SURFACE PATTERNING OF BIODEGRADABLE POLYMER IN TISSUE ENGINEERING BY APPLYING PHOTOLITHOGRAPHY

by

LEE-CHUN SU

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN MATERIAL SCIENCE AND ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

December 2007

ACKNOWLEDGEMENTS

The author would like to express her sincere appreciation to her major professor Dr. E. I. Meletis for his guidance and encouragement throughout this work. She would also like to thank Dr. J. Yang for many valuable suggestions and thank Dr. W. Hao for his participation in this research work as members of her examination committee.

She wishes to thank Dennis S. Bueno, Johnny Johnson, and Mark Cheng for their advice and assistance during the experiment of silicon processing in NanoFab at UT Arlington and MER at UT Austin.

She also would like to thank Dr. Lee for his effort on the mask design at ARRI.

She would like to acknowledge Jagannath Dey, Pankaj Jyoti Hazarika, and express her gratitude for their cooperation, advice, and friendship.

The author also wishes to express her appreciation of support and encouragement she received from Steven Hsu and family.

November 12 2007

ABSTRACT

SURFACE PATTERNING OF BIODEGRADABLE POLYMER IN TISSUE ENGINEERING BY APPLYING PHOTOLITHOGRAPHY

Publication No. _____

Lee-Chun Su, M.S.

The University of Texas at Arlington, 2007

Supervising Professor: Efstathios I. Meletis

The prevalence of cardiovascular disease and inherent problems with current surgical therapies has prompted major efforts in the field of vascular tissue engineering. While there have been significant advances towards creating functional blood vessel substitutes, the fabrication of a vascular graft with mechanical, structural and functional properties similar to native vessels has not yet been achieved.

We have presented an observation of a new biodegradable polymer utilized on the research of tissue engineering. Our idea was to create a structure to imitate blood vessel muscles in vitro and also to guide the cells when they start spreading and trying to connect with each other. Crosslinked urethano containing polyester (CUPE) is a

newly invented biodegradable polymer. It has good mechanical properties and biocompliance. The polymers were imprinted with two shapes of channels, namely a square shaped channel and a triangular channel, from silicon moulds, which were fabricated by two different etching methods. The imprinted polymers were then used as cell culture substrates. The result shows that cells were affected by the structure of the environment. As per the hypothesis, it is expected that the cells will be aligned in the channels as they proliferate. This was exemplified for the polymer sample patterned with triangle-shape walls since there is no space for cells to attach and grow on top of the wall. However, cells will eventually over accrete across the walls and cover the whole polymer surface.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

Table Page

CHAPTER 1

INTRODUCTION

Every year, millions of surgical procedures are performed in the United States for people who are suffering from cardiovascular disease. Organ transplantation and tissue reconstruction have successfully helped lots of patients, but only a fraction of those who could benefit from these therapies are treated due to a lack of tissue suitable for transplantation [1]. In most cases, saphenous vein grafts result in donor site morbidity and preclude the use of a transplant procedure for any subsequent coronary artery problems. Fortunately, as polymers were developed and used in the textile industry, many of these were tested empirically in man as vascular grafts, the first use being that of Vinyon-N by Voorhees et al. [2].

Large-diameter blood vessels have been successfully replaced with non-degradable polymeric materials such as Dacron and expanded polytetrafluorotethylene (ePTFE). In 1906, the first graft inserted to replace an excised segment of artery was performed, when Goyanes used the popliteal vein in situ to restore the continuity of the artery after excision of an aneurysm of the popliteal artery [3]. Large vessel grafting began with the work of Oudot [4] and Dubost [5] in the early 1950s with the use of harvested human aortas (homografts) for aortoiliac occlusive disease and abdominal aortic aneurysm. These were preserved subsequently in a variety of fashions from fresh antibiotic sterilization to freeze-drying. These grafts salvaged lives and limbs;

unfortunately, these materials are not applicable to small-diameter blood vessels (SDBVs). Poor patency is problematic due in part to incomplete endothelialization, thrombosis, and myointimal hyperplasia, particularly at the distal anastomosis [6].

Based on previous research, it is suggested that the compliance of the graft should be similar to that of the blood vessel to be replaced, as both an undercompliant and overcompliant graft may be detrimental for biomechanical adaptation [7, 8, 9]. To date, no graft has successfully achieved long-term patency when used for small-diameter blood vessel (SDBV) applications. Therefore, the development of SDBV grafts has been an area of intense research focus.

A tissue engineered approach to solving the problem of developing a suitable SDBV has been investigated for decades. It involves the isolation of the different cells, which make up the SDBV from the individual requiring a transplant and expansion of these cells in vitro. These cells can be arranged in the order that they grew in, inside a native SDBV on a suitable construct that acts as a scaffold to promote the growth, proliferation and differentiation of the cells. The construct is usually a porous biodegradable polymeric scaffold. As the cells proliferate on the scaffold, their arrangement on the scaffold should resemble that of a SDBV; when sufficient degree of cell growth is achieved, the entire construct is transplanted into the body to function as a graft. Ideally, the scaffold material should be similar to a SDBV in terms of its mechanical properties, in addition to being biodegradable and biocompatible. Significant challenges to cell transplantation include the design and fabrication of a suitable biodegradable cell scaffold that can promote cell adhesion, support cell growth,

proliferation, and differentiation, and guide the process of tissue development. Transport issues are very important for a tissue engineering scaffold and include nutrient delivery, waste removal, exclusion of materials or cells, protein transport, and cell migration, which, in turn, are governed by the scaffold's porous structure [10].

Biodegradable polymers have been widely used for this application. Cell affinity is one of the most important factors to be concerned with when biodegradable polymeric materials are utilized as cell scaffold in tissue engineering [11, 12]. Commonly, cell affinity includes two important factors: cell attachment and cell growth. One of the most important parameters, which modulate cell affinity, is the surface property of the polymers. The nature of the surface plays an important role in determining the composition of the adsorbed protein layer, which in turn regulates how cells respond to the material. Many studies have proved that hydrophilicity/ hydrophobicity [13], surface energy [14], charge [15, 16] and roughness [17] of the material surface influence the cell attachment and cell growth on the material to various degrees.

Both thermoplastic and thermosetting polymeric materials have been studied in medical device applications, including polyesters (Dacron), polytetrafluoroethylene(PTFE), phenolics, epoxies, polycarbonates, and a number of polyurethanes including Biomer, surethane, Tecoflex, Avcothane, Pellethane, and Mitrathane [18].

The material used here to make the cell scaffold is crosslinked urethane-containing polyester (CUPE). Traditionally, polyurethanes have been shown to exhibit

very favorable strength and elastic properties very similar to that of a native SDBV. However, polyurethanes suffer from the disadvantages of creep, moderate biocompatibility and slow degradability. On the other hand, crosslinked polyesters do not suffer from creep, have controlled degradation rates which can be achieved by varying the monomers and degree of crosslinking and show excellent biocompatibility. Crosslinked polyesters, however, do not have sufficient elasticity and strength. CUPEs were introduced to integrate the advantages of both families of polymers, having both good elasticity and strength without undergoing creep, controlled degradation and excellent biocompatibility.

From a material engineering point of view, patterning approaches have greatly profited by combining micro-fabrication technologies, such as photolithography, with biochemical functionalization. The ability to control shape and spreading of attached cells and cell-cell contacts through the form and dimension of the cell-adhesive patches with high precision is important. Several imprinting methods had been applied such as microcontact printing (μCP), microfluidic pattering (μFLP), and plasma polyerization combined with photolithography or laser ablation, photoimmobilization and photo-chemically generated patterns, etc. Bernard et al. [19] used the microcontact printing technique for patterning substrates. They took polydimethyl siloxane (PDMS) as stamps and activated the surface by means of silanization and subsequently, covalently immobilized antibodies specific to the adhesion-molecule NgCAM on to the stamp surface. These modified stamps were used to extract the NgCAM molecules from tissue homogenates and the affinity-purified molecules were subsequently printed on to clean

surfaces. Another example is performed by Hoffman and coworkers. It is demonstrated that plasma lithography can be a valuable alternative to other techniques [20]. Polyethylene terephthalate substrates are first coated with a non-fouling plasma polymer of tetraglyme (tetraethylene glycol dimethyl ether). Standard photolithography procedure is then applied. The resulting surface composed of tetraglyme polymer patches surrounded by PR is then coated by a fluorocarbon (FC) plasma-deposited polymer. Subsequently, the PR is lifted off and the final surface is composed of hydrophobic cell-adhesive patches in a non-interacting hydrophilic background. Smooth muscle cells (SMC) are seeded on these surfaces initially without serum in order to avoid cell spreading on the tetraglyme background. For longer time culture of SMC on patterns, 10% CS is added to the media 24h after seeding. Cells are shown to maintain pattern fidelity for at least 14 days [20].

Cellular developments such as proliferation, differentiation, migration or apoptosis are guided by multiple surface cues that are potentially remodeled during cell culture assays. The cell-responses are controlled by intra-cellular signaling pathways that are originally triggered by transmembrane proteins interact with the engineered surface [21]. The surface chemistry characterized by the type of cell-binding ligands (peptides, proteins, etc), their surface density [22, 23] and spatial distribution [21, 24, 25] as well as their conformation [26], have been demonstrated to be important surface cues.

CHAPTER 2

OBJECTIVE

Ideally, the present work is aimed at finding a way to promote the endothelialization of synthetic grafts by modifying the scaffold surface that will be attached to the graft lumen. This is expected to improve the thromboresistance of the grafts. The method applied to create the surface morphology is the photolithography technique. Additionally, the newly synthetic polymer, CUPE, is used in the research as cell substrate. The interaction of cells and patterned CUPE films is going to be the first investigation of our work in this area.

More specifically, the objectives of this research are: (i) to create a silicon mould with precise patterns from the mask by utilizing the technique of photolithography; (ii) to successfully imprint the trenches on silicon wafer onto CUPE films and characterize the films with SEM; (iii) to grow cells on the film surface and study the effect of channels to cell behaviors of growth.

CHAPTER 3

BACKGROUND AND LITERATURE SURVEY

3.1 Lithography Techniques

Didier et al. [27] have reviewed of surface engineering approaches to micropattern surfaces for cell-based assays. According to many researchers' efforts, it is proved that, in vitro, the interactions of anchorage-dependent cells with their environment are important. Cellular developments such as proliferation, differentiation, migration or apoptosis are guided by multiple surface cues that are potentially remodeled during cell culture assays. In order to be viable, anchorage-dependent cells require an adhesive surface to exert forces and consequently spread. The ability to constrain the spreading to a specific cell-surface contact area has been shown to dramatically affect cellular development [28, 29, 30].

Apart from its use in fundamental cell-surface investigations, arrays of living cells have found major applications in both cell-based sensors and drug discovery. Cell-based sensor devices contain living cells that monitor perturbations of the environment [31, 32, 33]. For instant, when dealing with wastewater processing, a facing issue is several different classes of chemicals, including electrophilic toxins, feeding into water treatment plants have been known to cause bioreactor degradation leading to system processing failures. Nancy Love et al. [34] have designed a cell-based microfluidic biosensor to detect the presence of electrophilic toxins in water streams entering

wastewater treatment plants in an effort to prevent failure of the bioreactor.

3.1.1 Patterning with Photolithography

In the photolithography process, geometric features drawn on a mask are transferred via UV illumination onto a substrate. A mask is generally made of a quartz (glass) plate coated with a thin layer of non-transparent chromium, and presents the desired geometric features. The design of the mask can be created with any computer-aided design (CAD) software. Such quartz chromium masks routinely allow feature resolution down to 1-2 μm. Before transferring the patterns from the mask onto the substrate one needs to spin coat the wafer with a thin layer of UV-sensitive polymer, named photoresist (PR). After spin coating, the PR-coated wafer is placed on a hot plate for baking to harden the PR by solvent degassing. And then, the wafer is brought in close with the mask (or in some cases a gap between the two surfaces is preferred), in a mask-aligner device. After the UV radiation, if a positive PR is used, the irradiated regions become soluble and are removed in the subsequent development process. On the other hand, a negative PR becomes insoluble when irradiated locally. Because of the development process, the surface of PR film is composed of patterns with “windows” providing access to the substrate (wafer) and a background protected with PR. As a final step, the PR is simply removed by dissolution in an organic solvent with sonication (“lift-off” process) [24, 35].

3.2 Process of Photolithography

Lithography is the process of defining the regions or patterns on the wafer where material is to be deposited or removed, or where dopants are to be introduced. One important aspect of lithography is photoresist processing, which is the process of covering areas that either need to be subsequently removed or retained with a light sensitive film known as photoresist. The process of material removal following a photolithographic process is known as etching.

Photoresist layers have two basic functions: 1) precise pattern formation; and 2) protection of the substrate from chemical attack during the etch process. Typical resists consist of three components: 1) the resin, which serves as the binder of the film; 2) the inhibitor or sensitizer, which is the photoactive ingredient; and 3) the solvent, which keeps the resist in liquid state until it is processed. The pattern formed by the resist layer actually results only after the uniformly distributed resist is removed, as explained in the following discussion on photoresist processing.

Photoresist processing, or simply resist processing, basically consists of six steps: 1) dehydration and priming; 2) resist coating; 3) soft baking; 4) exposure; 5) hard baking; and 6) development. Figure 1 briefly shows the process steps involved in photolithography.

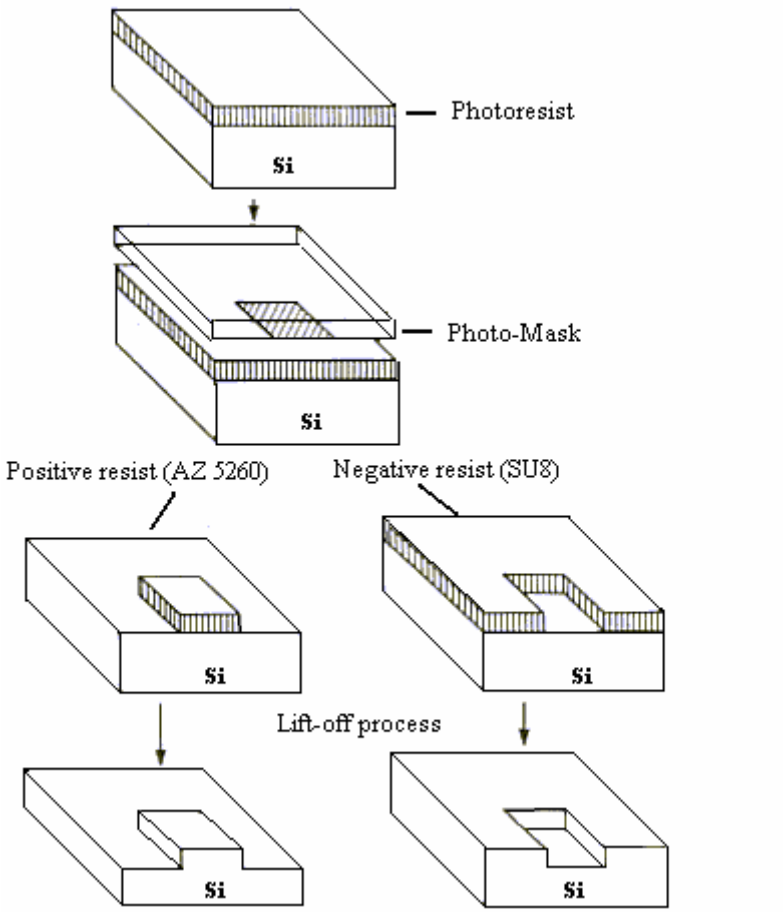


Figure 1 Exposure and development of negative and positive photoresists and the resulting etched film patterns

Prior to the application of resist to a wafer, the wafer must be free of moisture and contaminants, both of which cause a multitude of resist processing problems. Dehydration baking is performed to eliminate any moisture adsorbed by substrate surfaces, since hydrated substrates result in adhesion failures. The bake is usually performed from 400 °C to 800 °C. Convection ovens may be used for baking up to 400

°C, while furnace tubes are used for 800 °C baking. After dehydration baking, the wafer is coated with a pre-resist priming layer designed to enhance the adhesion properties of the wafer even further. One of the most common primers used for this purpose is hexamethyldisilazane (HMDS). Resist coating must follow as soon as possible after priming (within an hour after priming).

Resist coating, or the process itself of producing a uniform, adherent, and defect-free resist film of correct thickness over the wafer, is usually performed by spin-coating. Spin-coating consists of dispensing the resist solution over the wafer surface and rapidly spinning the wafer until it becomes dry. Most spin-coating processes are conducted at final spin speeds of 3000-7000 rpm for duration of 20-30 seconds.

Resist coating is followed by a soft bake, which is done to: 1) drive away the solvent from the spun-on resist; 2) improve the adhesion of the resist to the wafer; and 3) anneal the shear stresses introduced during the spin-coating. Soft baking may be performed using one of several types of ovens (e.g., convection, IR, hot plate). Soft-bake ovens must provide well-controlled and uniformly distributed temperatures and a bake environment that possesses a high degree of cleanliness. The recommended temperature range for soft baking is between 90 °C and100 °C, while the exposure time needs to be established based on the heating method used and the resulting properties of the soft-baked resist.

After a wafer has been coated with photoresist and subjected to soft baking, it has to undergo exposure to some form of radiation that will produce the pattern image on the resist. The pattern is formed on the wafer using a mask, which defines which

areas of the resist surface will be exposed to radiation and those that will be covered. The chemical properties of the resist regions struck by radiation change in a manner which depends on the type of resist used. Irradiated regions of positive photoresists will become more soluble in the developer, so positive resists form a positive image of the mask on the wafer. Negative resists form a negative image of the mask on the wafer because the exposed regions become less soluble in the developer.

Development, which is the process step that follows resist exposure, is done to leave behind the correct resist pattern on the wafer which will serve as the physical mask that covers areas on the wafer that need to be protected from chemical attack during subsequent etching, implantation, lift-off, and the like. The development process involves chemical reactions wherein unprotected parts of the resist get dissolved in the developer. A good development process has a short duration (less than a minute), results in minimum pattern distortion or swelling, keeps the original film thickness of protected areas intact, and recreates the intended pattern faithfully.

Development is carried out either by immersion developing, spray developing, or puddle developing. Regardless of method used, it should always be followed by rinsing and drying to ensure that the development action will not continue after the developer has been removed from the wafer surface.

Post-development inspection, as the name implies, is an inspection conducted after development to ensure that the resist processing steps conducted earlier have produced the desired results. This is typically done using an optical microscope, although SEM and laser-based systems are also used in some post-development

inspection tasks. Items that this inspection step checks for include the following: 1) use of the correct mask; 2) resist film quality; 3) adequate image definition; 4) dimensions of critical features; 5) defects and their densities; and 6) pattern registration [36].

3.3 Fabrication – Etching

In wafer fabrication, etching refers to a process by which material is removed from the wafer, i.e., either from the silicon substrate itself or from any film or layer of material on the wafer. There are two major types of etching: wet etching and dry etching.

Table 1 Brief comparison between two types of etching

	Wet	Dry
Method	Chemical Solutions	Ion Bombardment or Chemical Reactive
Environment and Equipment	Atmosphere, Bath	Vacuum Chamber
Advantage	1) Low cost, easy to implement 2) High etching rate 3) Good selectivity for most materials	1) Capable of defining small feature size (< 100 nm)
Disadvantage	1) Inadequate for defining feature size < 1um 2) Potential of chemical handling hazards 3) Wafer contamination issues	1) High cost, hard to implement 2) low throughput 3) Poor selectivity 4) Potential radiation damage
Directionality	Isotropic (Except for etching Crystalline Materials)	Anisotropic

3.3.1 Dry Etching

Dry etching can be associated with the use of plasma. Briefly speaking, plasma can be seen as a group of highly ionized gas, so that the “clouds” has a neutral electric charge, but at a local scale, you can see electric charges. Gases are made of molecules with no linking force between them. A gas naturally has a small ionization rate, this means a small quantity of ionized molecules compared to the total quantity of molecules in a given volume. These ions are created and ions recombine very fast inside the gas.

There are several ways of producing plasma from a gas. In every case, it is a question of energy brought to the molecules. If molecules are excited with a high energy, the link between its components will break up, and ions will separate. In microtechnology, plasmas are made either by increasing temperature, or by using electromagnetic excitation. In dry etching the second method is used [40].

Plasma is considered as a fourth state of matter. As the ionization is all but natural for the molecules forming the gas, it is chemically highly reactive. "Ionized" refers to presence of one or more free electrons, which are not bound to an atom or molecule. The free electric charges make the plasma electrically conductive so that it responds strongly to electromagnetic fields.

3.3.2 Reactive Ion Etching

Typically, during RIE process there are several kinds of gas that are used depending on different etched material [39]:

Table 2 Gas used during RIE process

Matrial	**Gas**	**Etching Rate (Å/min)**	**Mask**	**Selectivity**
Si (a-Si)	1) CF_4 2) SF_6 3) $BCl_2 + Cl_2$	~500	Resist Metal (Cr, Ni, Al)	~20:1 ~40:1
SiO_2	1) $CHF_3 + O_2$ 2) $CF_4 + H_2$	~200	Resist Metal (Cr, Ni, Al)	~10:1 ~30:1
Sl_3N_4	1) $CF_4 + O_2$ (H_2) 2) CHF_3	~100	Resist Metal (Cr, Ni, Al)	~10:1 ~20:1
GaAs	1) Cl_2 2) $Cl_2 + BCl_3$	~200	Sl_3N_4 Metal (Cr, Ni)	~10:1 ~20:1
InP	1) CH_4/H_2	~200	Sl_3N_4 Metal (Cr, Ni, Al)	~40:1
Al	1) Cl_2 2) $BCl_3 + Cl_2$	~300	Resist Sl_3N_4	~10:1
Resist/ Polymer	1) O_2	~500	Sl_3N_4 Metal (Cr, Ni)	~50:1

In RIE, the chamber top electrode is connected to the ground and the wafer is placed on the excitation electrode. The condition makes the “products” accelerated in the direction of the wafer. This has consequences on the etching process, especially if a more anisotropic etching is required.

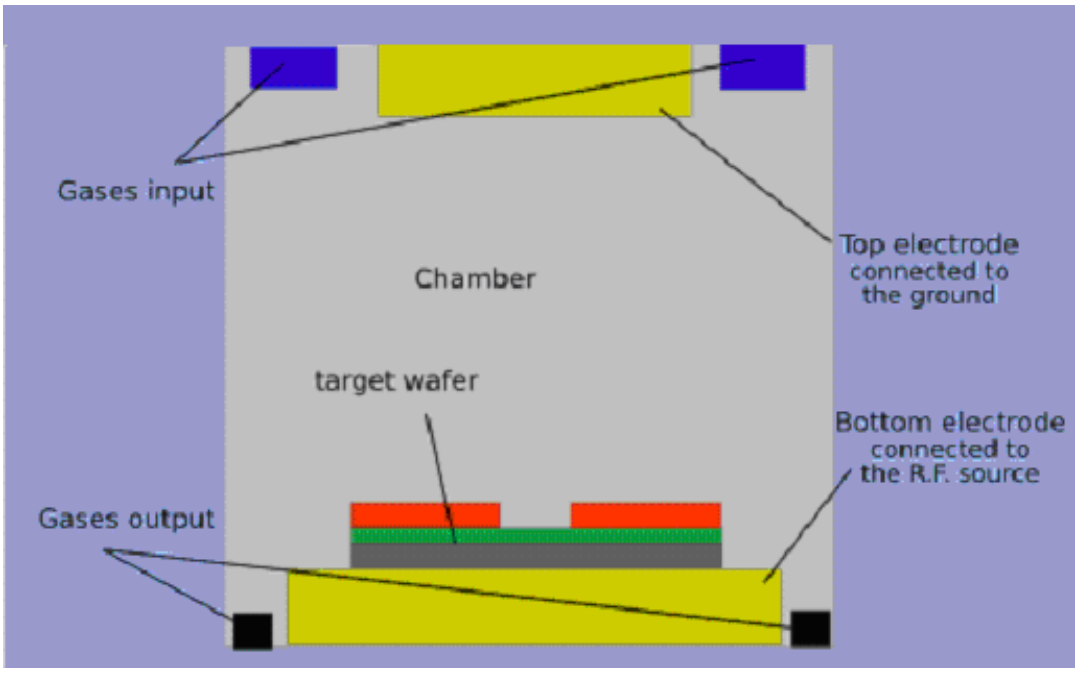


Figure 2 Schematics of RIE chamber

Since the wafer is connected to the R.F. signal, electrons are statistically more often in contact with the target than positive ions. Electrons are highly reactive species, so they are easily adsorbed by the target material and polarize it negatively. At the same time, the loss of electrons in the plasma makes a global positive charge in the ions "cloud". This produces an acceleration of the ions toward the wafer.

Figure 3 shows that RIE offers the possibility to set up anisotropic etching recipes [38]. This depends on the type of material to etch. If it has a poor capacity to adsorb electrons and polarize, RIE produces the same effects as PE (not much difference). On the contrary, a material with a high capacity to adsorb electrons allows the calibration of highly anisotropic recipes.

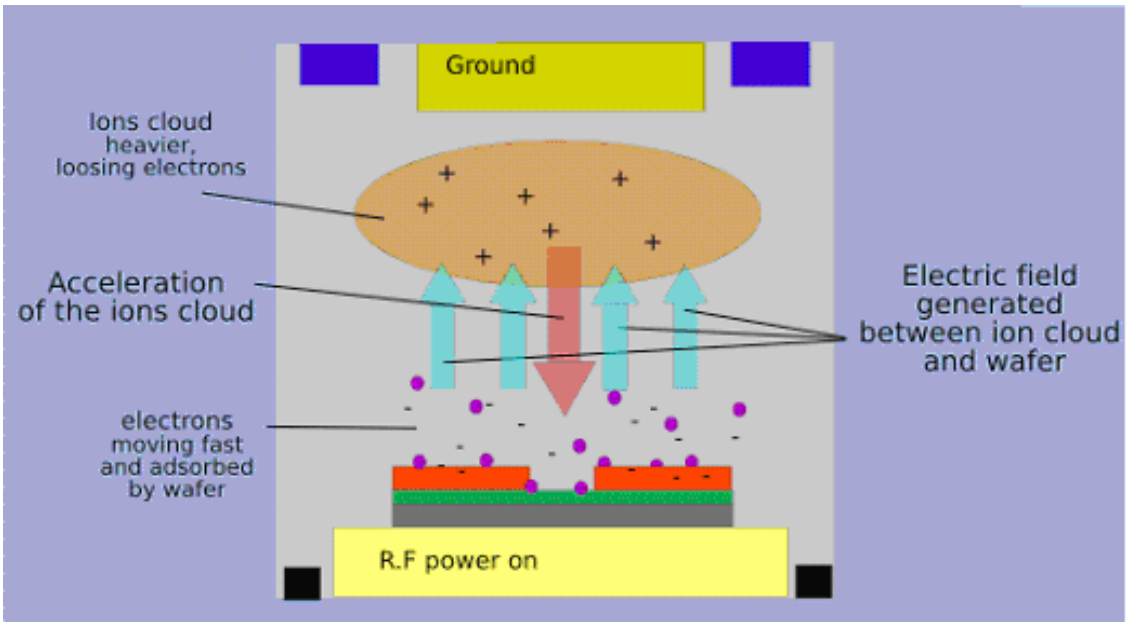


Figure 3 Schematics of the RIE effect

The following processes take place in the system during ion-enhanced etching [40]:

i. Active species generation: in RIE a glow discharge is used to generate from a suitable feed gas by electron-impact dissociation/ ionization the gas phase etching environment which consists pf radicals, positive and negative ions, electrons, and neutrals.

ii. Formation of a dc bias for ion acceleration: the material to be etched is placed on a high-frequency-driven (commonly 13.56 MHz) capacitatively coupled electrode. Since the electron mobility is much greater than the ion mobility, after ignition of the plasma the electrode acquires a negative charge. Therefore, the

electrode and material placed on the electrode will be exposed to energetic, positive ion bombardment.

iii. Transport of plasma-generated reactive intermediates from the bulk of the plasma to the surface of the material being etched: this occurs by diffusion which, for particular structures such as narrow deep trenches, can limit the etch rate.

iv. Adsorption step: reactive radicals adsorb on the surface of the material to be etched. This step can be strongly enhanced by concurrent ion bombardment which serves to produce “active sites” since it aids in the removal of the fluorinated surface layer which otherwise passivates the Si surface.

v. Reaction step: a reaction between the adsorbed species and the material to be etched must take place. In the case of fluorine-based etching of silicon chemical reactions between the fluorine atoms and the surface produces either volatile species (SiF_4) or their precursor. Because of the plasma-induced formation of reactive radicals the reaction rate is very large relative to reaction rates in non-plasma environments. The reaction step can be greatly enhanced by ion bombardment. For instance, chlorine atoms are known to adsorb readily on silicon surfaces but the spontaneous etch rate in a glow discharge without ion bombardment is very slow. Ion bombardment makes it possible for adsorbed chlorine atoms to attack more efficiently the backbonds of silicon and form a volatile $SiCl_4$ molecule.

vi. Desorption of volatile reaction product: The adsorptions of the reaction product into the gas phase is one of the most critical steps in the overall etching reaction. This requires that the reaction product have a high vapor pressure at the substrate temperature that, in RIE, is typically below 100°C. The removal of reaction product from the surface can be greatly accelerated by ion bombardment via sputtering.

vii. Pump out of volatile reaction product: This requires that the desorbed species diffuse from the etching surface into the bulk of the plasma and are pumped out. Otherwise plasma induced dissociation of product molecules will occur and redeposition can take place.

3.3.3 Deep Reactive Ion Etching

Deep Reactive Ion Etching is an extension of Reactive Ion Etching to indicate that the etching is anisotropic. This deep-silicon-etching machine can easily achieve etching rate in excess of 3 μm min^{-1} and selectivity to photo-masking materials greater than 70:1 [41]. In this technology plasma is generated by a magnetic field, and identified by a second magnetic field generator. The objective is to reach a high ionization rate in the gases to enhance the RIE effect [40].

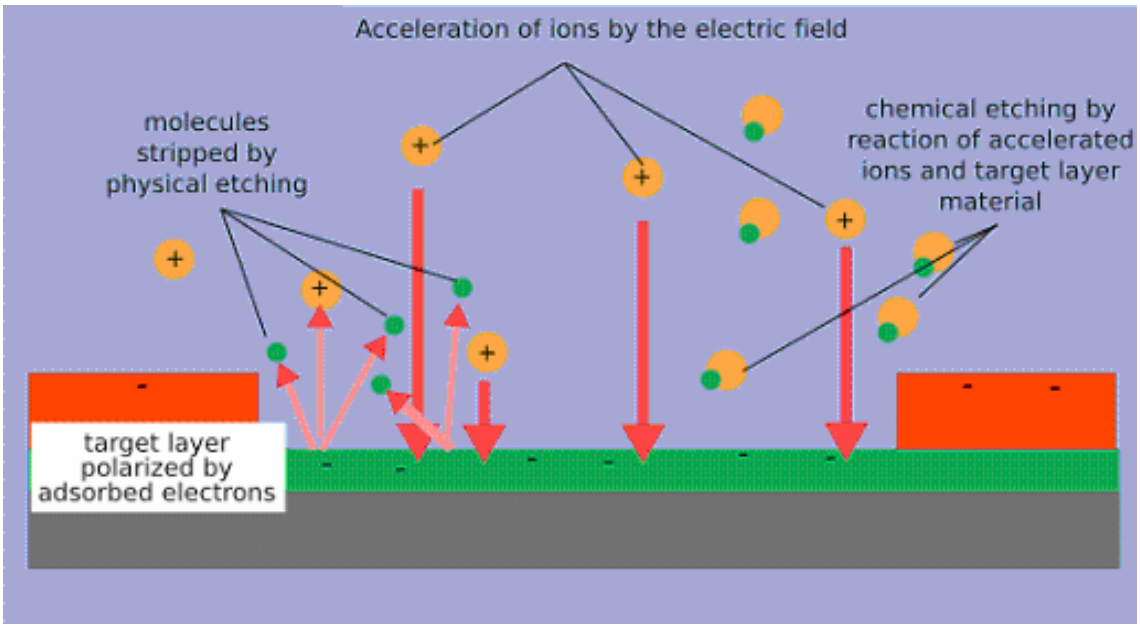


Figure 4 Schematics of the physical and vertically directed chemical etching in DRIE

Deep reactive-ion etching is a plasma-based dry etching technique characterized by a combination of physical sputtering with the chemical activity of reactive species. This enables the achievement of a highly anisotropic selectivity and also to create deep, steep-sided holes and trenches in wafers, with aspect rations of 20:1 or more [42].

Two effects are showing during the process. The first noticeable effect is the apparition of other etching phenomena due to the ions velocity: physical etching. The layer molecules are stripped away by impact of ions on the wafer. Physical etching is characterized by a very low selectivity, but is highly anisotropic. The second effect is that contrary to plasma etching, where ions move randomly above the target. In reactive ion etching they have a higher probability to have a movement in the direction given by the electric field. This makes a tendency to anisotropic etching [43,44].

3.3.4 Wet Etching

Wet Etching is an etching process that utilizes liquid chemicals or etchants to remove materials from the wafer, usually in specific patterns defined by photoresist masks on the wafer. The wafer can be immersed in a bath of etchant, which must be agitated to achieve good process control. For instance, buffered hydrofluoric acid (HF) was used commonly to etch silicon dioxide over a silicon substrate. Several anisotropic wet etchants are available for silicon [37]:

Table 3 Typical chemical etchants

Material	Gas	Etching Rate	Mask	Selectivity
Si (a-Si)	1) KOH 2) HNO_3 + H_2O + HF	~ 6 – 600 nm/min (anisotropic) ~ 100 nm/min	Resist	> 50:1
SiO2	1) HF 2) BHF	~ 10 – 1000 nm/min	Resist	> 50:1
SI3N4	1) HF 2) BHF 3) H_3PO_4	~ 100 nm/min ~ 100 nm/min ~ 10 nm/min	Resist SiO_2	> 50:1
GaAs	1) H_2SO_4 + H_2O_2 +H_2O 2) Br + CH_3OH	~ 10 um/min	Resist	> 50:1
Au	1) HCl + HNO_3 2) KI + I_2 +H_2O	~ 40 nm/min ~ 1 um/min	Resist	> 50:1
Al	1) HCl + H_2O 2) NaOH	~ 500 nm/min	Resist	> 50:1

Materials not covered by these masks are etched away by the chemicals while those covered by the masks are left almost intact. These masks were deposited on the wafer in the earlier wafer fab step.

A simple wet etching process may just consist of dissolution of the material to be removed in a liquid solvent, without changing the chemical nature of the dissolved material. In general, however, a wet etching process involves one or more chemical reactions that consume the original reactants and produce new species.

A basic wet etching process may be broken down into three basic steps: 1) diffusion of the etchant to the surface for removal; 2) reaction between the etchant and the material being removed; and 3) diffusion of the reaction byproducts from the reacted surface [38].

Reduction-oxidation (redox) reactions are commonly encountered in wafer fab wet etching processes, i.e., an oxide of the material to be etched is first formed, which is then dissolved, leading to the formation of new oxide, which is again dissolved, and so on until the material is consumed. Wet etching is generally isotropic (Figure 5). Its horizontal etching rate is the same as its vertical rate [37].

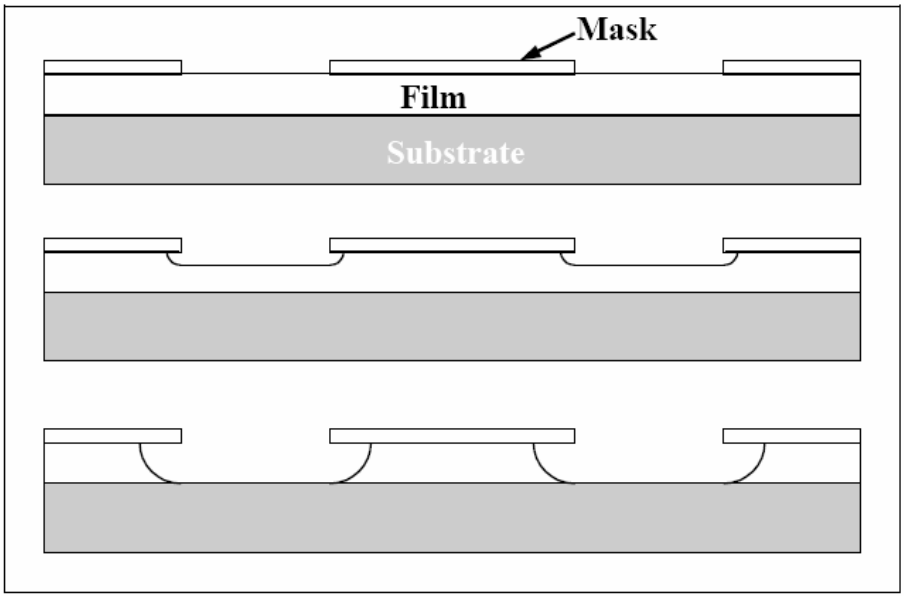


Figure 5 An isotropic wet etch on a silicon wafer.

However, on crystalline materials, etching rate is typically lower on the more densely packed surface than on that of loosely packed surface. For example, when etching Si wafer with diamond cubic lattice structure, we know the surface atom density on {111}> {100}> {110}, the etching rate of (100) will be 100 times than that of (111) [39]. Figure 6 below is a (100) silicon wafer with mask aligned in <100> direction. Amorphous area was easily etched away and the pattern lines are clearly aligned on the crystal phase.

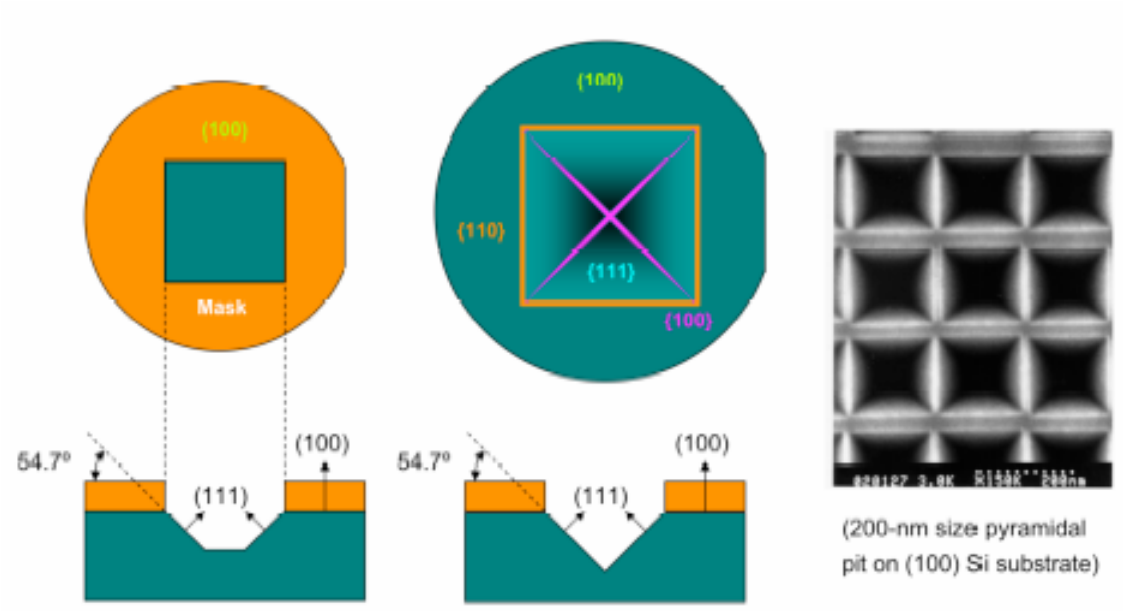


Figure 6 An anisotropic wet etch on a silicon wafer creates a cavity. The sides of the cavity are <111> planes and the horizontal ones are <100> planes. The yellow material is an etch mask, and the green material is silicon [39]

CHAPTER 4

EXPERIMENTAL PROCEDURES – MATERIALS AND METHODS

4.1 Materials

CUPE was synthesized in Dr. J. Yang's lab (Bioengineering Department); 4" p type (100) Si wafer; Negative photosensitive SU8 was purchased from MICRO CHEM. Positive photoresist AZ5209 and developer are from Shipley. The photo-Mask was designed in Stanford University by Micronic LRS-18 pattern generator with assistance by Dr. Lee (ARRI) and is shown in Figure 7.

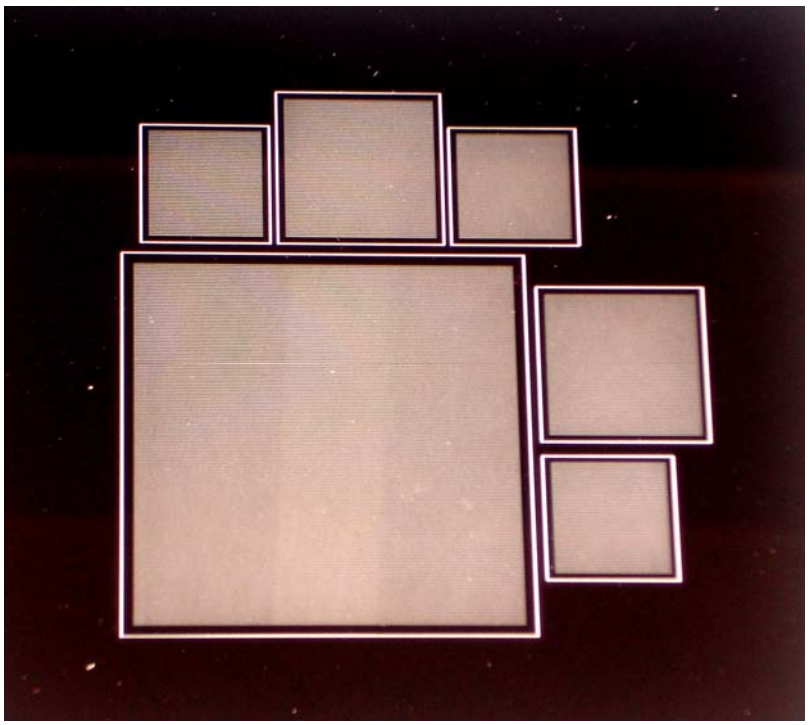


Figure 7 A photo image of designed mask of this research

4.2 Process of Photolithography

Table 4 describes the process of photolithography technique followed for this research work. Basically, the following procedure of this process is deep reactive ion etching (DRIE). Each step was operated in NanoFab at UTA.

Table 4 Process of photolithography

1	Clean wafers with chemicals	H_2SO_4: H_2O_2=5:1, 15 min
2	Dehydrate wafer surface	Bake wafer with hotplate 200 ℃ for 5 min
3	Spinning coating SU8 PR onto Si wafer	Two steps: a) Ramp to 500 rpm@ 100 r/s ,1 sec b) Ramp to 1100 rpm@ 300 r/s c) Spin @ 1100 rpm 30 sec
4	Soft back	Pre-bake: 65°C, 1 min Soft-bake: 95 °C, 3 min
5	Expose OAI Aligner	Expose dose 140 mJ/ cm^2 for 8 sec
6	Post Exposure bake	Pre-bake: 65°C, 1 min Soft-bake: 95 °C, 2 min
7	SU8 Developing	Dip wafer into SU8 developer for 3.5 min. Agitate every 10 sec.

Table 4-*continued*

8	DI water rinse and N_2 air dry	
9	Develop inspection	Check wafer for stress cracks
10	Hard back	140°C, 1 min
11	Measure step height	Use profilometer for the measurement

4.3 Pattern Formation

4.3.1 Micro-Fabricated Negative Patterning-Dry Etching

Negative image was presented on SU8 after photolithography process. A silica substrate is carried into a substrate carrying position in the DRIE main body, and then carried into a chamber by a robot arm. A reaction gas is introduced while the chamber is being evacuated; high frequency power is applied under applied pressure to generate plasma.

The etching rate was about 1.7 μm/min. The control setting for this research is attached below:

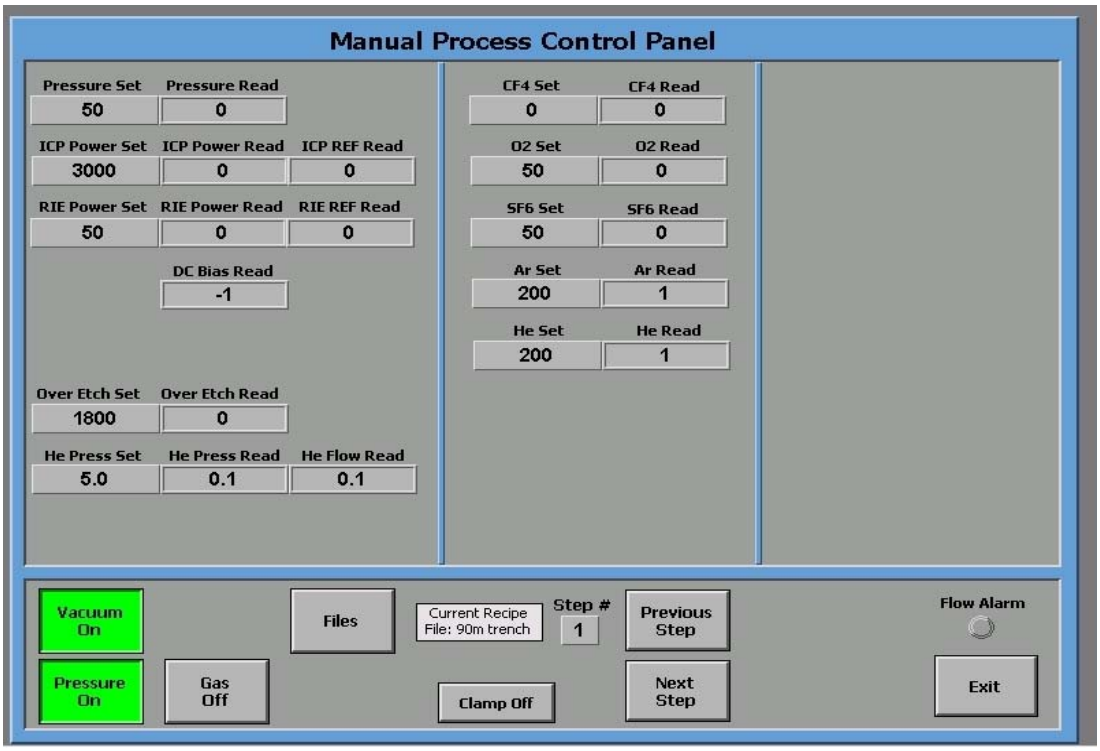


Figure 8 DRIE manual process control panel

4.3.2 Micro-Fabricated Positive Patterning-Wet Etching

In some cases, the masking material is photoresist that has been patterned using photolithography. Other situations require a more durable mask, such as silicon nitride. In this wet etching process, silicon nitride deposition was required to resist etchant. Potassium hydroxide (KOH) is the chemical etchant used here. It can achieve selectivity of 400 between <100> and <111> planes.

Table 5 Process of KOH wet etching

1	Remove SiO_2 with chemicals	Dip with HF and acetone for 15min respectively
2	HMDS deposition	Sit in HMDS oven for 20min
3	LPCVD (Low-pressure chemical vapor deposition) Nitride deposition	Put cleaned wafers in the nitride oven for 2.5-3 min to have about 16 nm of silicon nitride
4	Silicon Nitride thickness measurement	Woollam M-200 spectroscopic ellipsometers was used to examine the thickness
5	AZ5209 spin coating above silicon nitride	3000rpm, 30 sec to have ~1μm photoresist
6	Pre-bake	Pre-bake: 90 °C, 90 sec
7	Mask Aligner	Exposure light wavelength 360 nm with an intensity of 7.5 mW/cm, 15sec
8	Hard bake	120 °C, 20min
9	RIE etching	2 min
10	Remove photoresist	H_2SO_4: H_2O_2=2:1, 15min
11	BOE (buffered oxide etch) process for SiO_2 removal	NH_4F: HF=10:1, 3 min
12	KOH wet etching	3hr at 80°C

4.4 Polymer Information

The polymer that has been used is a cross-linked urethane-containing polyester (CUPE), which is prepared by the introduction of polyurethane linkages in a polyester backbone. The synthesis of the polymer is a simple and cost effective process, and is carried out in three steps; first, a linear pre-polymer that is polyester is formed. Second, a diisocyanate is used as a linker to introduce the urethane bonds into polyester. Last, the resulting linear urethane-containing linear polymers are crosslinked to form CUPEs.

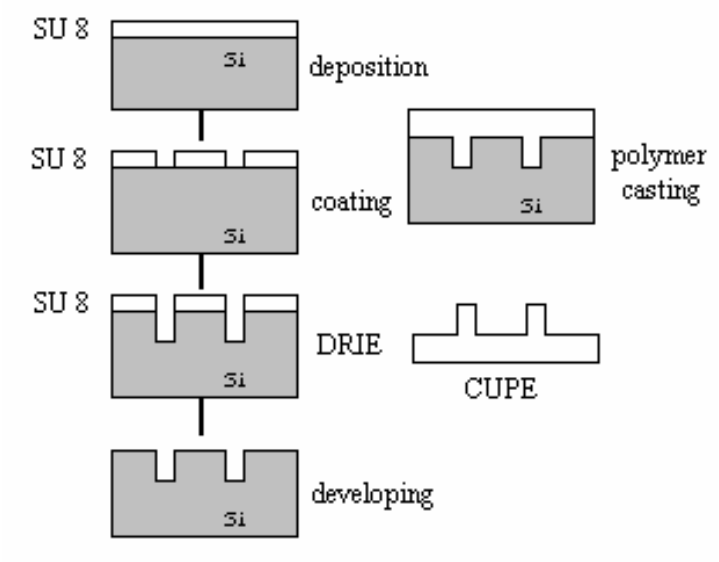
The polymer has excellent mechanical properties. It can also be easily cast into films of various shapes and sizes depending on the mould. The material is soft and proven to be easily patterned by casting the polymer on a designed pattern mould.

4.5 Film Fabrication

Solvent casting method was used to make the imprinted films [45]. Briefly, the polymer was dissolved in N, N'-dimethylformamide (DMF) to make a 10% concentration of the polymer in solution. This way the polymer solution will not be too viscous to flow into the channels. Continuously, the solution was then cast over the silicon mould and sitting at room temperature for 6 hr. After that, put it in the oven with ~80°C for 4 days to undergo polymerization reaction.

CUPE was cast onto silicon mould to imprint the designed image in order to produce channels on its surface. The schematic photos of the whole process for dry etching and wet etching are as follow (Figure 9):

Dry Etching



Wet Etching

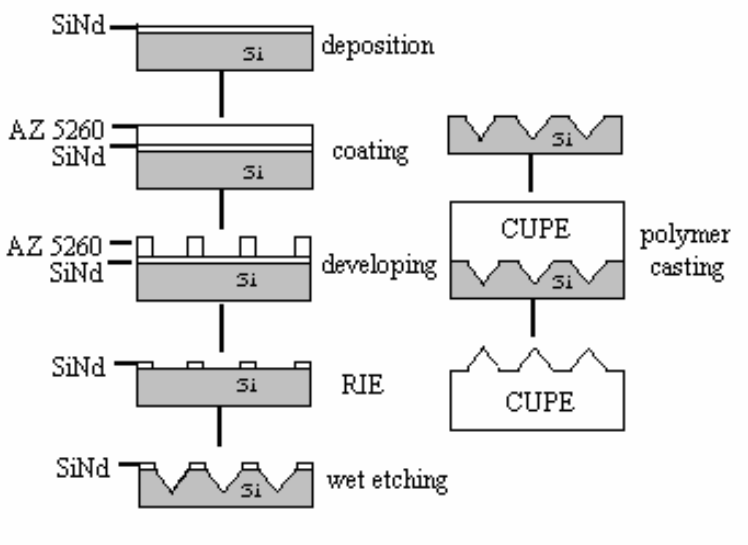


Figure 9 A schematic photo of film casting through Dry Etching and Wet Etching process

4.6 Cell Culture Information

Three different types of CUPE samples, namely, unpatterned CUPE, square patterned CUPE and triangular patterned CUPE were utilized in order to study the effect of the patterns in modulating cell growth. The unpatterned CUPE samples acted as the control. The samples were cut into discs of 9 mm diameter using a cork borer and placed in 35 mm diameter culture plates. Sterilization of the samples was carried out by treatment with 70% ethanol for 2 hours followed by exposure to Ultra-Violet (UV) for 1 hour. Following sterilization, the films were allowed to soak in Dulbecco's Modified Eagle's Medium (DMEM) for 24 hours prior to cell seeding.

3T3 fibroblasts were maintained in monolayer culture in a humidified atmosphere at 37 °C and 5% CO_2 for less than five passages in Dulbecos modified Eagles's media (DMEM) supplemented with 10% fetal calf serum. Passaging and preparation of single cell suspensions for sample seeding was achieved by enzymatic digestion using a 0.25% (v/v) trypsin. 0.02% (w/v) EDTA solution in phosphate buffered saline (PBS) pH 7.4. Cell numbers were determined using a hemocytometer and the samples were seeded with 5×10^3 cells in 4 ml of 3T3 complete medium on CUPE. The cells were then cultured statically at 37°C as above. Any non-attached cells were removed after the first day by washing the samples with 3 ml PBS for 5 min. at the end of 3 days of culture, the cells adhered to the surface of the films were fixed by treatment with 2% glutaraldehyde-PBS solution for 2 hours followed by sequential dehydration with different grades of ethanol. After freeze-drying for 24 hours, sputter coating and SEM observation ensued.

4.7 Characterization

KLA-Tencor Profilometer was used for surface roughness measurements of mask and produced patterns. Scanning Electron Microscope ZEISS Supra 55 VP was used to characterize the patterns after dry and wet etching on silicon wafers. SEM was also utilized to characterize the result of cell growth on polymer films. The preparations of cell cultures were sputter coated with gold atoms before examination in the SEM.

CHAPTER 5

RESULT AND DISCUSSION

5.1 Silicon Mold Patterning

The result of patterned silica substrate fabrication was observed under SEM. For the same mask design owing to two different etching procedures, KOH chemical etching and deep reactive ion etching, were applied. The produced channels result in two different groups of grooves and ridges. The following content is going to present the individual patterns produced from these two procedures.

5.1.1 Dry Etching Fabrication

Negative image was produced on SU8 negative photoresist layer after mask aligner. The thickness of SU8 was 20 μm. Etching rate and time determine the consequent microchannel patterns. Figure 10 is an optical micrograph taken by using Nomaski mode. It was taken during an inspection step. The purpose is to inspect the condition of pattern formation of the photoresist. If there are cracks on the photoresist the silicon underneath will not be well protected while going through the etching process. A good way to prevent thermal stress cracking on polymer surface is to gradually cool down the sample under room temperature after the step of hard baking.

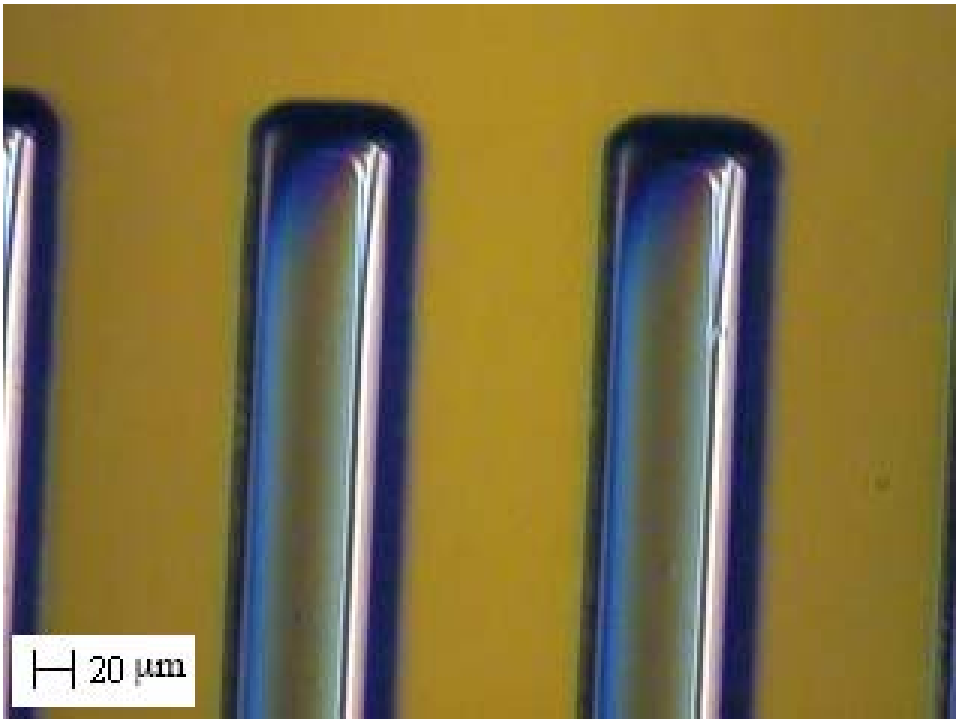


Figure 10 Optical-image of 4” silicon wafer covered with SU8 photoresist patterns

The channel fabrication was achieved after selectively etching on the silicon layers. In the etch step, ion bombardment promotes the preferential removal of the silicon atoms from all horizontal surfaces, allowing the profile to evolve in a highly anisotropic fashion. The alignment of patterns is shown in Figure 11. The wall of each channel is 36 μm high and 200 μm wide; every adjacent one is separated by 60 μm. Figure 12 is a part of a channel ridge at larger magnification. It was fabricated in plasma chamber under the setting shown in Figure 8.

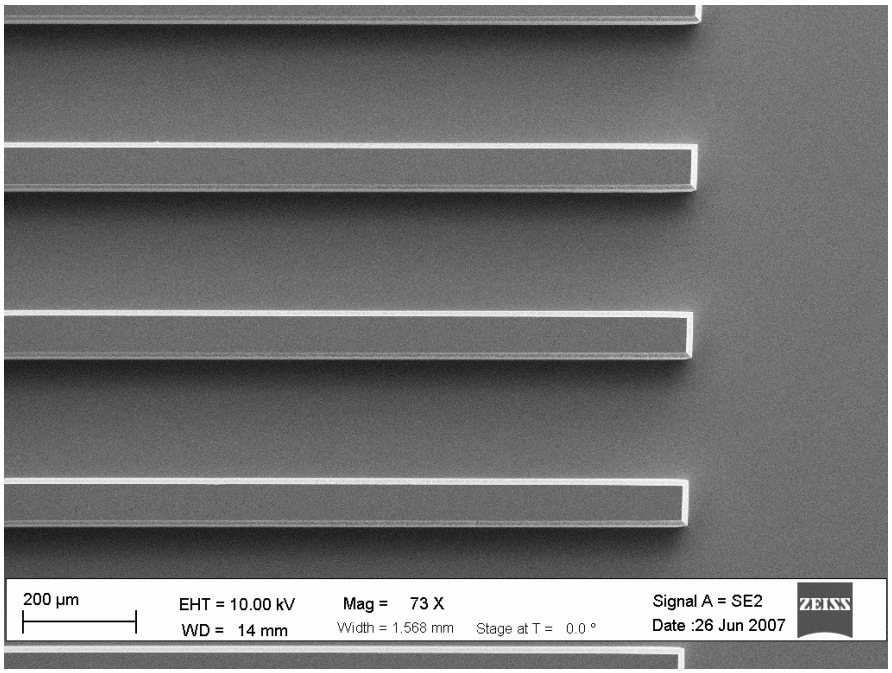


Figure 11 SEM micrograph of trapezoidal channels after deep reactive ion etching (1)

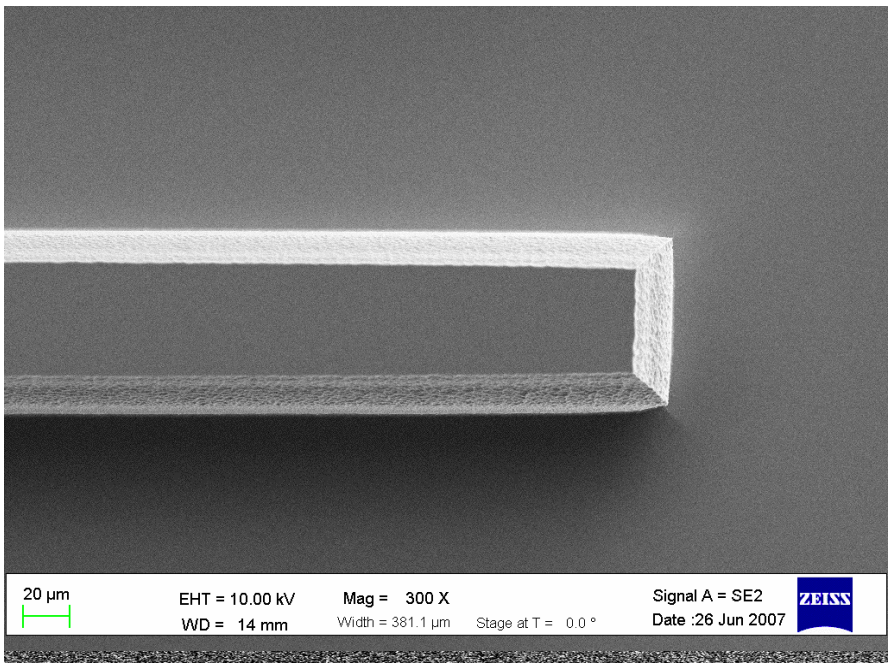


Figure 12 SEM micrograph of trapezoidal channels after deep reactive ion etching (2)

Several trails were run to find out the best plasma conditions for desired design. Etching behaviors are characterized by varying the ICP power, Ar or He mixing ratio, radio-frequency (rf) power, and chamber pressure. In addition, pressure, ICP power, gas flow ratio and rf power significantly influence etching rate and surface morphology. In particular, dc bias heavily influences the etching rates, suggesting that the ion-bombardment effect is an important factor of these etching processes. A smooth shinning surface will be produced under a good etching setting. Otherwise, it presents grimy because of damaged surface, as shown in Figure 13.

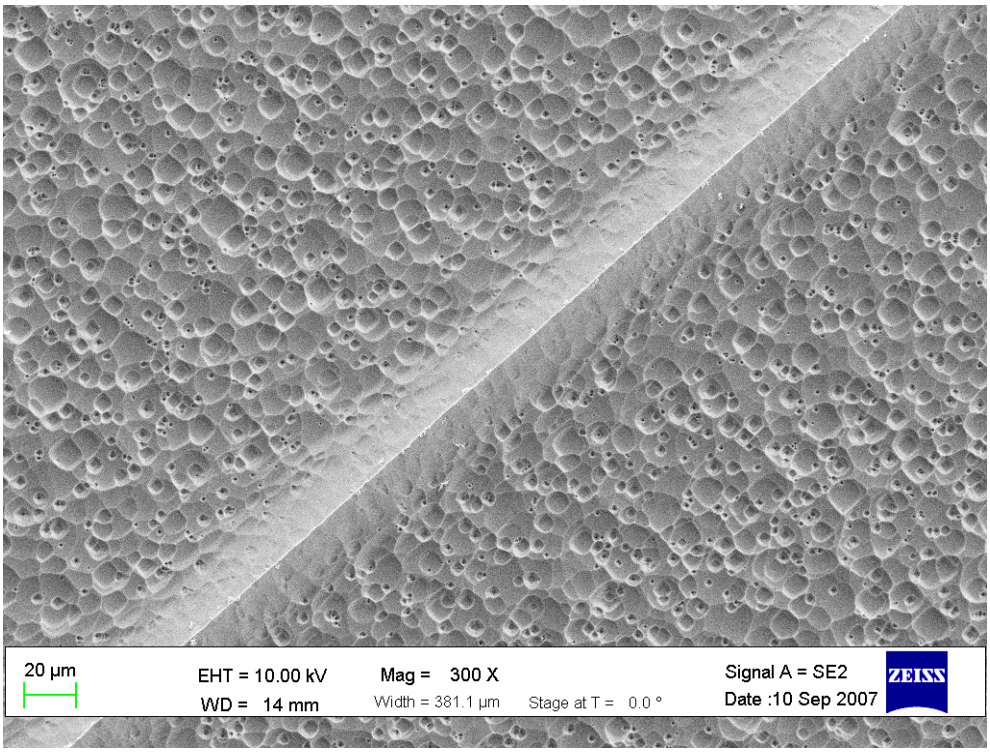


Figure 13 SEM micrograph of sample with surface damage by over-powerful bombardment under DRIE (1)

Figure 14 indicates that part of ridge surface, which was supposed to be protected by photoresist. That section was also etched away by over powerful ion bombardment. The masking material was not observed under the microscope right after the etching process was completed. It is speculated that the SU8 was damaged and sputtered off by the physical impact of ions. The film contamination can be effectively improved by having an ideal plasma processing.

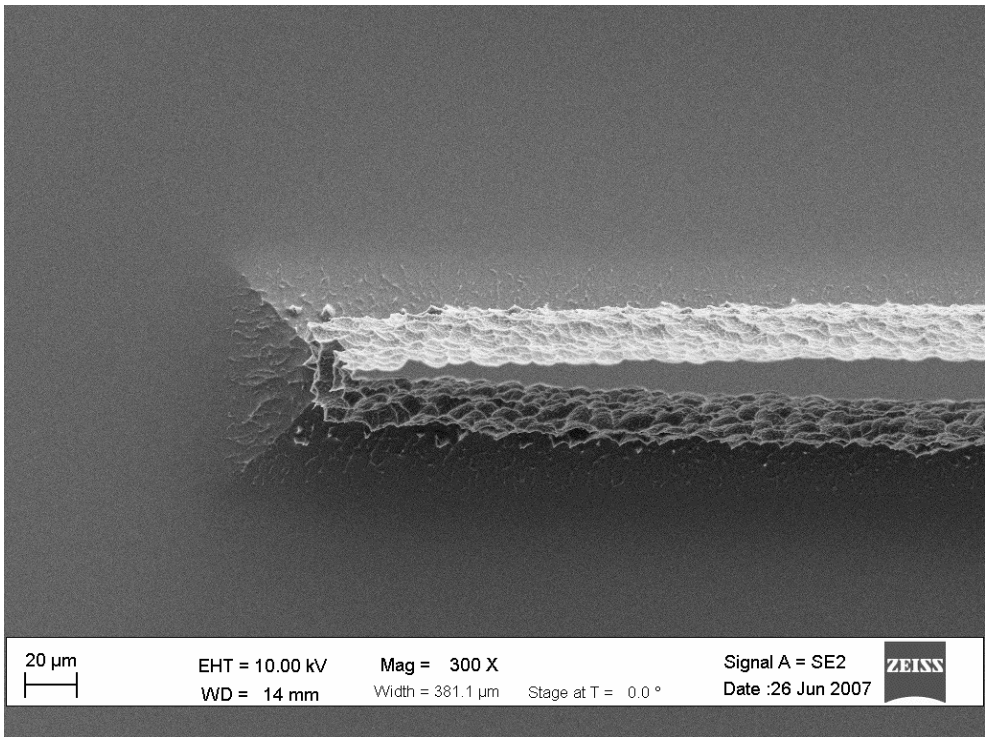


Figure 14 SEM micrograph of sample with surface damage by over-powerful bombardment under DRIE (2)

5.1.2 Wet Etching Fabrication

A (100) Si wafer was used as a substrate. Etching reaction took place along (111) to reveal the triangle-shaped grooves. This is due to the fact that (111) planes have the highest atomic density in the Si structure. The reaction took place from the surface. Si atoms will react with OH^- and form a layer of silicon dioxide. Then, the dioxide product will be dissolved away into the base solution. The overall reaction is as followed: $Si + OH^- + 2\ H_2O \rightarrow SiO_2(OH)_2^{2-} + 2\ H_{2(g)}$. Figure 15 is a SEM micrograph showing precise lines and smooth wall surface of silicon channels.

After wet etching positive (inverted) image was revealed with 65×100 μm in depth and width. Each groove is around 200 μm apart, as Figure 16.

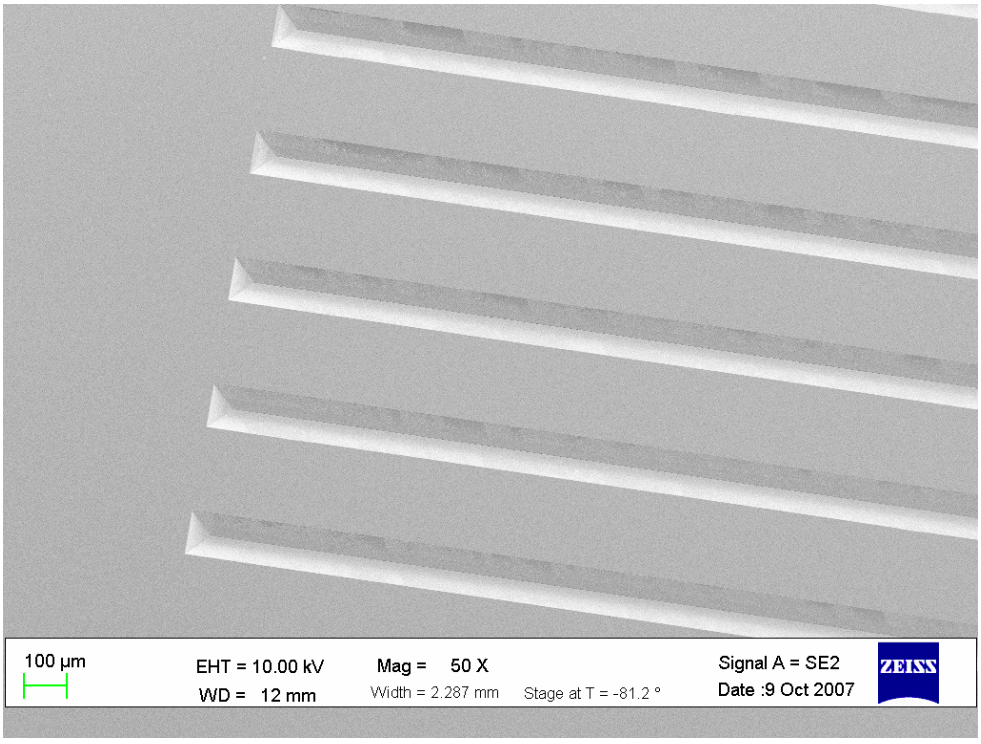


Figure 15 SEM micrograph of triangle channels after KOH wet etching

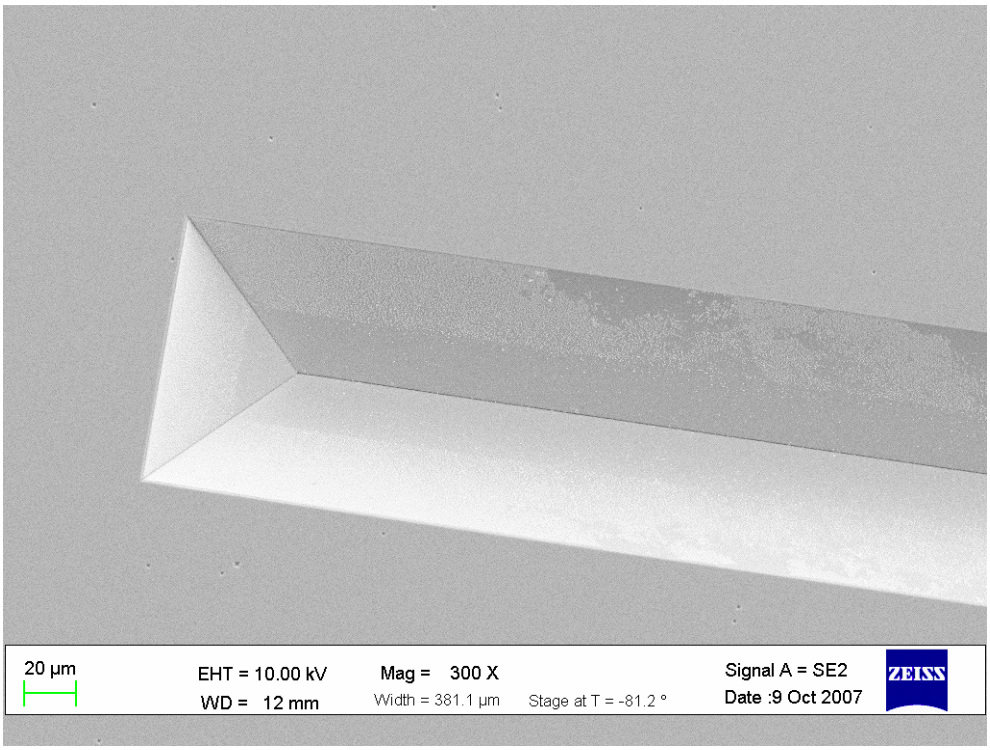


Figure 16 *SEM micrograph of triangle channels*

5.2 Modification on Surface Morphology of CUPE

Figure 17 is a SEM image of CUPE film before any surface modifications. The flat film was used as a control sample to compare the other two patterns. Figure 18 shows a cavity with a reversed trapezoidal cross-section of channel is the imprinted CUPE cast from the mould we have by DRIE dry etching. The polymer film shows precise size and shape of patterns on its surface. This indicates that the polymer is soft and its surface can be easily manipulated. The polymer films have good strength and elasticity. This reduces the damage to the films while they are being peeled off the surface of the silicon mold. Channel alignments are shown in Figure 19.

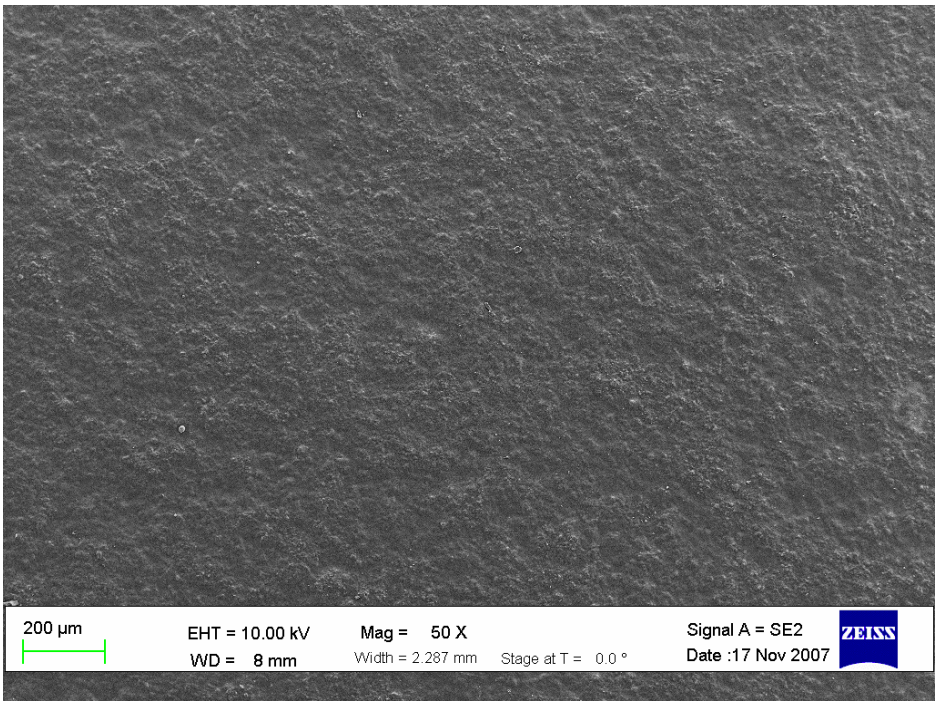


Figure 17 SEM micrograph of flat surface of CUPE film as a control sample

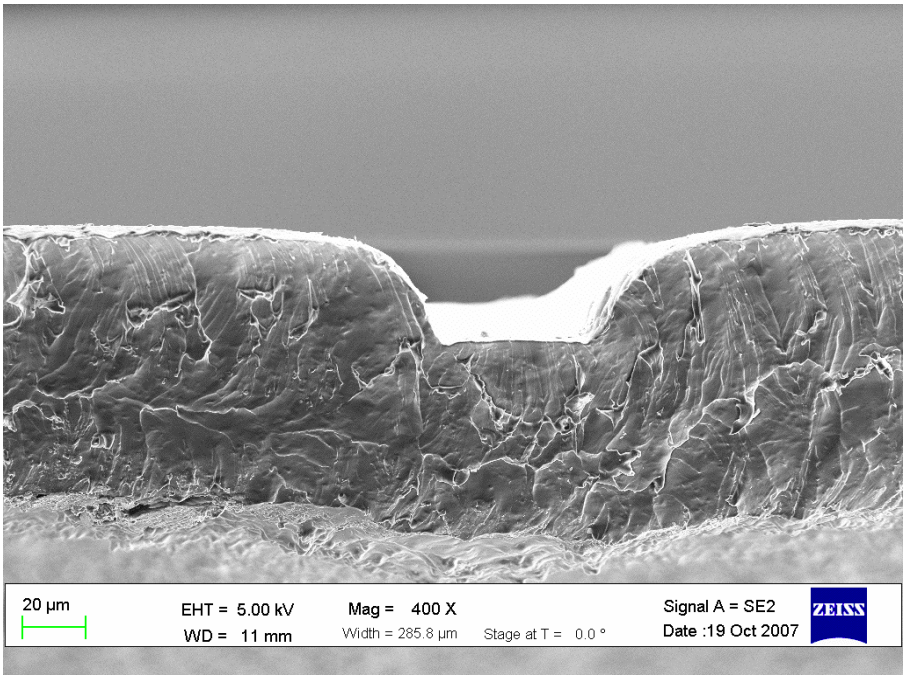


Figure 18 SEM micrograph of cross section of CUPE imprinted with trapezoidal channels

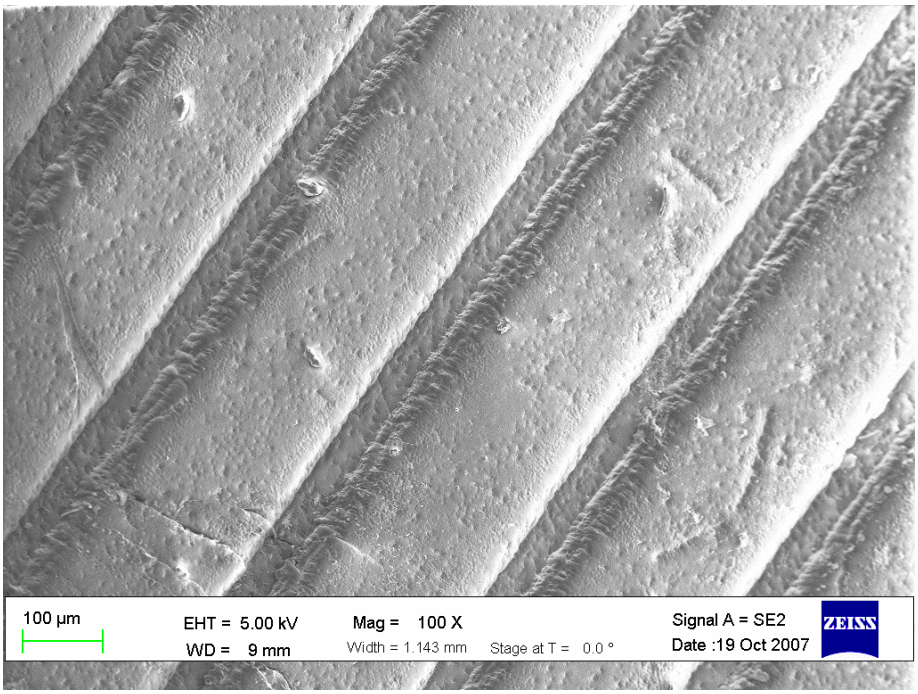


Figure 19 SEM micrograph of the over all trapezoidal channel alignment

The surface morphology results in trapezoidal channels with triangle-shape walls on the film after casting CUPE onto silicon patterns which was fabricated by wet etching. Figure 20 shows the cross section of the film. The structure of channels gives narrow area on ridges and wide area on the grooves. The size of each channel is 65×200 µm in depth and width. The channel alignment is shown from the top view of polymer, as Figure 21. It can be seen that the top areas of ridges are smaller on triangular walls than trapezoidal ones comparing with Figure 19.

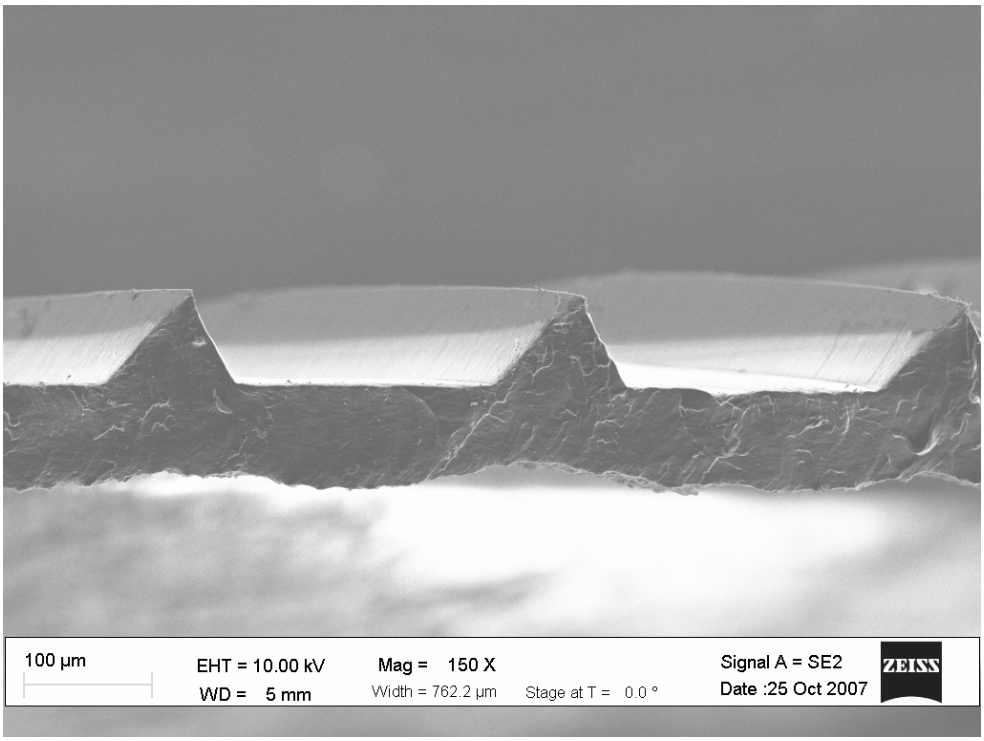


Figure 20 SEM micrograph of cross section of CUPE imprinted with triangle channels

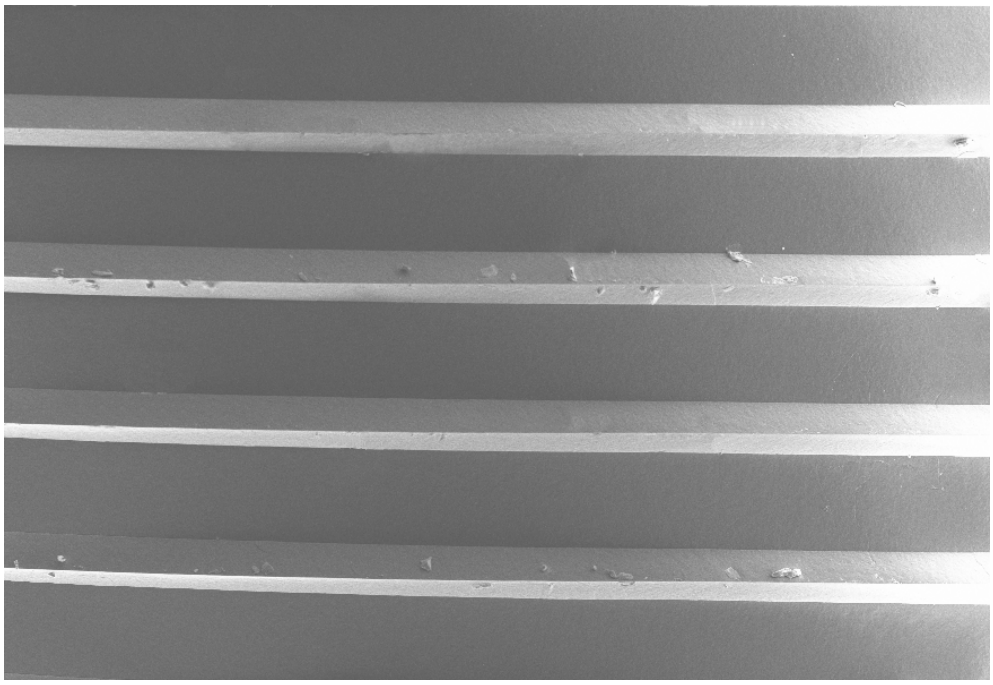


Figure 21 SEM micrograph of the over all triangle channel alignment

It is much easier to successfully get a polymeric film with the triangle-shape walls comparing the results produced by applying two different etching concepts. The narrow ridge gives less physical bonding between the intersection of CUPE film and silicon surface.

5.3 Cell Culture

Equal number of cells was seeded on CUPE polymer surfaces with different morphology to assess the effect of surface morphology on their growth. After 24 hours, the cells appeared to depict healthy growth on the CUPE control film containing no pattern (Figure 22). In Figure 23, a clear view of the cells maintaining the characteristic fibroblast like morphology can be observed. Cells grew all over the surface in a random manner after one day.

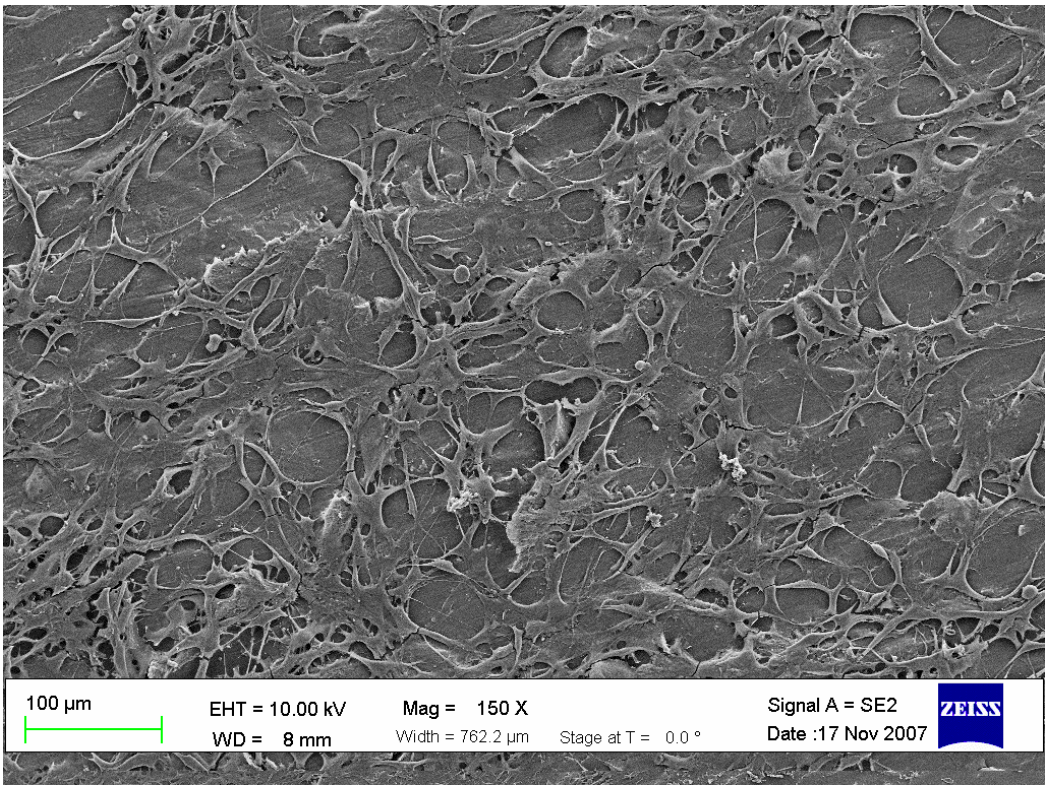


Figure 22 SEM micrograph of 3T3 fibroblast cells grew on CUPE flat film after one day (1)

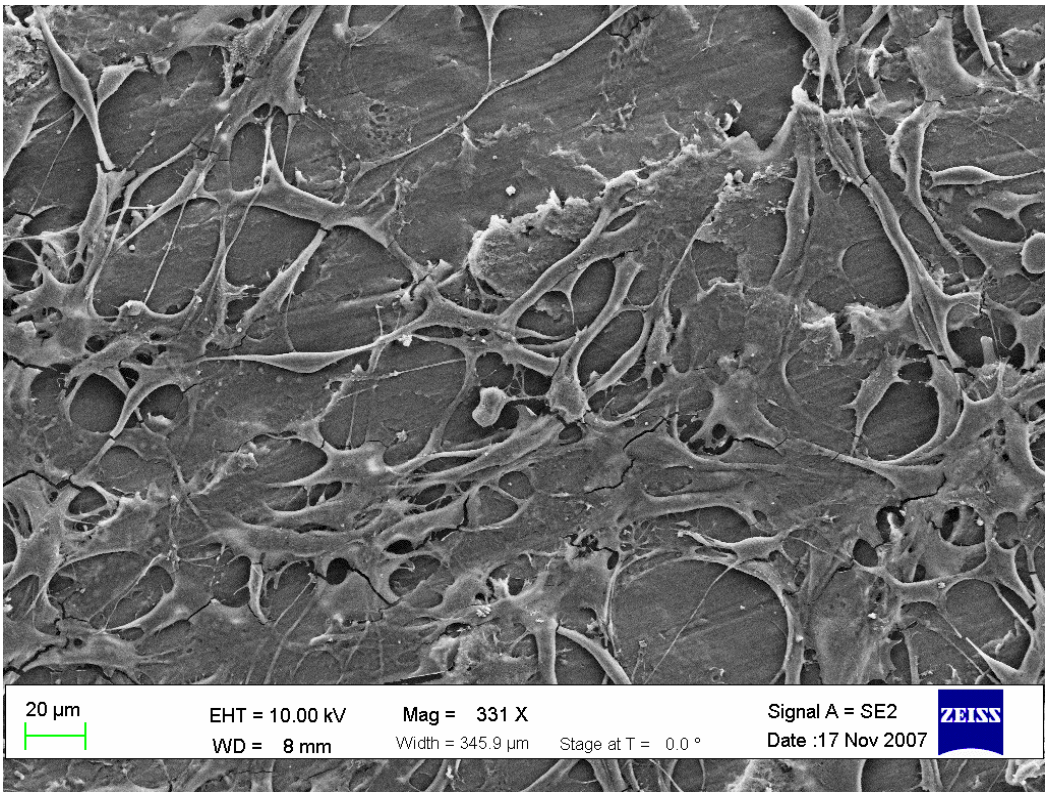


Figure 23 SEM micrograph of 3T3 fibroblast cells grew on CUPE flat film after one day (2)

On day 2, the cell growth on the control un-patterned film had become confluent, with the cells covering all parts of the surface, in no particular configuration (Figure 24).

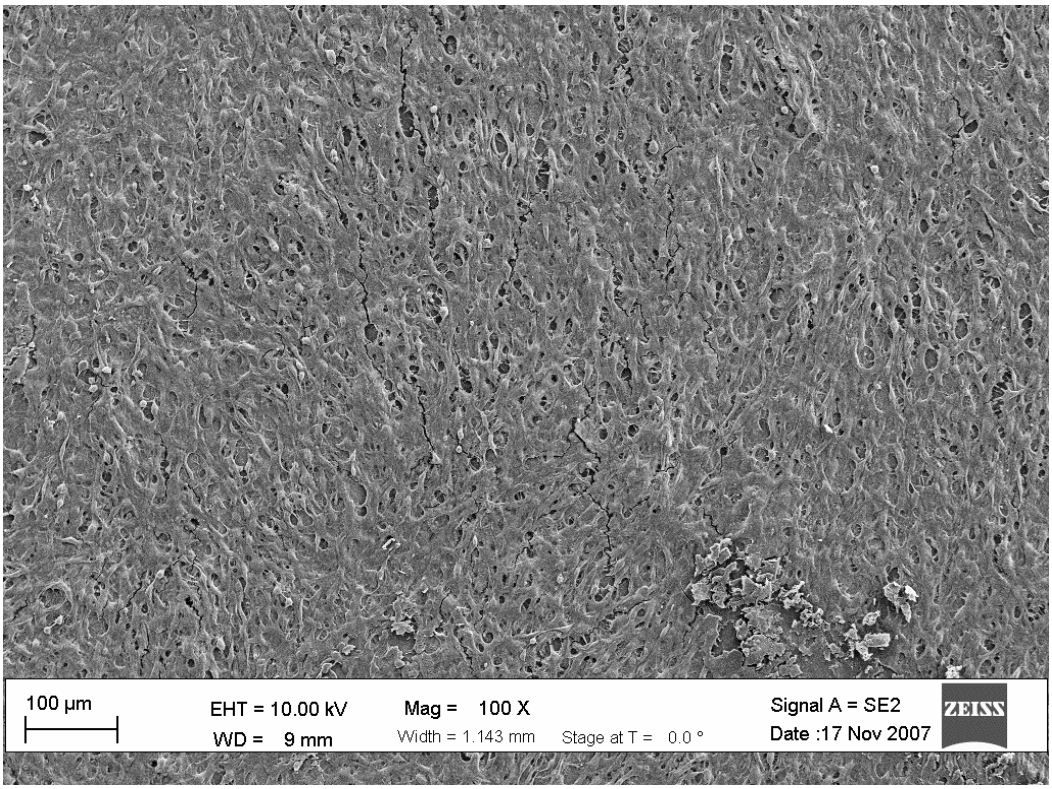


Figure 24 SEM micrograph of 3T3 fibroblast cells grew on CUPE flat film after two days

Healthy cell growth was also observed on the CUPE triangular and square patterned films. On the triangular patterned CUPE film, the cells did not grow in random directions. As shown in Figure 25, there is a distinct tendency of cells to grow along the direction of the channels. Cells, which have been attached onto polymer surface, were spreading out and trying to connect with each other (Figure 26). However, the triangular shaped walls did not permit the cells to grow across the channels and this barrier activity of the walls was observable in regions where there were cracks in the wall (Figure 27).

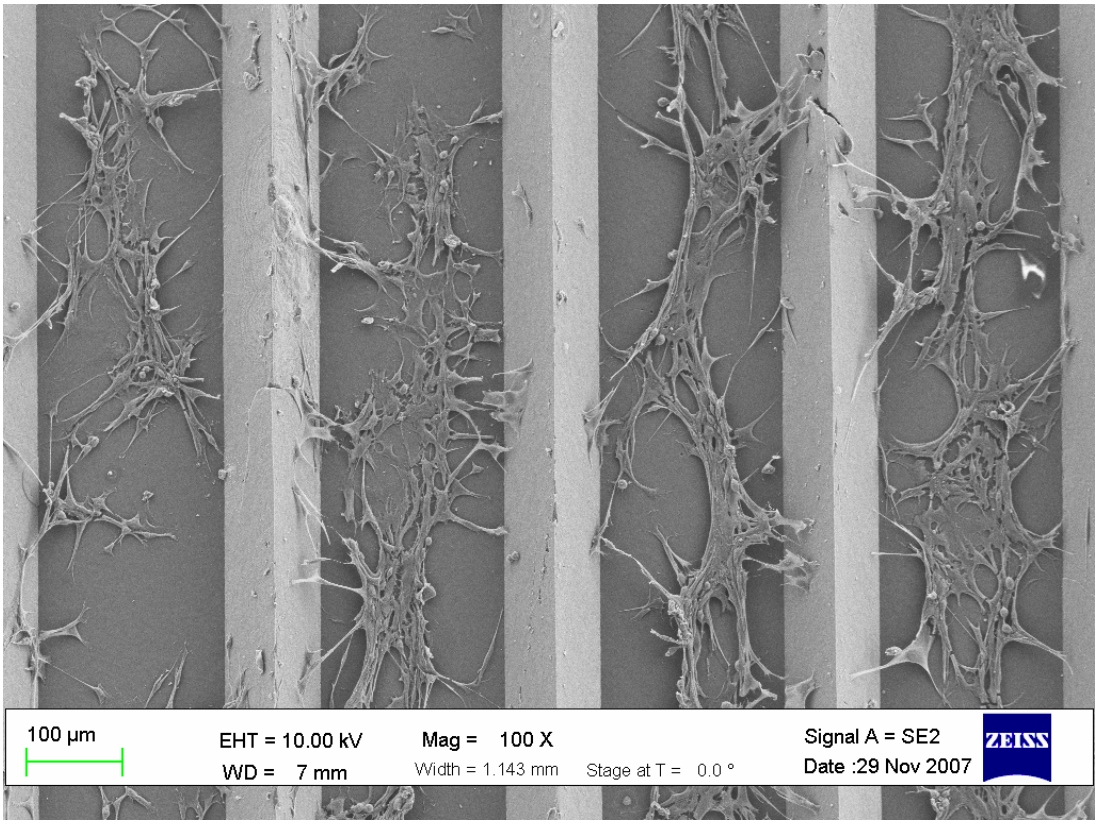


Figure 25 SEM micrograph of 3T3 fibroblast cells grew on CUPE film with triangular walls after one day (1)

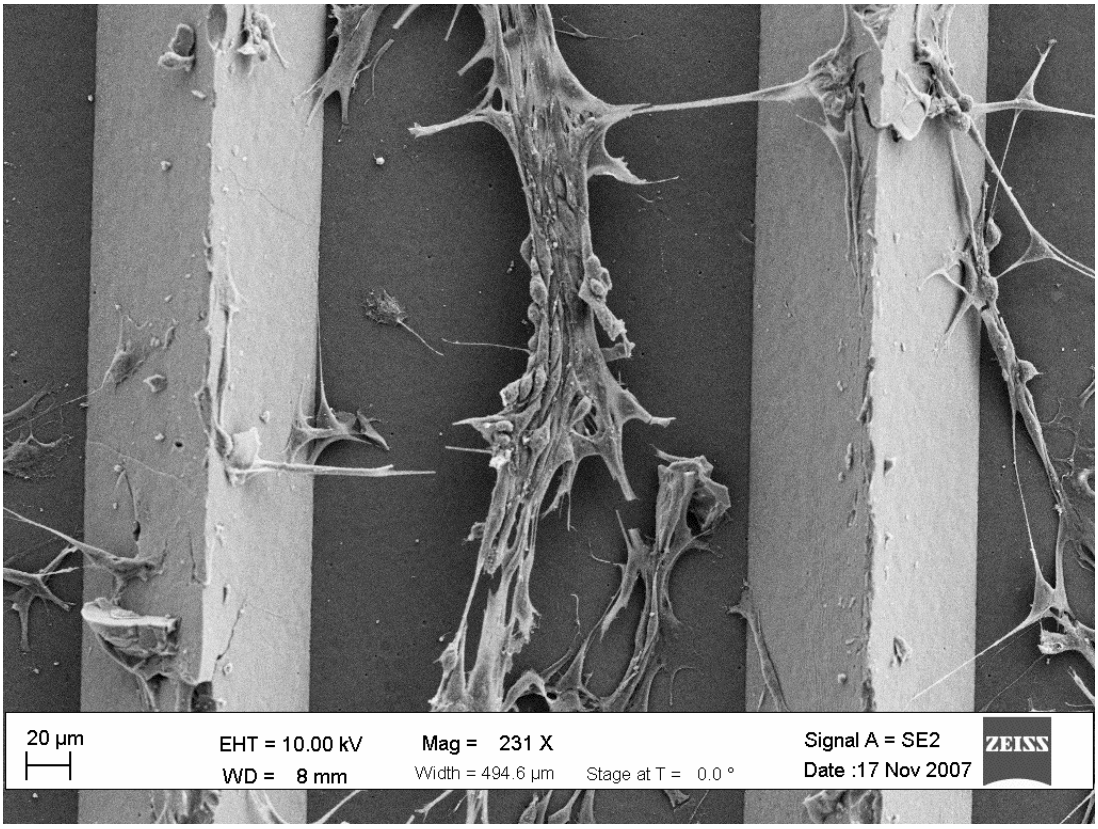


Figure 26 SEM micrograph of 3T3 fibroblast cells grew on CUPE film with triangular walls after one day (2)

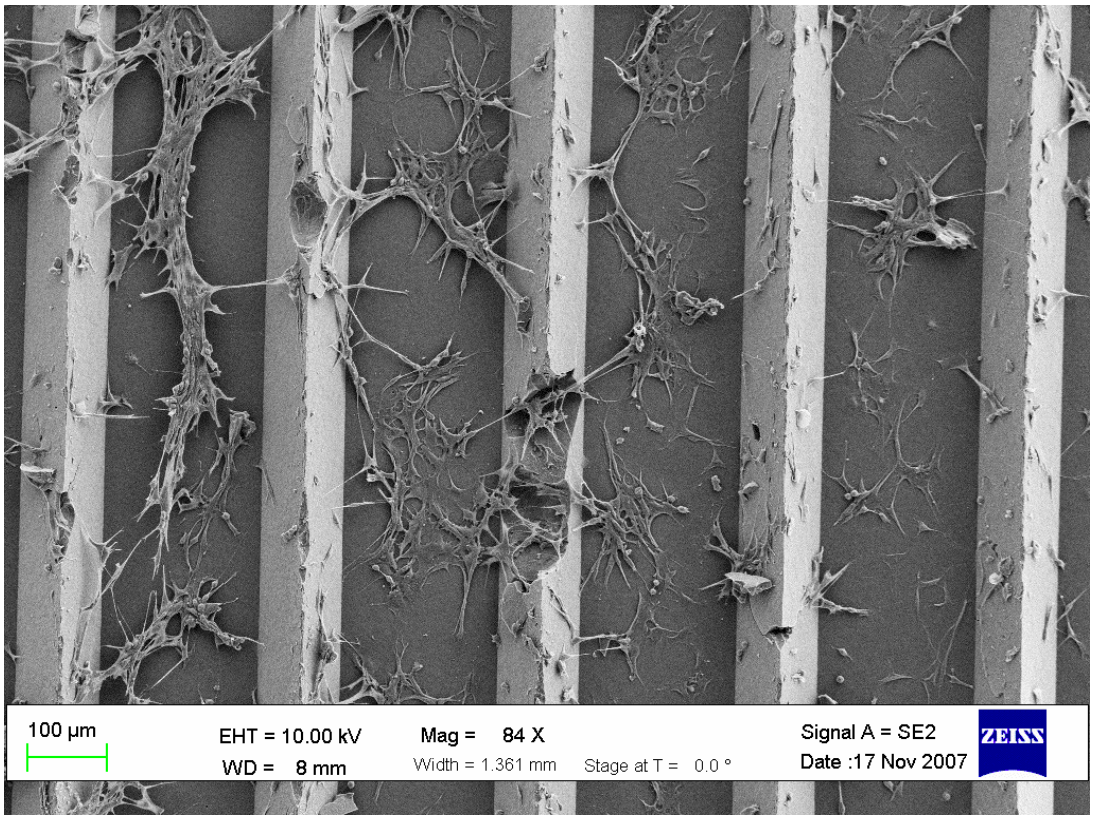


Figure 27 SEM micrograph of 3T3 fibroblast cells grew on CUPE film after one day with cracks on a triangular wall

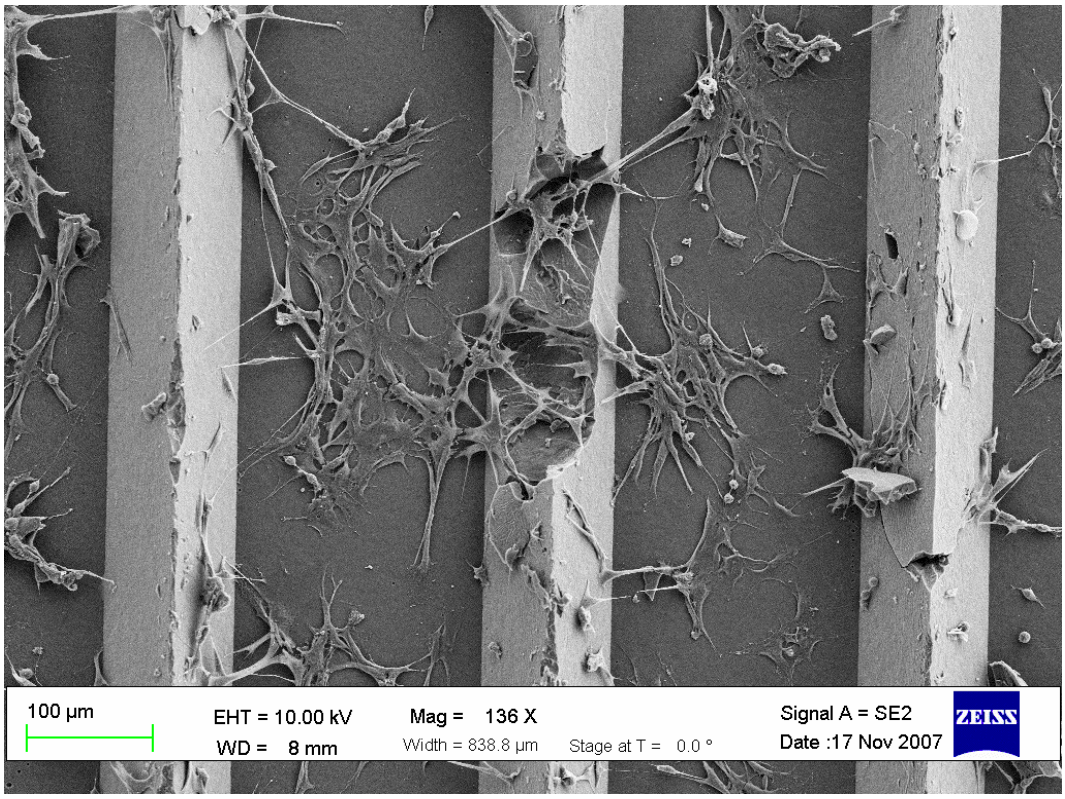


Figure 28 SEM micrograph of 3T3 fibroblast cells grew on CUPE film after one day with cracks on a triangular wall

Focusing on the crack region (Figure 28), the cells from adjacent channels were able to cross over and establish connections with each other. This is because the deformation of the channel decreased the wall depth. It results in a lower barrier effect of walls to cells. After two days, it can be seen that cells are filled out all the area on the bottoms of the trenches, but are still separated by top of the triangular walls (Figure 29, 30).

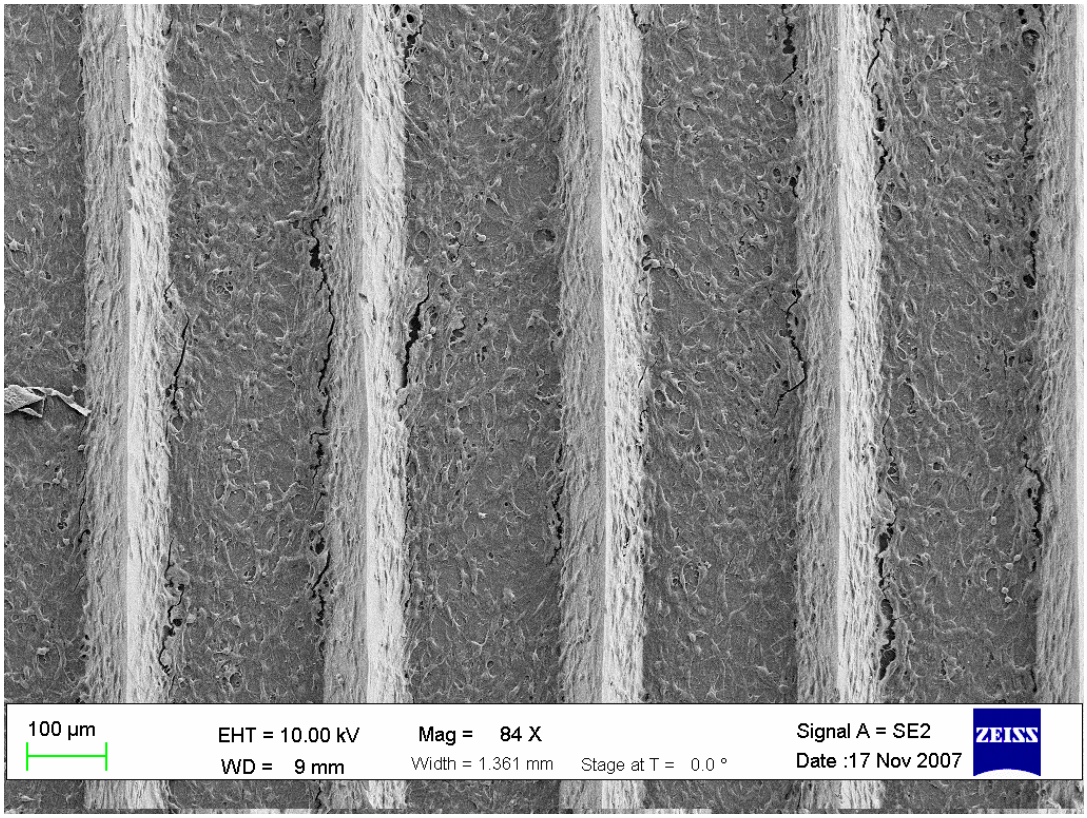


Figure 29 SEM micrograph of 3T3 fibroblast cells grew on CUPE film with triangular walls after two days (1)

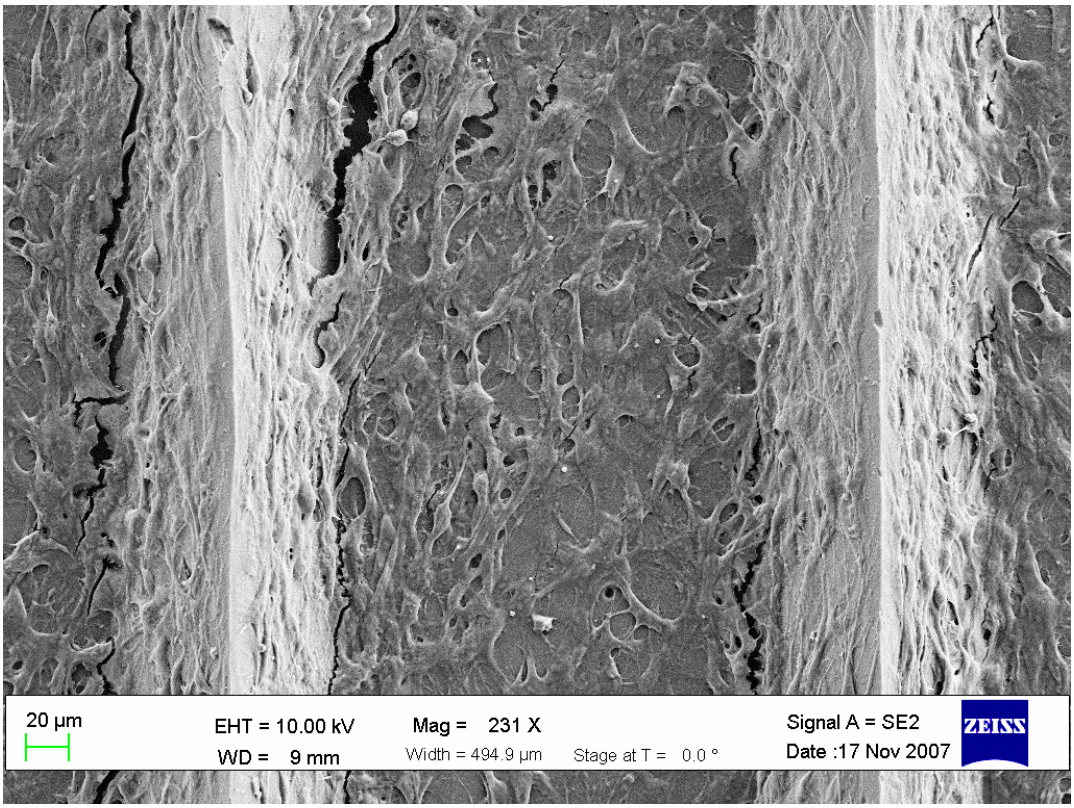


Figure 30 SEM micrograph of 3T3 fibroblast cells grew on CUPE film with triangular walls after two days (2)

Figure 31 shows the cell attachment on the sides of walls. A tendency was found that the direction of cell membrane deformation is along the direction of channels as well. It can be seen that cells still were not able to cross over to reach the cells on the other side of wall yet.

The ability of the triangular patterned surface to cause the cells to align along the channels may be explained by taking into account the dimensions and shape of the walls. A typical 3T3 fibroblast has dimensions of 40-50 microns after spreading. With a height of 65 microns, the walls of the triangular pattern are high enough to prevent the infiltration of the cells from one channel to another. The shape of the walls also plays a

major role in regulating the direction of the cell growth. The triangular walls have a sharp edge at their highest point. This sharp edge does not allow the cells to grow at the edge of the wall, thereby isolating them further from each other.

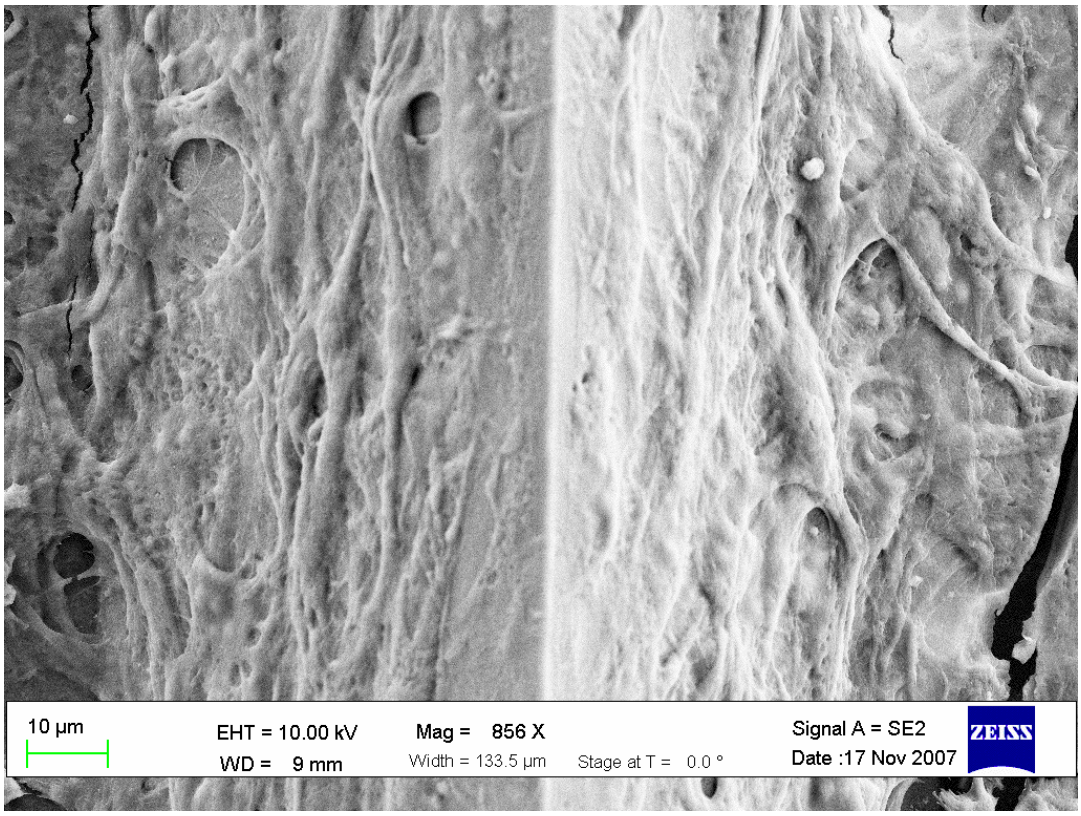


Figure 31 SEM micrograph of 3T3 fibroblast cells grew on the sides of CUPE triangular wall

Although, a similar barrier effect was expected, from the CUPE polymer film with the trapezoidal pattern imprint, cells were able to grow haphazardly in all directions. The observations are shown by SEM-images (Figure 32, 33). With the same cell growing time as the other films after two days, most area of this sample was covered. The results for the trapezoidal imprint polymer films were similar to that of the

control film, although the number of attached cells was considerably lower on the patterned film. The reasons might be, first, the trapezoidal wall is only about 36 microns. Cells can be relatively easy to “climb up” the groove and grow on the ridges; another possible reason is regarding to the wide space on the ridges. It is believed that the imprinting process gives less difference in roughness between the different parts of the substrate, i.e. wall, top and bottom of a groove, as compared to lithographic processes [46].

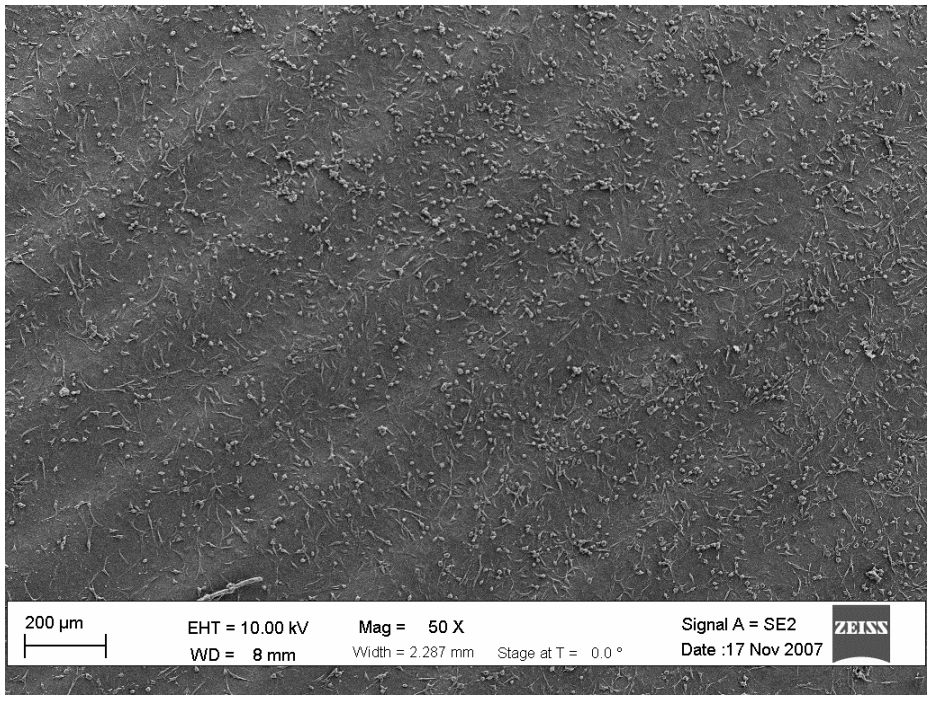


Figure 32 SEM micrograph of cells grew on trapezoidal walls after two days (1)

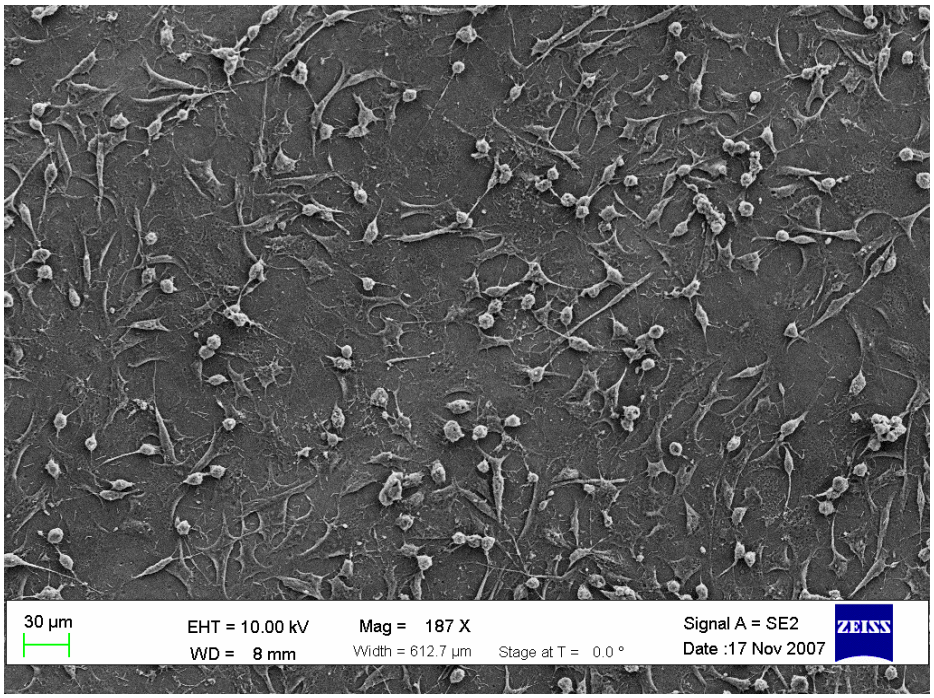


Figure 33 SEM micrograph of cells grew on trapezoidal walls after two days (2)

CHAPTER 6

SUMMARY

Organ transplantation and tissue reconstruction are useful solutions for people suffering from cardiovascular disease. Previous research indicated that the compliance of the graft should be similar to that of the blood vessel to be replaced.

We have presented an observation of a new biodegradable polymer applied to the research of tissue engineering. Our idea was to create a structure to imitate blood vessel muscles in vitro and also to guide the growth of endothelial cells when they start spreading and trying to connect with each other. CUPE is a newly invented biodegradable polymer. It has great mechanical properties and biocompliance. The polymers were imprinted with two shapes of channels from silicon moulds, which were fabricated by two different etching methods as cell culture scaffolds. SEM micrographs showed the results of cell growth.

After 2 days in culture, numerous cells had spread out on the polymer surface. Most cells were found to align along the imprinted micropatterns. The SEM-images of the samples showed that the cells growth occurs mainly along the channels. Some of the cells from both sides of wall attended to connect with each other but inhibited by the height of the wall. It can be seen that the polymer surface will be completely covered by cells after more days.

Comparing three kinds of patterns: flat surface, channels with triangular and trapezoidal walls, 3T3 cells demonstrated obvious tendency of being guided by channels on the sample with triangular walls. It is conjectured that the narrow space on the top of triangles straitens cell growth. Moreover, owing to the higher depth of triangular walls, it is more difficult for cell to connect with each other from two sides of walls when the cell membrane started to spread out. However, with the same days of cell growth, all trapezoidal walls were covered. The polymer surface cannot be seen anymore under the top view of SEM. The results of this study show that 3T3 fibroblast cells can be guided by micro-sized structures on a polymer surface. Cells also demonstrated high preference for the scaffold material, CUPE.

REFERENCES

1) *R. Langer and J.P. Vacanti, "Tissue engineering," Science 1993; 260, 920-926.*
2) *Voorhees AB Jr, Jaretzke AL III, Blakemore AH: The use of tubes constructed from Vinyon-"N" cloth in bridging arterial defects. Ann Surg 1952; 135: 332.*
3) *Goyanes J. Substitution plastica de las artenas por las venas, o arterioplastica venosa, aplicada, como nuevo metodo, al tratamiento de los aneurismas. El Siglio Medico 1906; 53: 546.*
4) *Oudot J. La greffe vasculaire dans les thromboses du carre-four aortique. Press Méd 1951;59:235–6.*
5) *Dubost C, Allary M, Oeconomos N. Resection of an aneurysm of the abdominal aorta: reestablishment of continuity by a preserved human arterial graft, with result after five months. Arch Surg 1952;64:405–408.*
6) *Nillason, L. E., and Langer, R.S. Advances in tissue engineering of blood vessels and other tissues. Transplant. Immunol 1997; 5: 303.*
7) *Tiwari, A., Cheng, K. S., Salacinski, H., Hamilton, G., and Seifalian, A. M. Improving the patency of vascular bypass grafts: The role of suture materials and surgical techniques on reducing anastomotic compliance mismatch. Eur. J. Vasc. Endovasc. Surg 2003; 25: 287.*

8) *Teebken, O. E., and Haverich A. Tissue engineering a small diameter vascular grafts. Eur. J. Vasc. Endovasc. Surg 2002; 23: 275.*

9) *He, H., and Matsuda, T. Arterial replacement with compliant hierarchic hybrid vascular graft: Biomechancial adaptation and failure. Tissue eng2002; 8: 213.*

10) *Peters MC, Mooney DJ. Synthetic extracellular matrices for cell transplantation. Mater Sci Forum 1997; 250:43–52.*

11) *Wang SG, Yang J, Cai Q, Shi GX, Bei JZ. Research progress of biomaterial and cells scaffold for tissue engineering use. Chin J Plast Surg 2000; 16(6): 328–30.*

12) *Yang J, Bei JZ, Wang SG. Cells scaffold for tissue engineering and improvement of cells affinity between the cells and cells scaffold. J Funct Polym 2000; 13(4): 455–60.*

13) *WebbK, HladyVand TrescoPA, Relative importance of surface wettabilityandchargedfunctional groups onNIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. JBiomedMater 1998; 41: 422–430.*

14) *DawR, CandanS, BeckAJ andDevlinAJ, Plasmacopolymer surfacesofacrylicacid/1,7 octadiene:surface characterization and the attachment of ROS 17/2.8 osteoblast-like cells. Biomaterials 1998; 19: 1717–1725.*

15) *DawR, CandanS, BeckAJ andDevlinAJ, Plasmacopolymer surfacesofacrylicacid/1,7 octadiene:surfacecharacterization and the*

attachment of ROS 17/2.8 osteoblast-like cells. Biomaterials 1998; 19: 1717–1725.

16) *VanWachemPB, HogtAH, BeugelingT, FeijenJ, BantjesA, DetmersJP and Van AkenWG, Adhesion of cultured humanendothelial cells onto methacrylate polymers with varyingsurfacewettabilityandcharge. Biomaterials 1987; 8: 323–328.*

17) *ChehroudiB,GouldTRLandBrunetteDM, Titanium-coated micromachinedgroovesofdifferentdimensionsaffectepithe- lial andconnective-tissuecells differentlyinvivo. JBiomed MaterRes 1990; 24: 1202–1219.*

18) *M. Crombeza, b, P. Chevalliera, b, R.C. -Gaudreaulta, c, E. Petitclerca, c, D. Mantovania, b and G. Larochea, b, Improving arterial prosthesis neo-endothelialization: Application of a proactive VEGF construct onto PTFE surfaces. 26; 35: 7402-7409.*

19) *Bernard A, Fitzli D, Sonderegger P, Delamarche E, Michel B, Bosshard HR, et al. Affinity capture of proteins from solution and their dissociation by contact priniting. Nat Biotechnol 2001; 19: 866-869.*

20) *Goessl A, Bowen Pope DF, Hoffman AS, Control of shape ad size of vascular smooth muscle cells in vitro by plasma lithography. J Biomed Mater Res 2001; 57: 15-24.*

21) *Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110: 637-687.*

22) *Michael KE, Vernekar VN, Keselowsky BG, Meredith JC, Latour RA, Garcia AJ. Adsorption-induced conformational changes in fibronectin due to interactions with well-defined surface chemistries. Langmuir 2003; 19: 8033-8040.*

23) *Healy KE, Thomas CH, Rezania A, Kim JE, McKeown PJ, Lom B, et al. Kinetics of bone cell organization and mineralization on materials with patterned surface chemistry. Biomaterials 1996; 17: 195-208.*

24) *Maheshqari G, Brown G, Lauffenburger DA, Wells A, Griffith LG. Cell adhesion and motility depend on nanoscale RGD clustering. J Cell Sci 2000; 113 (Pt 10): 1677-1686.*

25) *Rajagopalan P, Marganski WA, Brown XQ, Wong JY. Direct comparison of the spread area, contractility, and migration of balb/c 3T3 fibroblasts adhered to fibronectin- and RGD-modified substrata. Biopbys J 2004: 87: 8718-8727.*

26) *Luk YY, Kato M, Mrksich M. Self-assembled monolayers of alkanethiolates presenting mannitol groups are inert to protein adsorption and cell attachment. Langmuir 2000; 16: 9604-9608.*

27) *Didier Falconnet, Gabor Csucs, H. Michelle Grandin, Marcus Textro. "Surface engineering approaches to micropattern surfaces for cell-based assays." Biomaterials 2005; 27: 3044-3063.*

28) *Chen CS. Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997; 276: 1425-1428.*

29) *McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS, Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Develop Cell 2004; 6: 483-495.*

30) *Thomas CH, Collier JH, Sfeir CS, Healy KE. Engineering gene expression and protein synthesis by modulation of nuclear shape. Proc Natl Acad Sci USA 2002; 99: 1972-1978.*

31) *Park TH, Shuler ML. Integration of cell culture and microfabrication technology. Biotechnol Prog 2003; 19: 243-253.*

32) *Jung DR, Kapur R, Adams T, Giuliano KA, Mrksich M, Craighead HG, et al. Topograpical and physicochemical modification of material surface to enable patterning of living cells. Crit Rev Biotechnol 2001; 21: 111-154.*

33) *Iegler C. Cell-based biosensors. Fresenius' J Anal Chem 2000; 366: 552-559.*

34) *Nancy Love, Brian Love, Virginia Polytechnic Institute and State University Laurie Locascio, John Travis, NIST. Cell-based sensors for screening toxins*

35) *Kleinfeld D, Kahler KH, Hockberger PE. Controlled outgrowth of dissociated neurons on patterned substrates. J Neurosci 1988; 8: 4098-4120.*

36) *S. Wolf and R.N. Tauber, Silicon Processing for the VLSI Era Volume 1- Process Thchnology Sec Edition.*

37) www.mrsec.harvard.edu/education/ap298r2004/

38) *http://www.dbanks.demon.co.uk/ueng/wetetch.html*

39) *http://www.siliconfaroast.com/dryetch.htm*

40) *Stephen M. Rossnagel, Jerome J. Cuomo and William D. Westwood, HANDBOOK OF PLASMA PROCESSING TECHNOLOGY Fundamentals, Etching, Deposition, and Surface Interactions.*

41) *Ayon A A, Braff R, Lin C C, Sawin H H and Schmidt M A, Characterization of a time multiplexed inductively coupled plasma etcher. J. Electrochem. Soc 1999; 146: 339.*

42) *http://matthieu.lagouge.free.fr*

43) *http://en.wikipedia.org/wiki/Deep_reactive_ion_etching*

44) *http://matthieu.lagouge.free.fr/microtechnology/dry_etch.html*

45) *U. Siemann. Solvent cast technology – a versatile tool for thin film production Progr Colloid Polym Sci 2005; 130: 1–14*

46) *Fredric Johansson, Patrick Carlberg, Nils Danielsen, Lars Montelius, Martin Kanje. Axonal outgrowth on nano-imprinted patterns 2006; 27: 1251-1258*

BIOGRAPHICAL INFORMATION

Lee-Chun, Su was born on April 20, 1980, in Pescadores, Taiwan. In 2000, she left for Taichung to study in Feng Chia University of Fiber and Composite Materials and she got her bachelor degree in 2004.

In 2005 she began graduate work in the Department of Material Science and Engineering at University of Texas at Arlington, USA. She is currently a candidate for the degree of Master of Science in Material Science and Engineering to be awarded at the fall commencement, 2007.