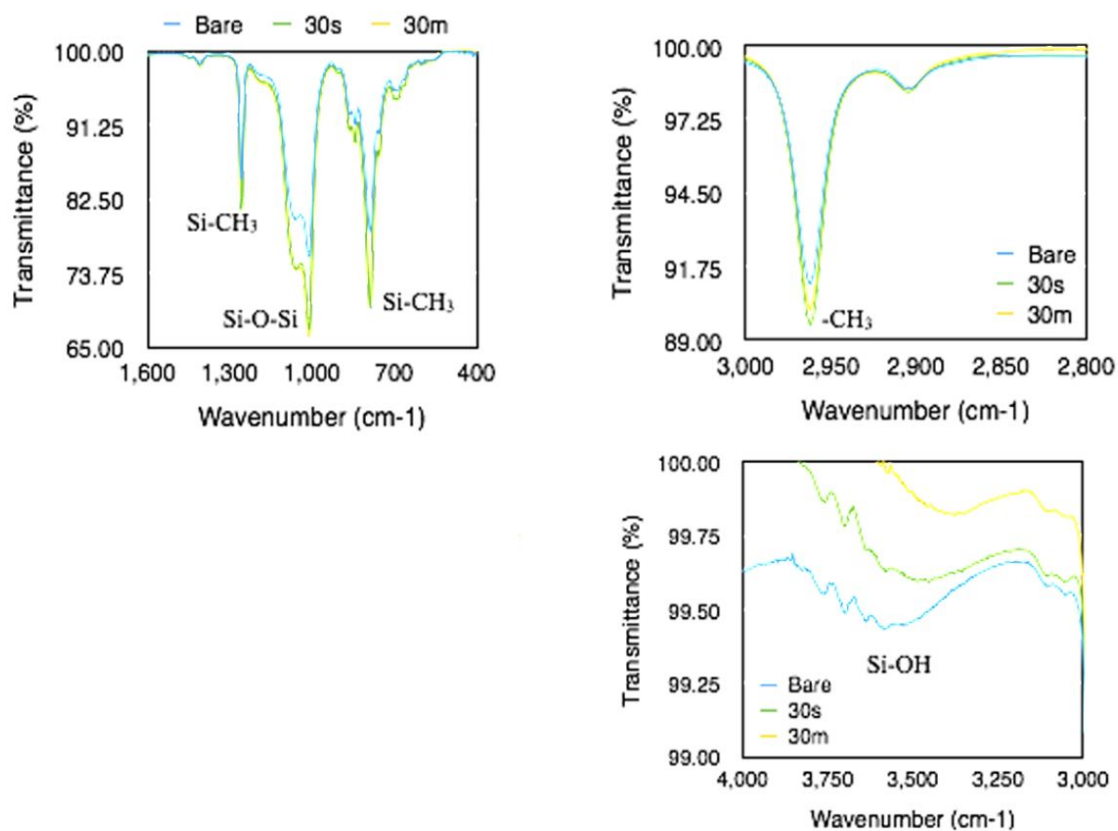
Figure 4.8: Closed up look at the peaks of 10:1 PDMS transimttance spectrum

Figure 4.1 shows close look at peaks that occurring in various wavenumbers. The peak that occurs at 790 cm^{-1} is known to be the Si-CH bonds. This bond is the combination of CH rocking and Si-C stretching bond. Another important peak occurs at 1000 to 1050 cm^{-1} that corresponds to Si-O-Si stretching bond. The peak that occurs at 1300 cm^{-1} referred to as Si-CH3 deformation bond.

Peaks occurring at 2950 to 2960 cm^{-1} are pointing to the asymmetric CH$_3$ stretching in Si-CH$_3$ bonds that are existed at the surface of PDMS substrates. Moreover, we can observe a small amplitude peak of Si-OH stretching at wavenumbers ranging form $\sim$ 3100 to 3500 cm^{-1}.
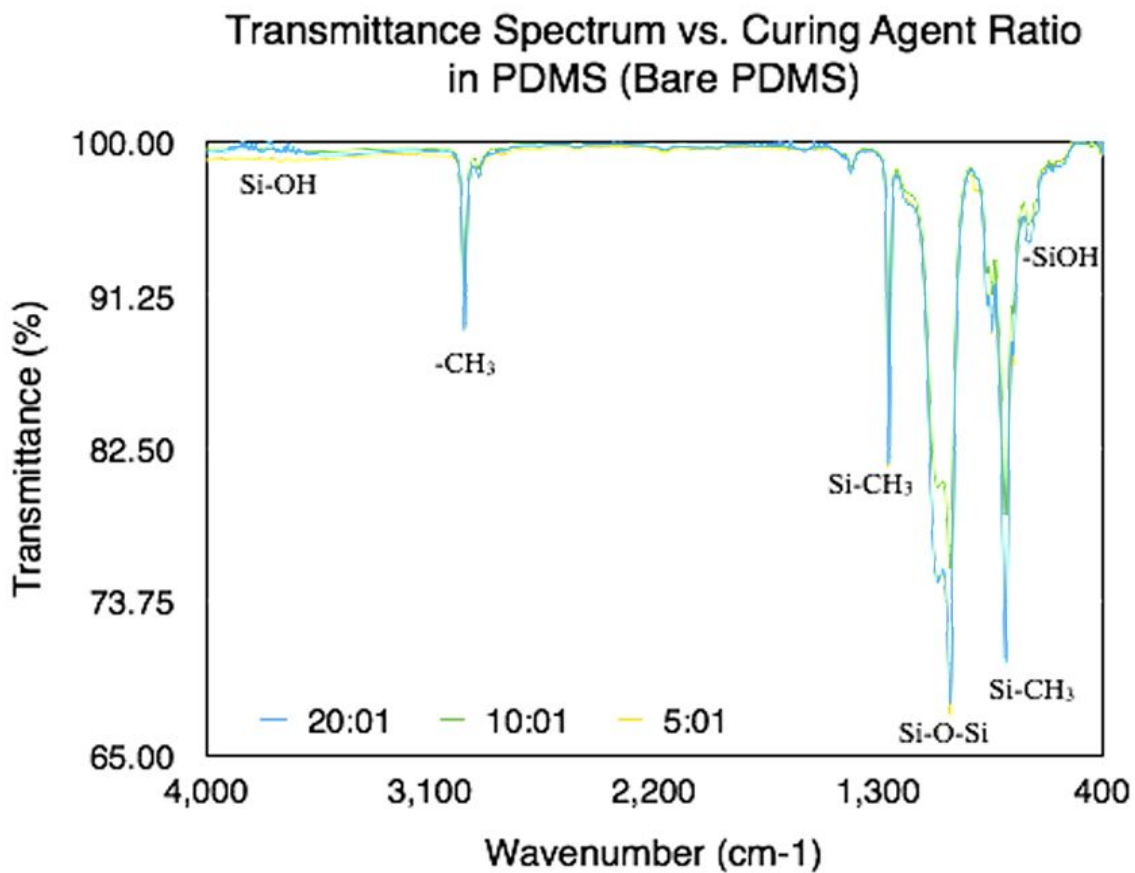

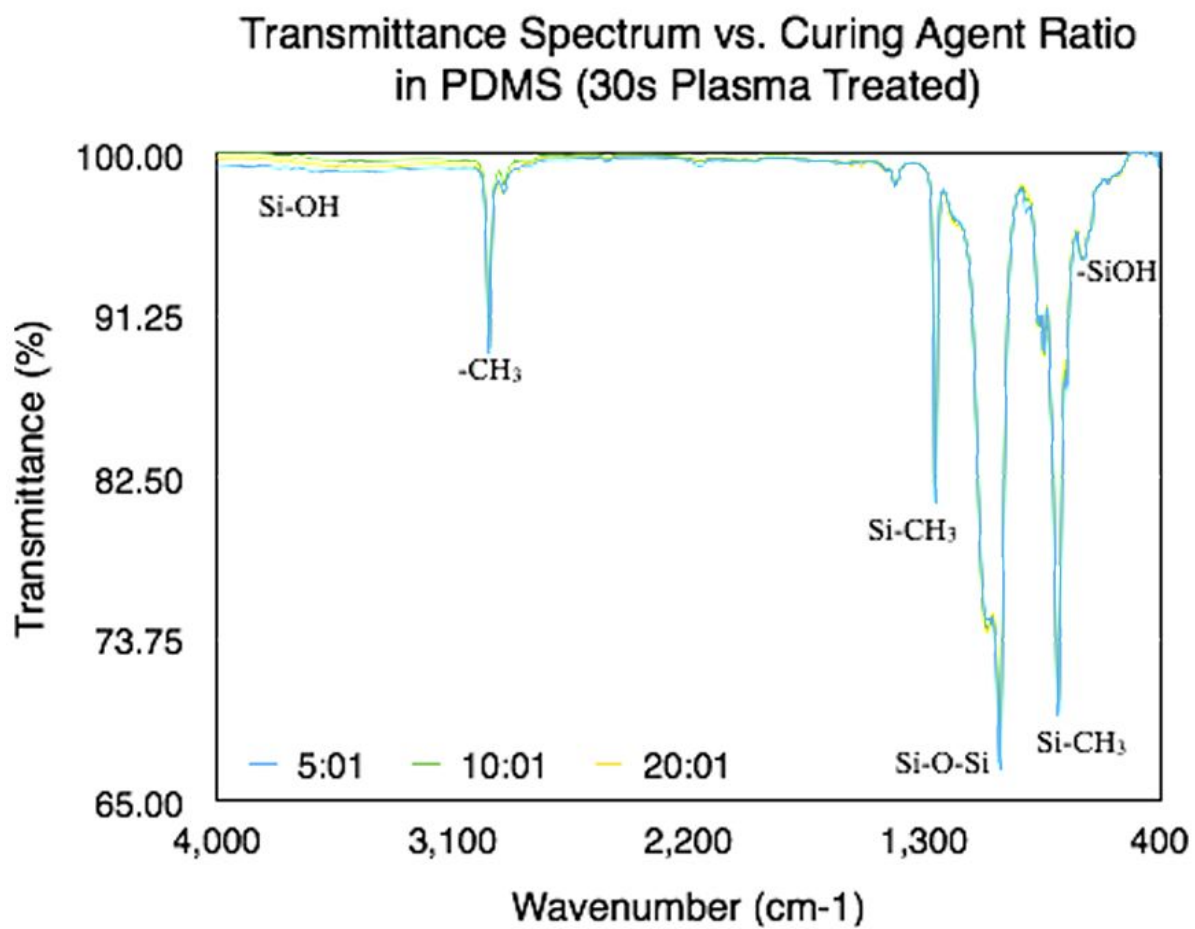**Figure 4.9: Bare PDMS transmittance spectrum for different curing agent ratio**

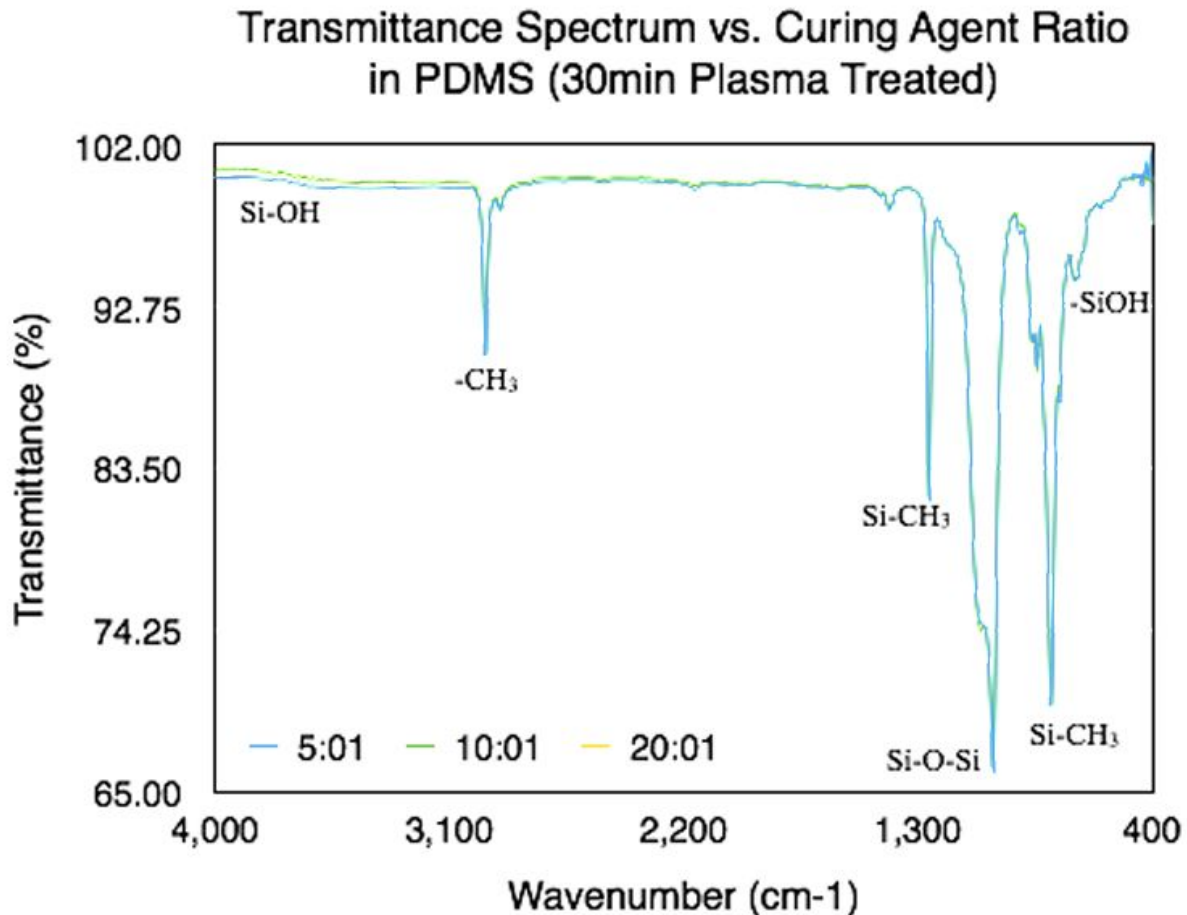**Figure 4.10: 30 sec plasma treated PDMS transmittance spectrum for different curing agent ratio**

Transmittance Spectrum vs. Curing Agent Ratio in PDMS (30min Plasma Treated)

Figure 4.11: 30min plasma treated PDMS transmittance spectrum for different curing agent ratio

3. 2. 3   Topography of PDMS

Atomic Force Microscopy (AFM) imaging was completed with an Agilent and PicoView imaging system cantilevers with a resonant frequency of 10 Hz and a nominal force constant of 0.06 N/ m. The topography images were taken in contact mode for both

bare PDMS and plasma etched PDMS surface. 3D images were obtained using Gwyddion 2.20 analysis software. As it can be seen from Figure 4.12 topography of surface after only 30 seconds of plasma treatment using RF plasma etcher at 200 RF power.
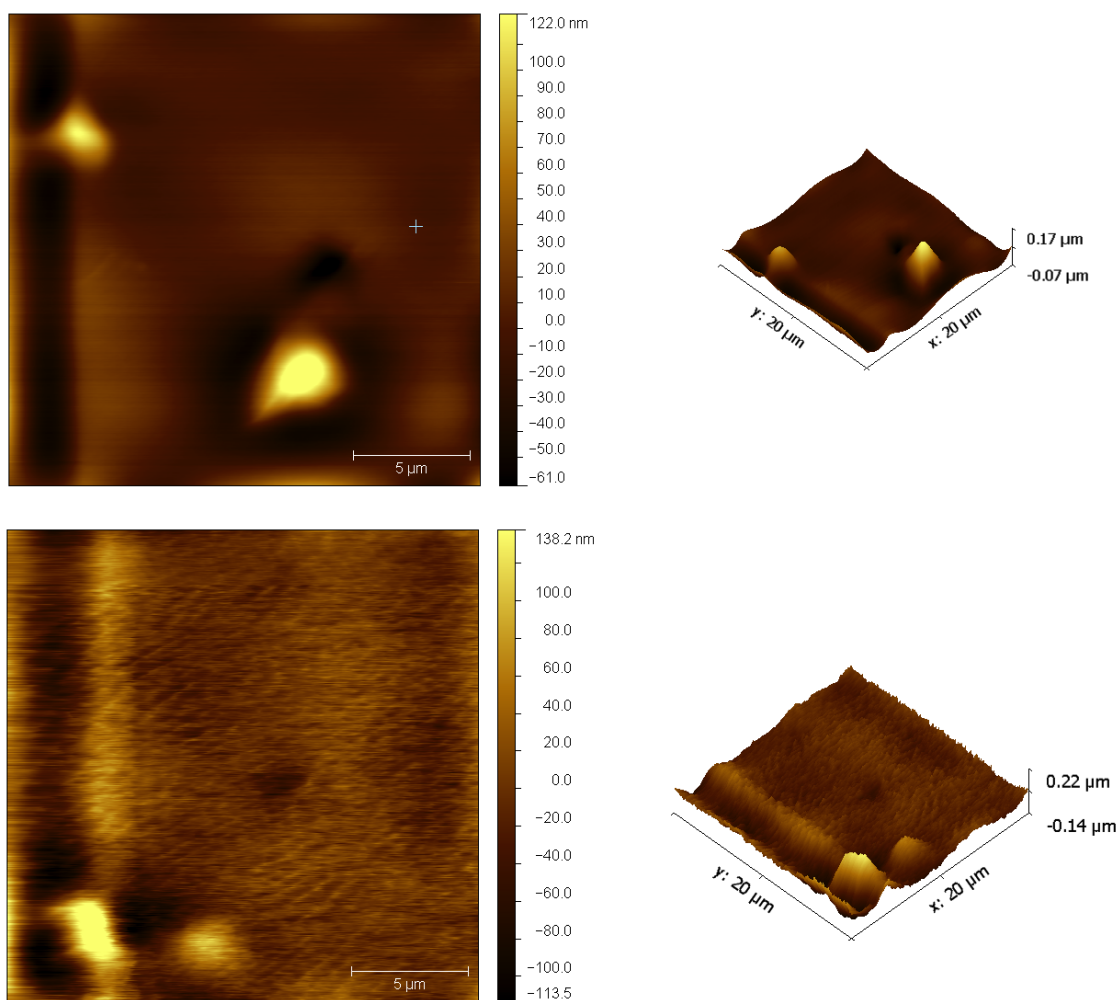

Figure 4.12: Topography image of PDMS surface before and after plasma treatment. Top) bare, Bottom) Plasma treated PDMS

4. 4 Cell Culturing and Migration

Initially, cell culturing over flat surface PDMS substrate was studied and investigated. In Figure 4.13 PDMS substrates were made in glass petri dish. They were washed with hot water followed by autoclaving. These substrates were plasma treated for 30min. Figure a shows bare PDMS, b is the collagen coated PDMS surface and c is the collagen coated. Our observation shows that 30min plasma treatment causes mechanical changes in the surface of PDMS and probably changes surface chemistry by turning the PDMS into glass like substrates as we see cracks in the substrates.
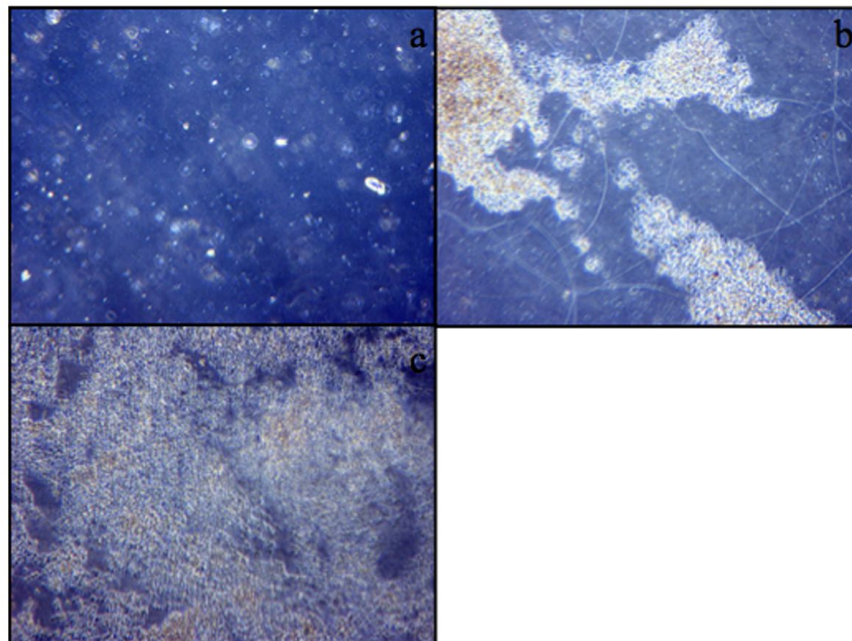

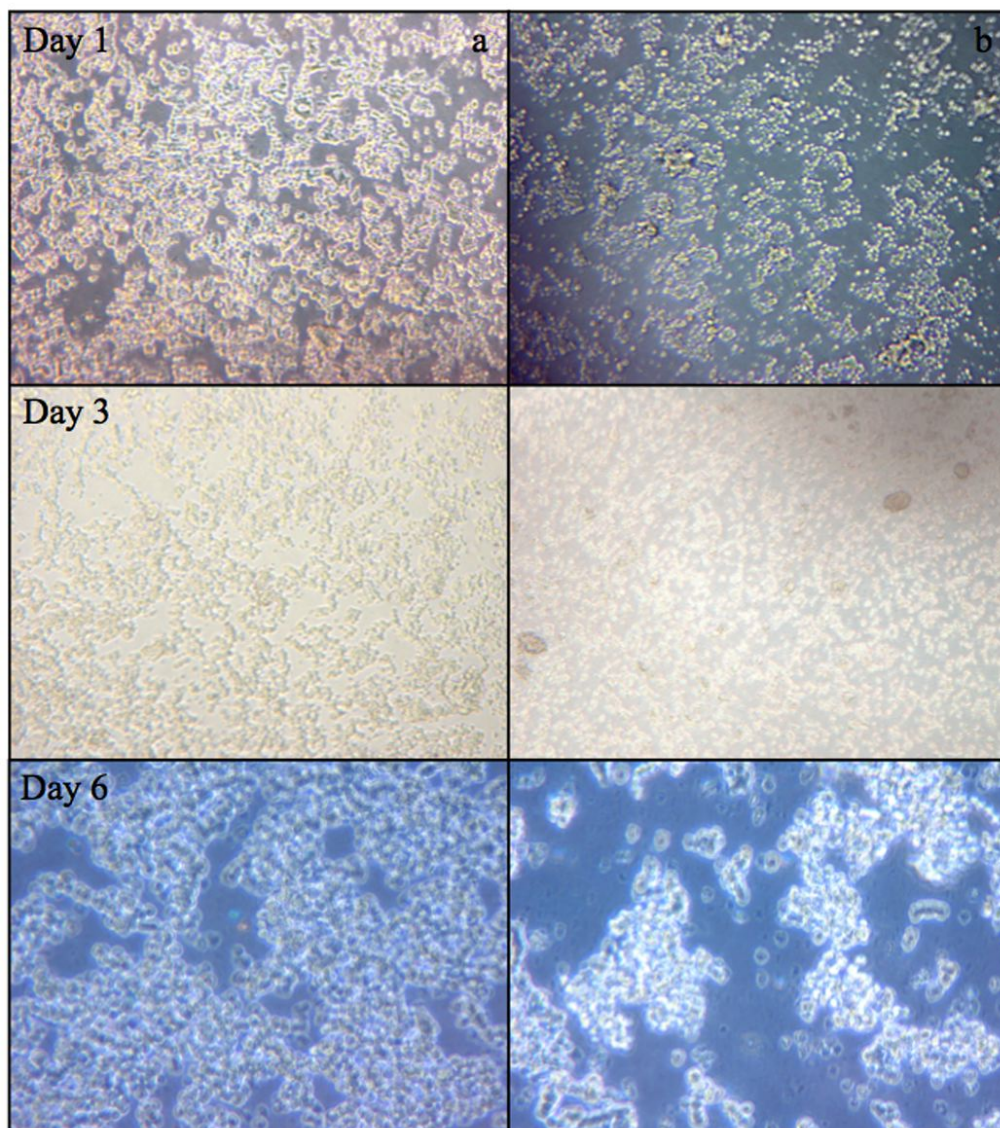**Figure 4.13: 30min plasma treated PDMS substrate a) bare b) collagen c) PDL treated PDMS**

Figure 4.14: Variiton of PDL concentration a) 0.1 ug/mL PDL b) 0.4 ug/mL PDL

After optimizing the plasma treatment time and finding the most suitable time that could hydrophilize the surface without changing surface chemistry, we coated our

substrates with PDL to provide a proper mechanism for the cells adhesion and growth. As it can be seen from Figure 4.14 increasing PDL concentrations may enhance the cells adhesion however in our studies we decided to use 0.1ug/mL PDL as it provide good enough platform for cell adhesion and growth. Additionally, it is more cost effective.
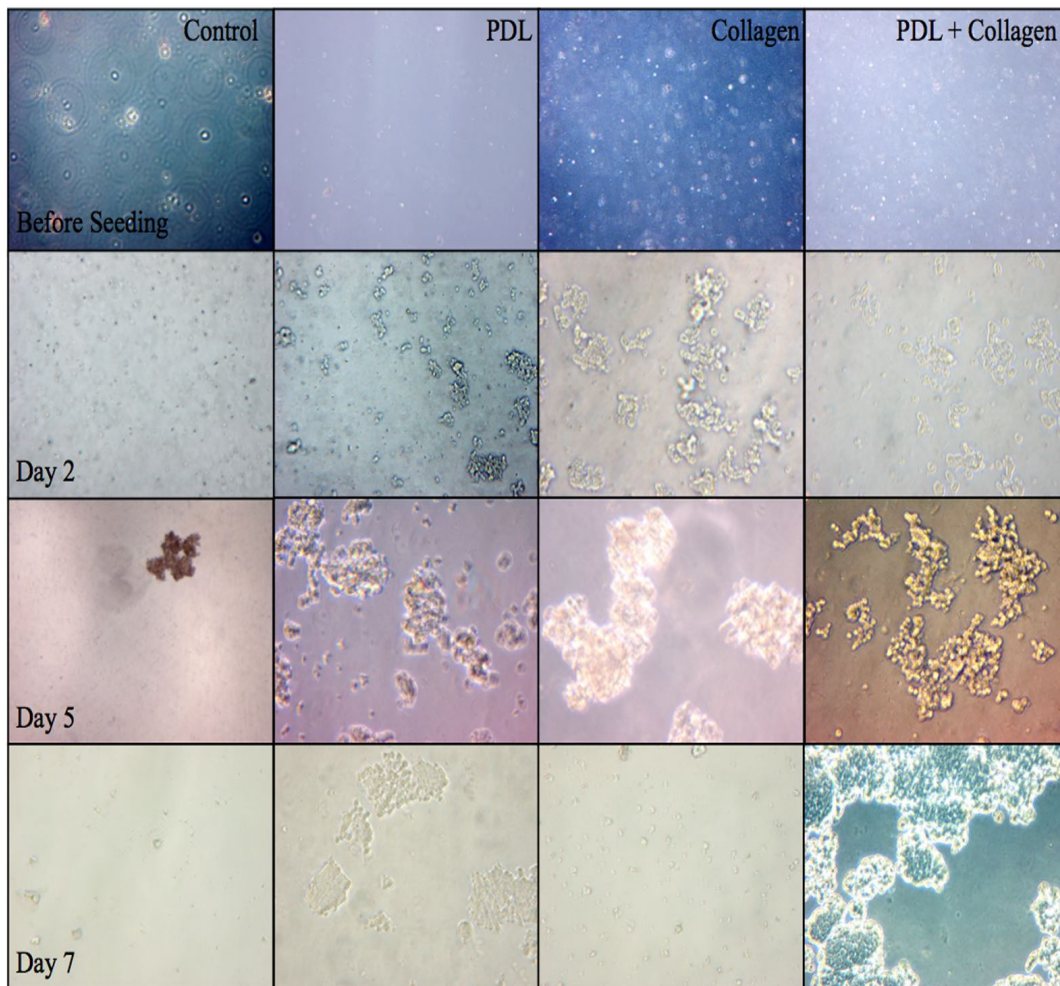

Figure 4.15: Cell culturing on a) PDL b) Collagen c) PDL coated with collagen

In this experiment, we are using same concentration for the same material and comparing adhesion of cells using collagen versus PDL coating. These two materials are the most commonly used for adhesion of cells to the surface. Aim of this experiment to choose the material that can provide the better adhesion hence we are comparing cell adhesion, viability and growth on PDL versus Collagen versus PDL coated collagen PDMS surface. Samples were plasma etch and followed by the immediate coating of surfaces with collagen and PDL and incubated over night. Then, cells suspension was added to petri dishes and incubated in the incubator. Cells are been fed every other day. As it can be seen form the obtained optical microscope images, cells are adhering vey well to both PDL and PDL coated collagen substrates.
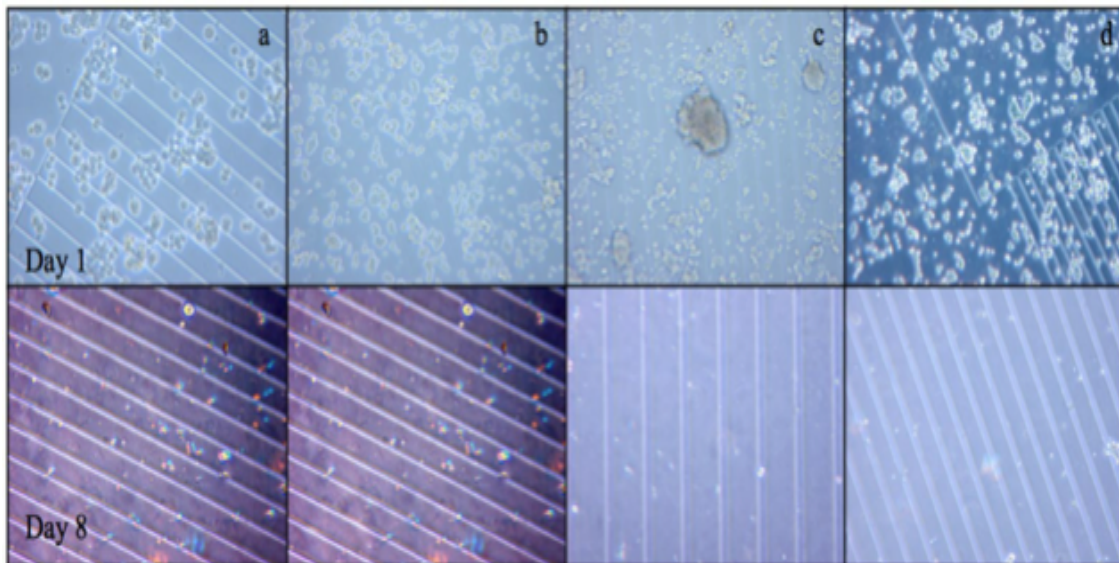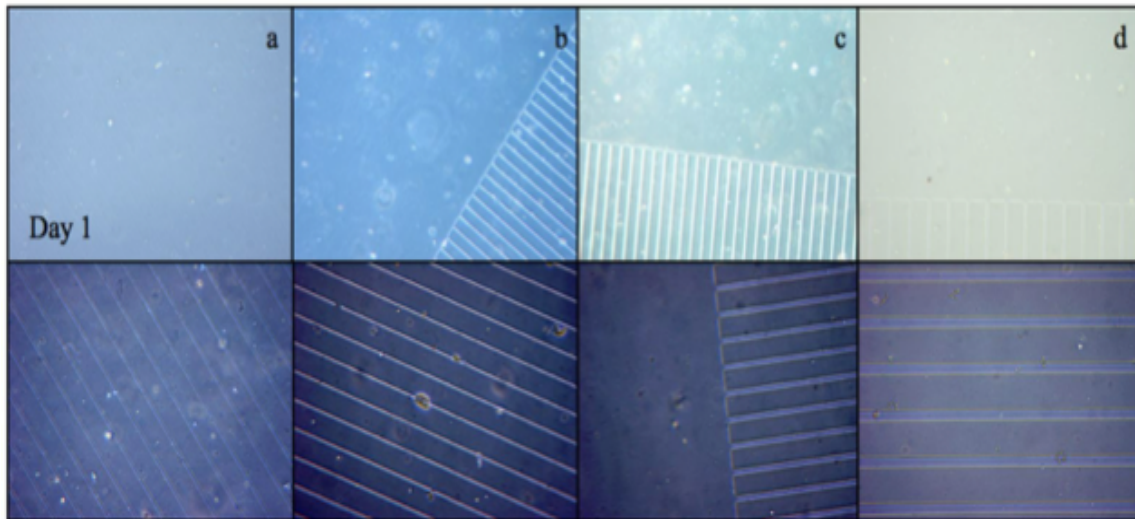

Figure 4.16: Full suspension

Figure 4.17: Pipeting cells on seeding area a) 2um b) 5um c) 10um d) 15um

Figure 4.18 shows the results obtained from culturing cells using inkjet printing. All the substrates were washed with hot water, autoclave and then plasma treated for 30sec via plasma etcher. Immediately, after plasma treatment they were PDL coated and kept overnight. After 24h incubation period, cells were seeded at the designated seeding area. After seeding the cells and media we realize that although some of the cells adhere well to the seeding regions, majority move to other region of channels.

Hence, we had to find other methods to make sure cells are only seeded at the seeding area and they grow towards the cannels opening or adhere at the beginning of channels and start growing towards the other end of the channels. Figure 4.19 demonstrates that to tackle this issue we attached cylindrical wall on the seeding area and coated all other regions with PDL except the seeding area.
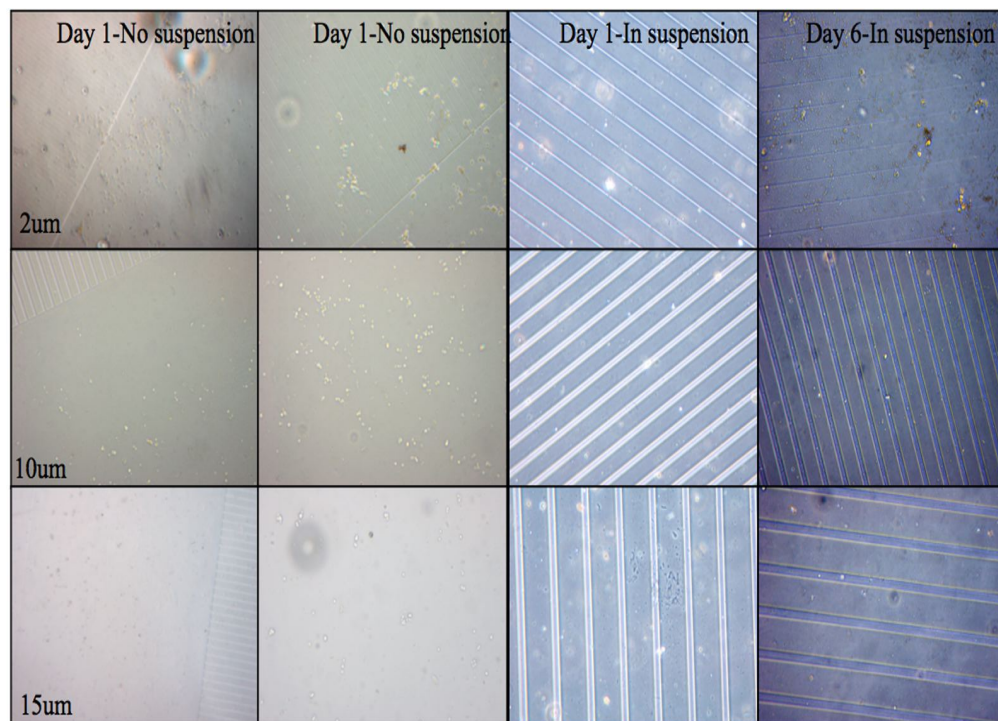
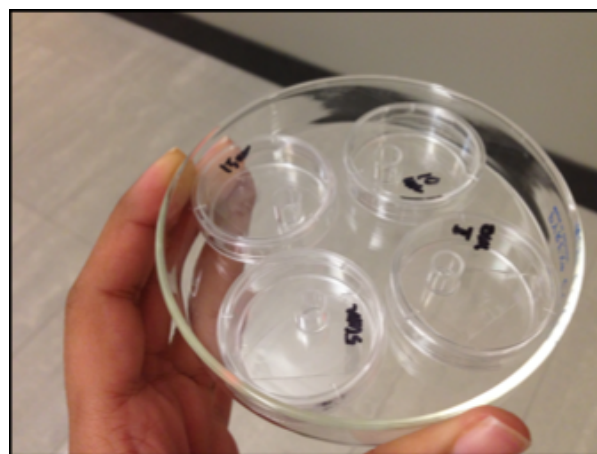**Figure 4.18: Inkjet printing of 2um, 5um and 15um**

Figure 4.19: Cyliner wall attached on the seeding areas to aid cell seeding step

CHAPTER 5:

CONCLUSION AND FUTURE RESEARCH

Performing plasma treatment on PDMS surface has shown that increasing plasma treatment time increase surface contact angle, meaning that as the plasma etch time increases surface hydrophilicity doesn't improve. This is in agreement with the theoretical understanding of surface modified PDMS. Also our studies on various curing agent ration suggested that 10:1 ratio PDMS substrates have the lowest contact angles in other words it is more hydrophilic comparing to other ratios under studies. Additional studies using atomic force microscope (AFM) confirmed that only 30sec of plasma oxidation cause topographical change in surface properties and hence in hydrophobicity of bare PDMS surface. Contact angle measured on 30sec plasma treated PDMS substrate was approximately zero hence; all of the experiments performed were treated for 30sec with plasma etcher.

Fabrication of soft micro-channels using SU-8 resist provides a better support for cell culturing and growth within the channels. However, SU-8 mold fabrication seems to be challenging. Additionally, fabrication of channels using positive resist S-1818 has its own challenging especially when it comes to cell culturing and seeding cells at the designated area for seeding.

In third phase of this project we did a study to find the best protocol that could help and promote adherence of PC-12 cell line on flat PDMS surface. Results of our

studies on collagen, PDL and PDL plus collagen coated PDMS substrates suggests that of Cell culturing on PDL and PDL coated with collagen provides a better viable environments for cell adherence and growth.

There are many various aspects to research in this field that can be further studied and investigated. Among possible improvements are designing a mask suitable for negative resist so we can easily obtain the channel depth desire and to eliminates or minimize the errors added up in fabrication process. To further study and research effect of micro-channels dimension on axonal or neurite outgrowth and Schwann cell migration. Studies had shown that certain micro and nano structure on the surface or in the grooves could help to enhance cell growth and migrations. One of the major studies that we suggest to do is to investigate effect of surface morphology in cell adhesion, growth and migration.

Moreover, extracellular excitation of neuron using gold particles or other materials can be utilized to excite neurons and provides potential gradient for neurons regrowth and migration.

Lastly, these fabricated 2D soft micro-channels can be rolled up to form 3D high-density channels for neuron migration and studies.

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