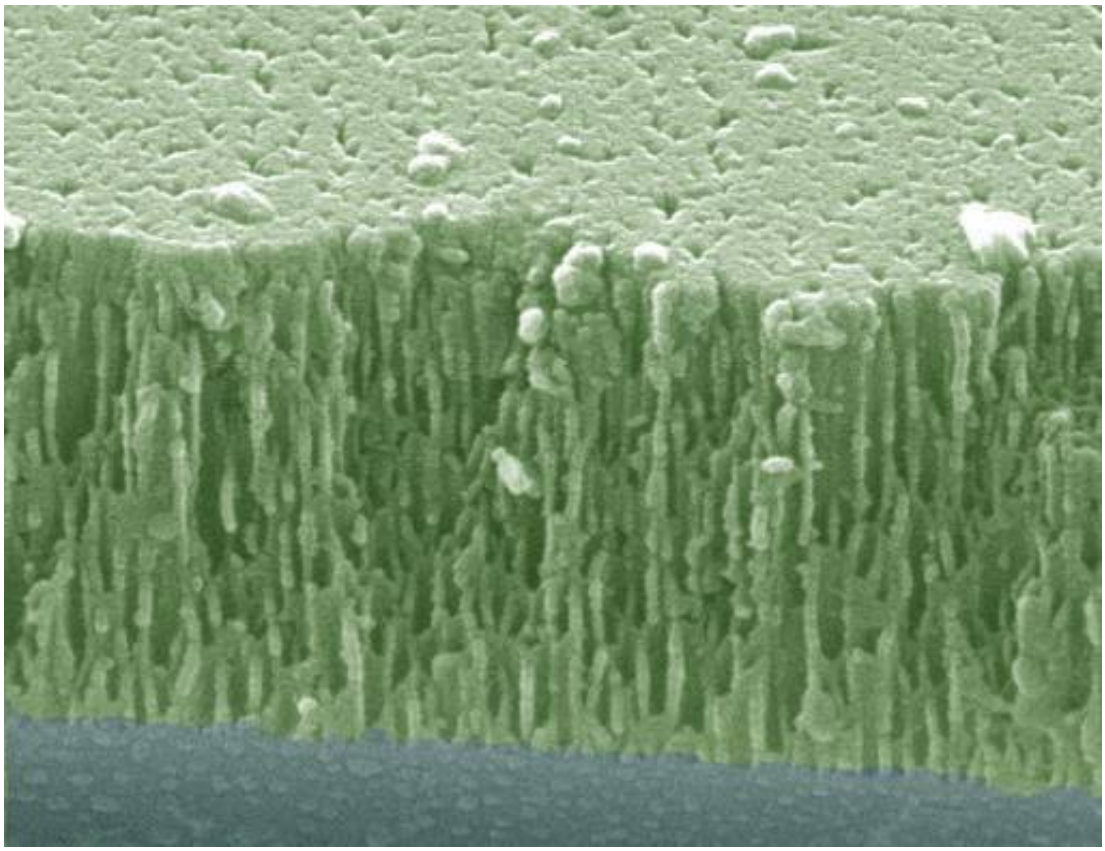
Chapter 1



Introduction to porous silicon and its potential in biomaterial and photonic applications

1. Introduction to biomaterials

Research on materials interfaced with biological systems for evaluating, treating, replacing or enhancing any function of the body (so called biomaterials) is an inter-disciplinary field. Driven by the identification of newer and more complex diseases and disorders, there is an increasing demand for biomaterials that are suitable for a range of practical applications and these demands had, in turn, motivated significant research efforts over the years generating significant advances in materials synthesis and evaluation[1-4].

Biomaterials can be described as natural or synthetic materials that are used as substitutes for implants, drug delivery or biosensors within the living body[1]. There are several key attributes to the use biomaterials in the body. Biomaterials can be engineered to feature material properties which mirror the ones of the faulty body part the biomaterial is replacing. Secondly, new additional properties such as increase in structural strength and anti-biofouling capabilities can be integrated into those artificial materials. Thirdly, hardly any constraints remain today in terms of the size and shape that the material can be manufactured. Furthermore, degradable biomaterials are available where the degradation kinetics can be tuned from days[5] to years[6]. This last point is very useful, especially for regenerative medicine, tissue engineering and drug delivery. Some common biomaterial applications have been summarised by Ratner et.al and are as shown in table 1[1].

Medical uses	Non-medical uses
Artery grafts	Array for DNA and diagnostics
Breast implant	Bioremediation materials
Cochlear implant	Biosensors
Ear drainage tube	Bioseparations, chromatography
Dental implant	Bio-fouling resistant materials
Feeding tube	Biomimetics for new materials
Hydrocephalous shunt	Cell culture
Introocular lens	Control release for agriculture
Joints (hip, knee, shoulder)	Electrophoresis materials
Keratoprosthesis	Fuel cells (biomass)
Left ventricular assist device (LVAD)	MEMS
Medical heart valve	Muscles (artificial) and actuator
Nerve guidance tube	Nanofabrication
Ophthalmic drug delivery device	NEMS
Pacemaker	Neural computer/biocomputer
Renal dialyzer	Smart clothing for biowarfare
Stent	Yeast array chip
Tissue adhesives	
Urinary catheter	
Valve, heart	
Wound healing	
X-ray guide	
Zirconium knee joint	

Table 1. Common uses for biomaterials (listed from A-Z)[1]

However, before any material can be used as a biomaterial, there are certain prerequisites for consideration. Gelain et al., proposes ten essential conditions for a material before it can be used as a biomaterial. These points are as summarised below[7]:

1. Good biocompatibility
2. Amenable surface chemistry
3. Controllable degradation rate
4. Displaying low or no cytotoxicity
5. Promote good cell-substrate interaction
6. None or little immune response
7. Economical

8. Ease in transportation and use
9. Chemically compatible with aqueous environment
10. Permits the integration with other material in the body

While many of these outlined conditions are interconnected in a number of ways, the most significant of all these factors, arguably, for motivating and influencing the application of biomaterials is biocompatibility. This can be described as the ability of a material to perform with an appropriate host response in a specific application and is often the most considered criteria during biomaterial design[1, 3, 8]. Immune responses from the host to biomaterial can occur with materials having poor biocompatibility. Normally, upon introduction of the biomaterial into the host, nonspecific protein adsorption occurs nearly immediately while this response is not commonly observed in normal physiological entities found within the body. In such an event of nonspecific protein adsorption, commonly termed as foreign body reaction, the result is either be a tolerance to the material or an inflammation at the biomaterial interface. Any inflammations could well frustrate attempts to efficiently serve its functions or, in worse scenarios, cause physiological damage. Nonspecific protein binding on the biomaterial surface is generally regarded as the initial precursor to subsequent immune responses mounted by the body[9]. Due to many physiological implications that have led to implant failures, the subject of foreign body reactions to biomaterials had been reviewed extensively in literature[9-12].

One of the advantages of using a well selected biomaterial platform is its ability to elicit responses that may closely mimic those expected from natural materials found within the human body[2, 7]. It is possible to produce biomaterials platforms that can integrate into the body to perform a function similar to its natural counterpart. Secondly, due to its mimicking

properties, these biomimetic materials are often well-suited for characterising and studying cellular behavior *in vitro*, a situation that had previously been proven rather difficult to observe within the living body. This study of cellular behavior is important for the successful integration and higher durability of biomaterial implants.

Another important consideration for biomaterials involves surface modifications allowing new chemical moieties and physical attributes to be incorporated onto the surfaces. These surface modifications can aid in transforming a biologically inert material into a bioactive material having good cell specificity and this is useful for devices that are required to interface directly with cells. The most common approach in transforming the material involves the coupling of specific protein layers or self-assembling peptide scaffolds on the material surface to promote and enhance specific cell adhesion and tissue bioactivity. One good example is the use of the oligopeptide, arginine-glycine-aspartic acid (RGD) peptide[13]. This peptide moiety is found in a number of extracellular matrix proteins, such as collagen[14] and fibronectin[15] and upon introduction on the surface, these short oligopeptides help to direct adhesion and promote bioactivity[16] or help reduce immune response to the implant[15]. Biomaterials can also be fabricated in such a way that the surface will present to the adhering cells 3-D topographical features similar to those found in the native system, with examples such as hydroxyapatites[8, 17].

While cell adhesion and attachments are strongly encouraged in certain biomaterial applications, other purposes such as drug delivery and biosensors may require a low biofouling surface to discourage cell adhesion or protein adsorption. It is possible to fabricate low-fouling surface by incorporating functional groups such as poly(ethylene glycol) (PEG)[18] or phospholipids[19] on these surfaces which will strongly discourage cell or protein adhesion.

When selecting a biomaterial platform, it is also vital for the candidate to possess either low or negligible toxicity. This is especially important in biodegradable systems where degradation products are released during the degradation phase. These molecules can be either inert or might trigger certain immune responses in the host. For the latter scenario, the triggering of immune responses might possibly result in adverse effects on the well-being of the recipient of the bioimplant. It is therefore imperative to select for a system in which the effects from the degradation are either minimized or manageable. Finally, depending on the purpose of intended use of the biomaterial, degradation of the biomaterial can be often tuned to occur over short (hours to days) or long time frames (months to years).

1.2 Cell-surface interactions

During the designing and selecting of a biomaterial, one of the most crucial considerations will be the nature of cell-surface interaction. It has been well documented that many cells phenotypes are *anchorage dependent*, i.e. it is necessary for them to bind to surfaces in order to survive and proliferate. But binding onto the surface alone does not necessarily guarantee the desired cellular outcome. It has been shown in the past that the process of spreading and differentiation upon adhesion are as equally important[20,21]. Cell shape had been reported influencing cell vitality[21] and even gene expression[22,23]. Unfortunately there is a current lack of knowledge in regards to the cellular response to biomaterials and this has often been attributed to difficulties of correlating the net effect of both surface chemistry and topography on cellular behaviour[24]. Furthermore, the current state of affairs is also complicated by the use of different cell types by different authors, which renders direct comparison and correlations of data rather daunting even on the same biomaterial surface. As mentioned earlier, it is of utmost importance that any biomaterial must possess negligible toxic or injurious effects on top of the ability to promote a desirable cellular response. However, prior to designing a biomaterial for cells and tissue engineering, it is necessary to first understand and appreciate the events occurring during initial cell-surface interactions.

In principle, the response of individual cells to the biomaterial can be described as being dependent on how well this material mimics the natural environment. It is important to note that the final cellular outcome can often be decided from the initial cell-surface interaction[25]. Investigation into this early interaction is pivotal since the early stage of cell development has been identified having cursors contributing to the final cellular outcome and behaviour[26,27]. As such, in order to develop an optimal biomaterial, it will be necessary to first understand the fundamental workings of cell-surface interaction regardless of material. Only with this

apprehension of initial cell-surface interactions can the fate of the final outcome be controlled by introducing a series of externally induced factors. For example, it was found that the actual spacing of integrin binding ligands presented on a surface can change and influence final cell morphology and behaviour almost immediately after cells were seeded onto the surface[27,28].

The actual event of cell adhesion on the surface is a multi-stage process[25]. As cellular/protein contacts on surfaces are nearly unavoidable due to the administering environment, upon initial exposure of the biomaterial to a biological environment, the surface undergoes rapid adsorption of small fast diffusing proteins (Vroman effect) such as fibrin[29,30]. This initial adsorption of small proteins on the surface that will later dictate and regulate the way cells are attached on the surface[30,31]. The conformation and the quantity of these proteins on the biomaterial in the initial stages also influence the quantity of preliminary cellular attachment. The potency from these adsorbed proteins to cell attachment is dependent on physiochemical features such surface energy, geometry/topography and also the pH of the environment and surface chemical composition[24,32].

Weak cellular attachments on the surface occur almost immediately upon the fast protein adsorption and preliminary attachment points as small as $0.01\mu m^2$ have been reported[33]. Within minutes, gels of polysaccharides and larger fibrous proteins such as fibronectin and collagen are secreted from cells via exocytosis[34] and these proteins start to deposit on the surface following the initial phase of small protein adsorption and weak cell attachment. These large fibrous proteins together with polysaccharide gel and glycosoaminoglycan ultimately form an embedding network on the surface known as the extra-cellular matrix (ECM). Cellular attachment to the surface is further strengthened by the adhesion of surface

transmembrane adhesion receptors (integrins) and their corresponding ECM proteins[27]. The assembly of the complexes that bridge between the ECM and the cytoskeleton of the cell are further mediated by a series of integrin proteins present on the surface of the cell[35,36]. It has been well demonstrated that upon binding to cell adhesive ECM epitopes (RGD, YIGSR, etc.), several intracellular anchor proteins are also recruited to the adhesion site, ultimately inducing the assembly of focal complexes/focal adhesions points and the associated cytoskeleton[37]. However, the actual biochemical and biophysical aspects of these processes are still largely unknown[27].

In comparison to the cellular attachment phase, the adhesion phase spans across hours. Upon attachment to the surface, the cells will undergo a subsequent flattening by non-specific electrostatic forces such as Van der Waals and by passive formation of ligand-receptor complexes[33]. The cell flattening on the surface resembles the flattening of a liquid droplet on a highly wettable surface and this process helps expand the contact area between the cell and the surface. Further strengthening of the flattened cell is then achieved by extensive attachment of adhesion proteins such as fibronectin, collagen and laminin to the integrin ligands from the cell membrane.

The next phase of cell-surface adhesion is the spreading, differentiation and the subsequent proliferation of the cells and this can have a time frame spanning from couple of hours or even days. During this phase, the cells undergo extensive topological rearrangement of the plasma membrane to flatten out on the surface. The extension can occur either through the formation of a series of lamellipodia (thin actin-rich veils) or by fingerlike projections termed as filopodia[38, 39]. Both types of extensions will involve polymerisation of actin filaments in conjunction with extensive reorganisation of the plasma membrane. The extent of this

extension plays an important role for the differentiation of the cells on the surface. If a cell fails to attain an ideal shape on the surface, it is possible that the situation may retard the differentiation and proliferation[23]. The extent of spreading and how well the cells differentiate and proliferate on the surface in vitro is in turn dependent on how well the ECM is presented on the surface[38].

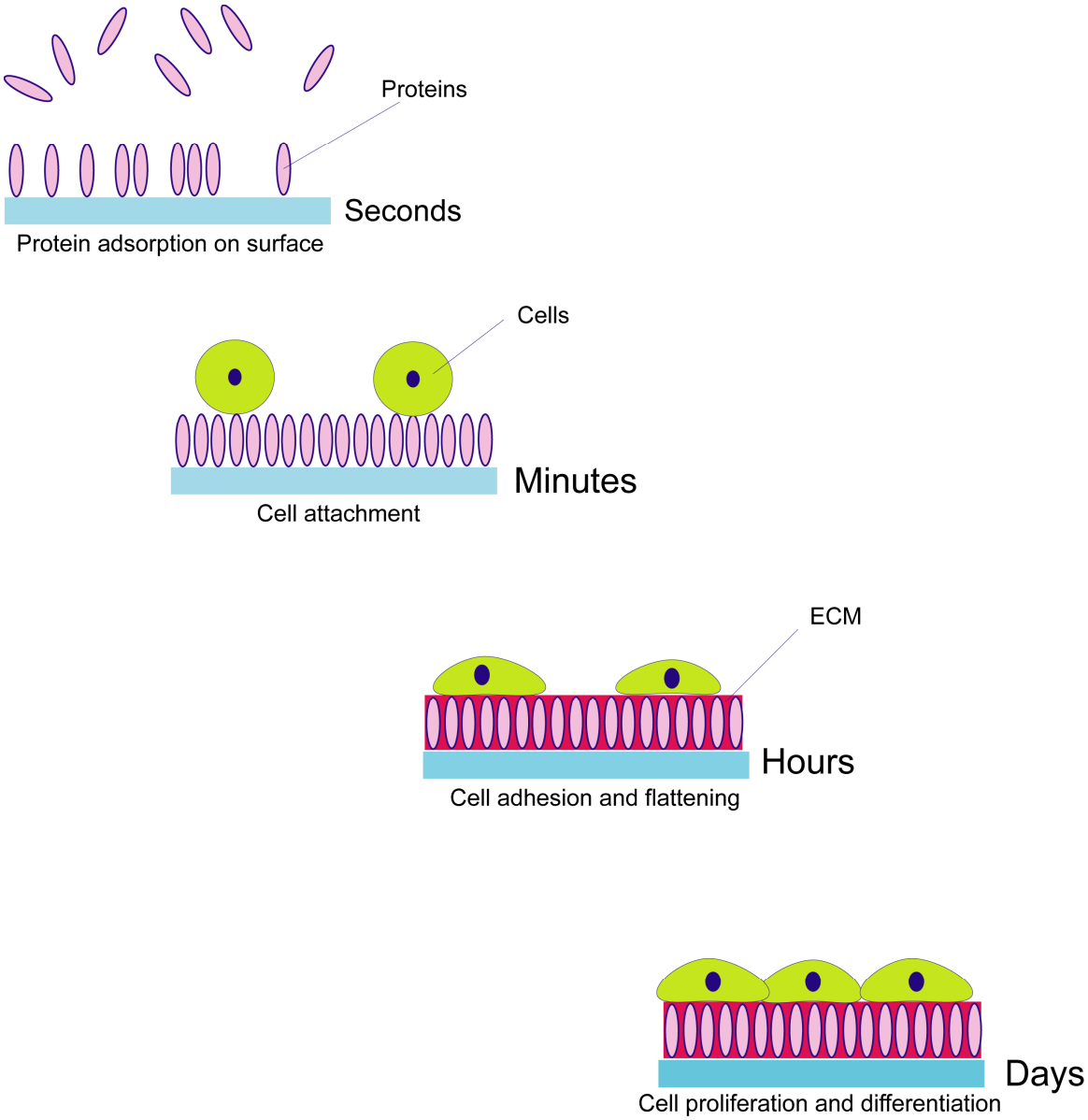


Figure 1.1 - Schematic of the series of events after introduction of cells onto a biomaterial surface.

In a sense, the organisation and the potency in the ECM presentation directly controls the spreading, differentiation and proliferation of the cell. And this organisation and assembly of

ECM is in turn affected by many surface properties such as wettability, surface roughness and topography. On a surface where the ECM presentation to the cells is unfavorable, this can results in a less than desirable outcome in terms of the cellular response.

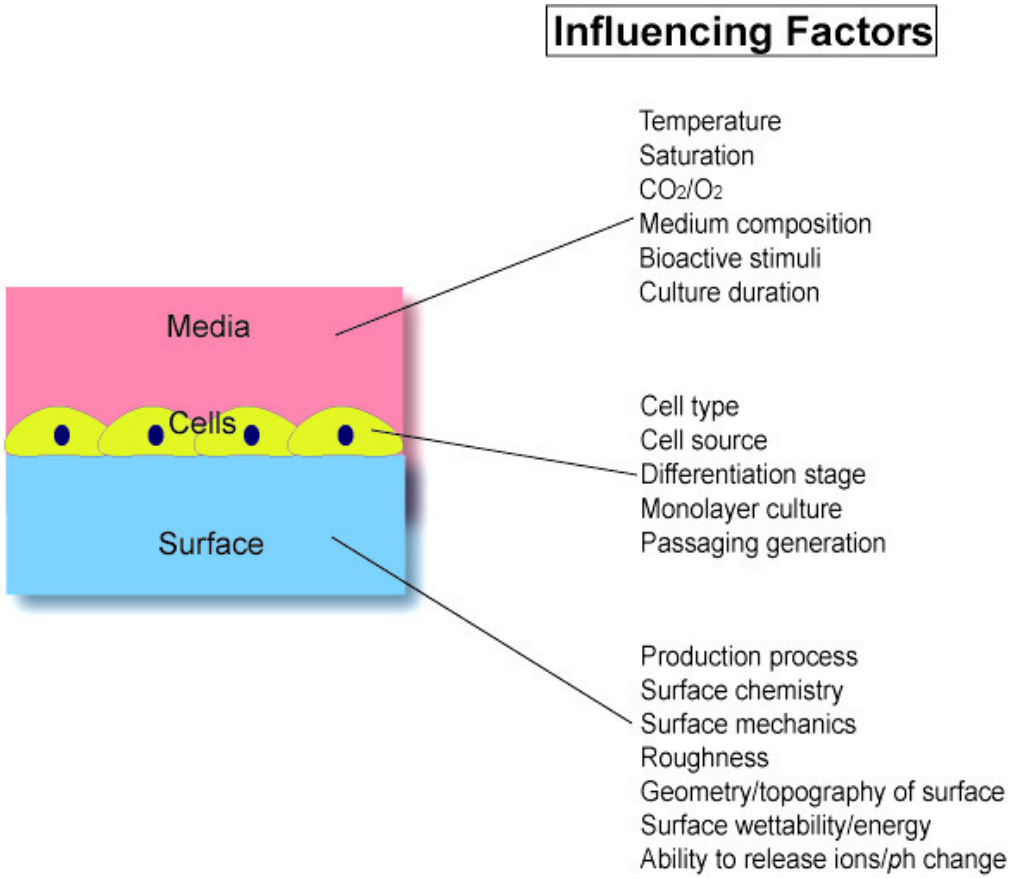


Figure 1.2 - A listing of the various factors that can potentially influence the outcome of cells growing on biomaterial surfaces.

In designing and selecting an appropriate biomaterial platform, there are also many external factors that can also influence cell development. Some of those more commonly known factors are listed in figure 1.2. In all these considered factors, the most important ones are arguably those concerning the physicochemical dynamics on the surface. While issues from the media conditions and cells culture can be easy amended and rectified in-situ, changing the conditions of the surface can prove difficult especially after cells have contacted the surface.

Thus, in order to design a sound biomaterial platform that will promise favourable cell-surface interactions, it will be important for us to evaluate some of the surface properties that can potentially influence cellular outcome. After all, biomaterial design is about striking the right balance between topography, chemistry and substrate mechanics in order to optimize cell response. In this thesis, one of the most important physicochemical dynamics, surface topography, will be further addressed.

1.3 The influence of surface topography and roughness on cell adhesion

One of the most important physicochemical factors affecting cell adhesion is the three-dimensional topography of the surface. In brief, this can be described as the size, the shape and the surface texture of the material. Two main categories commonly used to describe the surface texture during cell-surface interaction studies are topography and roughness[40]. Surface roughness is interpreted as a random pattern of features that are much smaller than the cell while topography describes patterns of features deliberately presented from the surface to the adhering cells. It was first reported early last century that anisotropic features on surfaces can induce cell morphological changes[41] and this paradigm has since generated significant interest in the scientific community[42-46]. It was also later discovered that the propagation and alignment of cells can be precisely controlled on a surface by inducing them to follow certain surface contours. This phenomenon is widely known as contact guidance and so far, there are many reports using contact guidance to describe the cellular behavior in relation to surface topography[40,47,48].

When a growing adherent cell first comes into contact with the surface, it reorganises its cytoskeleton to adapt to any topography features that are present under its contact area in order to attain a most energetically favored state. As such, the cellular morphology often changes upon contact with a textured surface and these physical changes in shape can cause metabolic changes in the cells[22]. The ways cells respond towards surface topography/roughness is different for every cell phenotype[49-51]. Most mesenchymal cell types such as epithelial, fibroblast and osteoblast, often spread in directions following the texture of the surface[3,52]. Interestingly, on rough surfaces, these cell lines often complete their adherence process to the surface much earlier than when seeded onto polished surfaces

and this suggest that rough surface can closely mimic the natural environment for these cell line[52]. The alignment of these elongating cells has also been found following the contours of a texture surface much closer than spherically shaped cells. The importance of surface topography is further reinforced by the fact that some cell types such as endothelial cells are unable to assume their native morphology *in vivo* when cultured on untextured surfaces in vitro. Only by presenting an appropriately textured surface such as micron-scale lines and ridges, normal cell morphology is observed[3]. There are also instances where surface topography had been held accountable for inducing cell apoptosis[21].

Osteoblastic cells in particular have been used in studies of the effects of biomaterial surface topography on cell behavior. The main driving impetus for using osteoblast cell is to develop a bone substitute material that can effectively mimic the tensile strength of the bone and thus promote and improve bone regeneration in vitro. Various reports have demonstrated that osteoblast cells are sensitive to the topographical features of the material at varying degrees[43,53,54]. However, the direct correlation is rather vague due to the fact that there is no general consensus concerning the proper representation of the surface and the cells[32,55], i.e. differences in the selection of topographical features and cell lineage renders direct comparisons difficult.

With the altering of topographical features on a surface, it is important to note that surface wettability/energy is also being changed. This change in wettability must be taken in account when describing cell-surface interaction for it has been reported in the past that there is a relatively narrow margin in wettability in which cell adhesion is encouraged[56-58]. It has also been demonstrated that apart from topographical influence over cell-surface interactions,

wettability can have an effect on intracellular interactions[58] and this is pivotal to tissue development.

1.3.1 Linear topographical patterning

The fabrication of linear lines has been employed extensively and is one of most common approaches used to describe the effects of physical cues on cell response[40,54,59-63]. Generally, this includes the fabrication of well-aligned linear grooves and ridges with interspacing width/height dimensions spanning from several microns[40,59,62,63] to tens of nanometers[60, 64, 65]. Attaining such linear features on the surface is readily achieved by conventional methods such as deep reactive etching[66] or embossing[40]. If the width of these grooves and the interspacing of these ridges have dimensions larger than the width of the cell, the surface patterns will effectively "entrap" the cell within the groove thus confining cellular orientation and intracellular interaction. This forces the cells to grow in an alignment following the direction of these grooves[59]. Under such topography, it is still possible for the cells to retain a similar cellular morphology comparable to those cultured on flat surface, despite of their overall tissue alignment. If the interspacing of these grooves and ridges are smaller than the width of the cell, cellular extension and extracellular projections can still be observed following the general direction of the grooves[60]. The general response of cells growing on these linearly patterned surfaces is the reorganisation in both the cytoskeletal network and focal adhesion points[67,68]. These cellular changes were also reported to be more prominent for grooves with interspatial dimensions of between 1-5 microns as these physical features are generally smaller than the width of the adhering cells. Past studies using 1-5 micron linear surface patterns had demonstrated that the cellular focal adhesion points of the cell, regions of adhesive structures that actually connect the cell membrane to the ECM, will

preferentially align along the top ridges of the patterns[59,62] or even increase surface contact area[68]. This alignment of the focal adhesion points and subsequent cellular propagation can then be carefully guided along the surface by specifying the direction of the grooves and ridges.

One potential weakness in using these micron-size linear features to control cell-surface interaction is the fact that most mammalian cells have an average width of 10-20 μm, and thus any micron size features under the cellular contact area to the surface would certainly be felt by adhering cells. While this will almost certainly guarantee a cellular response, the actual sensitivity range to topographical change which can be felt by the adhering cell has not been properly addressed. With the present realization that cell sensitivity begins at the submicron/nanometer regime[43,49], micron size patterning on the surface in the past have seemingly achieved little in addressing the actual cell-surface mechanism other than achieving a precisely tuned surface orientation. Only a few investigations using nanoscale grooves have been performed[60,69-71] and a cellular response to grooves with spacing as low as 70 nm has been reported[60]. So far, theories such as the non-uniformities in protein adsorption resulting from an uneven surface energies distribution[72] or specific condensation of actin and vinculin along the grooves/ridges boundaries[46] has been proposed to be responsible for contact guidance. However, their findings have been are rather inconclusive and the sequence of events that leads to cell orientation still remain largely unknown. To this point, the behavior and the actual physical range by which cells will response to topographical cues and the description of how each variation in dimension of topographical feature will influence cellular morphogenesis has not been elucidated.

Another problem faced with linear patterning for addressing surface effects is that these surfaces were often prepared having only a limited range in the variation of the topographical changes[60,69,71]. As such, many authors often had to use large sample numbers to capture a range of feature sizes[60], arguably a tedious undertaking. Gradients of patterns encompassing a wide range of dimensions would allow the investigation of the cellular response to a range of feature sizes on a single sample.

1.3.2 Influence of porous topography on cell behavior

Porous materials have a significant advantage in the field of cell culture and tissue engineering. Porous scaffolds can permit a good flow of bioactive nutrients throughout the material and this is found to be useful in cell differentiation and development. Presently, materials such as hydroxyapatite[4,73,74], chitosan[75,76] and porous alumina[77-79] are some of the more common porous materials that have been used to interpret and explain cell-surface interaction. In conjugation with bioactive molecules promoting cell adhesion, these surfaces have proved to be very successful in encouraging cellular differentiation and overall tissue development. Cellular studies performed on these surfaces have often been done to evaluate the collective cellular development but the actual individual cellular responses to pores size or porosity has been overlooked.

The influence of topography to cellular behavior has also been clearly demonstrated in the past from porous substrates[50,80-82]. This importance of porous materials for tissue engineering has been realized in the literature. For example, 3-D interconnected porous structures have been identified promoting tissue development in vitro[80,81]. Some cell types, such as

osteoblasts, are notoriously indifferent to smooth non-tailored substrate surfaces, but native-like morphology can be achieved on porous substrates[24,32]. As such, porous surfaces had been used frequently for promoting good osteoblast development in vitro and specific pore size regions had already been identified as crucial for a stable interfacing between the osteoblast cell and porous substrates[79].

The description of the "porosity effect" has often been misappropriated with respect to cells adhering on 3D porous polymeric scaffolds such as electrospun fibers[83-85] and polymeric sponges[86,87]. While these materials are correctly labeled as porous, their observations for cell-surface interaction are rather inconclusive. Firstly, the pores derived from electrospun fibers and sponges are extremely disorganised and the surface of the material presented to the adhering cell can be very uneven (from a few hundred nanometers to tens of microns). This renders a detailed correlation between pore size and cell morphology very difficult. Secondly, with regards to large micron size pockets, cells are often observed residing along the individual fibers[83] or in the case of the polymeric sponges, entrapped within the porous structures. Any correlation of cell behavior to porosity in these cases is meaningless unless the topographical aspect from the specific area under which the cells actually resided is taken into consideration as well. As such, in order to correctly correlate cell behavior on porous material, 2D porous surfaces are more appropriate compared to 3D models.

Porous alumina has been utilised successfully in the past to study the direct effects of porosity on cell behavior[78,79]. An interesting finding is that porous alumina with pore sizes of less than 100nm was found to aid adhesion and stimulate metabolic functions of osteoblasts[88,89]. Hepatocytes cultured on porous alumina with pore sizes of 200 nm have been observed to form numerous filopodia extensions from the cell body in order to achieve

better adhesion[90]. However, hepatocytes cultured on pore sizes of 60 nm and less did not exhibit such characteristics[90]. The use of porous alumina is handicapped by the fact that the range of attainable pore size on this material is rather limited (10-450 nm) and it is non-biodegradable in aqueous environment. Due to this limitation and the need for large number of samples, achieving a comprehensive study of cell responses on a wide range of different pore size using porous alumina can be time-consuming and thus stays unlikely.

1.4 Porous silicon

In order to effectively characterise cell-surface sensitivity, it is imperative that the substrate surface be biocompatible as well as able to be fine tuned in terms of the topography at nanometer/submicron range. Furthermore, it would be desirable if a topographical gradient can be easily tailored on such a biomaterial. Such topographical gradient can then be used to understand cell surface interactions without the need for many samples.

To fulfill these criteria, we have decided to select pSi as the surface platform to perform a series of cell-surface studies. pSi is an biodegradable/biocompatible material derived from the porosification of silicon surface via electrochemically anodisation under hydrofluoric acid (HF). Over the past decade, pSi has attracted a lot of attention in the areas of electronic and optoelectronic applications[91,92], biosensors[93-95], biomaterial applications[96-98]. The overall thickness of the porous layer from the wafer is dependent on the conductivity of the silicon substrate, the anodic current density and the duration of anodization. And by controlling the applied anodisation current and the concentration of HF, pore sizes on the surface can be fine tuned from a couple of nanometers to several microns and the porosity attainable ranges from 20-80%[99].

An important feature of pSi is the ease of performing chemical modification on the surface. After etching, analysis of the material had shows that the presence of chemical elements are Si and H on the pSi surface[100] confirming that the surface is predominantly hydride terminated and. Upon oxidation of the surface, it is possible to introduce a wide range of chemical moieties on the surface by methods such as hydrosilyation and silanisation[18,101]. Chemical moieties can also influence the degradation behavior of the porous layer and the

overall rate of degradation is often described as a net effect of both porosity and chemistry[82]. Under aqueous conditions, the degradation can be tuned between couple of hours to months. Due to its biocompatibility[96,98,102,103] and low level of toxicity from its subsequent degradation in aqueous environments to silicic acid[104], pSi surfaces has have already been reported in the past as an excellent biomaterial candidate. Being of a porous material, it also permits offloading of biomolecules throughout the entire porous film, or, in the case of pSi membranes, the flow of nutrients from one side to the other. This can be a very attractive feature for tissue engineering.

In recent years, pSi's ability to serve as reflectors of wavelengths with narrow photonic bandgaps[105-107] has gained much research interest. Through controlled modulations of porosity and pore size, a refractive index modulation of the porous layer can be easily achieved, allowing the fabrication of photonic membranes with good bandwidth confinement properties. These surfaces are excellent reflectors and there are already some attempts to integrate these photonic materials into biodevices[108,109].

1.4.1 Formation of porous silicon (pSi)

Pore formation during anodisation occurs anisotropically and the level of porosity is dependent on the applied current and duration. More importantly, regions that are already porosified will stay unaffected throughout the rest of the anodisation process.
The formation of pSi layer is described below[110];

1. The dissolution reaction stochastically initiates at sites where electronic holes (sites where there is an absence of electron due to the doping) are supplied

2. The effective valence of dissolution of the silicon atom is 2
3. The micropores grow preferentially towards the (103) directions
4. The dissolution reaction occurs at the tip of micropores
5. Inner surfaces of pores are automatically passivated by hydrogen atoms generated during etching

Several mechanisms for the dissolution chemistry of silicon have been proposed with the general consensus that electronic holes are prerequisites for pore formation and electropolishing. During the process of pore formation, two hydrogens are liberated for every Si atom and two electrons are consumed. The reaction is as shown below[111]:

$$\mathbf{Si + 6HF \rightarrow H_2SiF_6 + H_2 + 2H^+ + 2e^-} \qquad \mathbf{(1)}$$

Upon etching, the surface is generally covered by SiH, SiH_2 and SiH_3 surface groups and these have rather limited stability. Further stabilization can be achieved by a variety of ways. Firstly, the surface can be oxidised via rapid thermal oxidation[112] and flux of ozone[113]. The exposure of a halogen vapour has also been reported to oxidise the pSi surface[114]. Nitridation of pSi surface under a rapid thermal annealing in the presence of nitrogen or ammonium was reported to cover the surface with oxynitride functional groups[115]. The profile of a typical pSi film is as shown in figure 1.3.

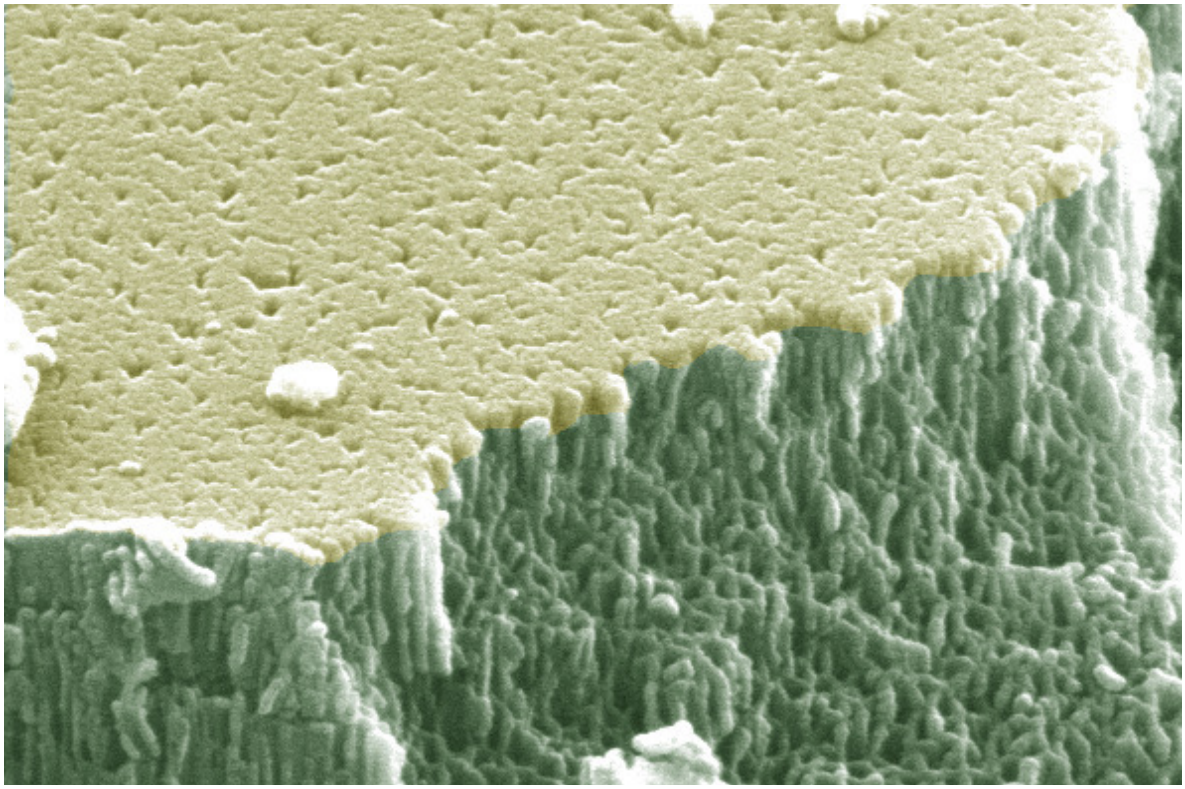


Figure 1.3 - SEM of a partially broken pSi film revealing an array of straight pores.

1.4.2 Factors influencing the formation of pSi

In brief, the formation of pSi and the topographical/profile outcome can be influenced by the following parameters:

1. *Wafer type*

   There are two types of wafers used in pSi fabrication, n-type (negative) and p-type (positive). N-type silicon wafers are doped with Group VA elements such as phosphorous, antimony or arsenic in order to increase the number of free electrons within the material, thus giving it an overall negative charge. These free electrons in turn act as current carriers in the material. P-type

silicon wafers, on the other hand, are doped with Group IIIA trivalent elements such as boron or aluminium to form "electronic holes" within the material (see figure 1.4). These electron gaps in the material will act as current carriers. The nature of the wafer type influences the way the electrochemical anodisation is performed. During the fabrication of n-type pSi, light must be supplied for the generation of hole/electron pairs which in turn drives the anodisation process while illumination is not necessary for p-type pSi[99].

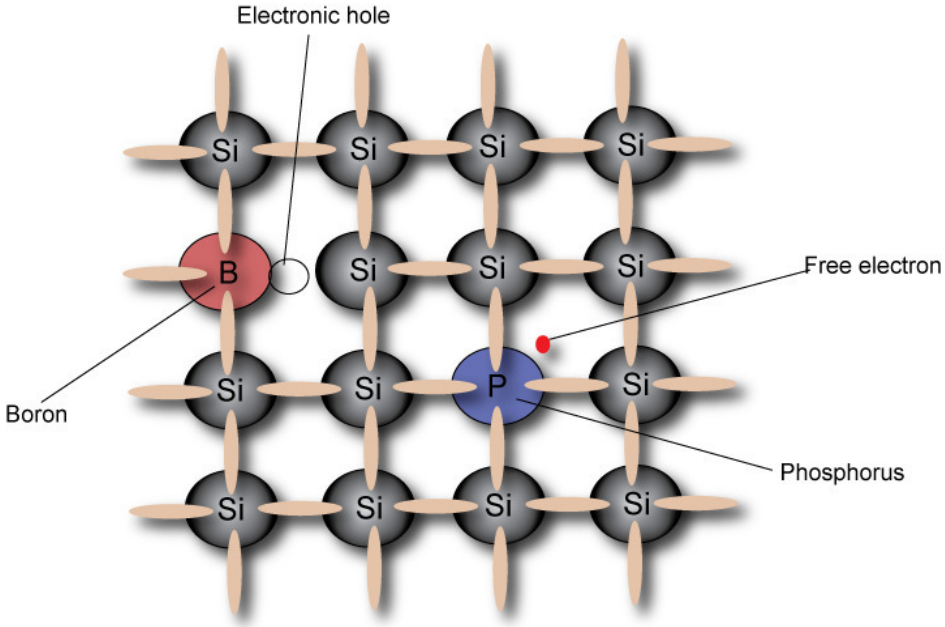


Figure 1.4 – An illustration for both boron (p-type) and phosphorous (n-type) doping within the silicon lattice. Boron doped silicon contains electronic holes that can act as current carriers. The current carriers for phosphorous doped silicon are the free surplus electrons.

2. *The doping level and resistivity of the silicon substrate*

The resistivity of the silicon wafer is dependent on the level of doping; the higher the concentration of the dopant, the less resistive the material. And the resistivity, in turn, influences the porosity range and surface morphology.

Wafers with high resistivity (low doping) often give rise to brittle pSi films and a small controllable range of porosity[99]. However, the pore profiles on high resistivity wafers can have good depth-width aspect ratio in an anisotropic fashion. Wafers with low resistivity (highly doped), in contrast, allow for a wider range in pore sizes to be obtained by anodisation with good film stability but a less ordered microarchitecture.

3. *Current density and concentration of HF*

The current density applied during the electrochemical anodisation directly determines the size of the pore formed. Generally, larger current density applied to the surface will give rise to large pores and while lower current density will produce smaller pores. This is also influenced by the concentration of the HF used during the anodisation. The higher the concentration of HF used, the lower the porosity. Thus, to obtain a desirable pSi pore profile, it is important to optimize both the concentration of HF in the electrolyte solution and the current density applied[99,116,117].

4. *Duration of anodisation*

The thickness of the pSi film is dependent on the duration of the anodisation, the current applied and the concentration of HF. In principle, the longer the anodisation time, the thicker the film. However, it is important to note that in long anodisation duration, there will be a depletion of fluoride ion in the electrolyte and it might be necessary to replenish HF. Secondly, since pore formation proceeds in an anisotropic fashion into the material, as the layer thickens during lengthy anodisation, the deeper regions within the material

will be less accessible to the HF electrolyte. This can result in narrowing of pore size in deeper regions of the pSi film[99].

1.4.3 Porosity and thickness determination

Porosity and thickness are some of the most important aspect of characterising pSi. The determination of the porosity simply involves measuring the change in weight of silicon wafer before etching (m_1), just after etching (m_2) and the weight after complete dissolution of the layer under alkaline conditions (m_3). All these parameters are fitted into the following equation[118]:

$$\textbf{Porosity (\%)} = (m_1 - m_2) / (m_1 - m_3)^{118} \quad \textbf{(2)}$$

From there, it is possible to determine the thickness of the layer in the following equation[63]:

$$\textbf{Thickness} = (m_1 - m_3) / (S \times d)^{118} \quad \textbf{(3)}$$

Where d is the density of the bulk silicon wafer and S is the area exposed to HF during etching. Samples with porosity between 20% and 75% can be easily fabricated. But due to fact that pSi is unable to withstand large capillary forces exert onto it, it is quite difficult to handle pSi at higher porosity. However, techniques such as supercritical drying[119] has been applied to tackle the issue of structural collapse.

1.4.4 Multilayered pSi films

The direct modulation of current density has allowed for the fabrication of multilayered structures in pSi films (see figure 1.5) and this permits for photonic mirrors to be tailored into pSi films. By stacking alternating layers of different porosity/refractive index into the film, these multilayered materials can control and manipulate electromagnetic wave propagations in a similar manner to the way semiconductor chips direct the electron movement by defining energy bands[124]. Multilayered pSi can be produced by (1) by periodically varying the the current density during the electrochemical anodisation (2) by using periodically doped substrates while maintaining a constant etching parameter[125,126]. Photonic crystals derived from pSi are excellent reflectors of light as the light confinement can be easily fine tuned by current density modulation. Interesting, apart from applications in the field of optics, these multilayered pSi films have also been utilized for biosensing[93, 127]. Furthermore, these periodically stacked layers are very useful in controlled release systems for they offer realising different rates of degradation within a single pSi sample[128, 129]. A multilayered pSi film will erode and release the entrapped drug in an aqueous environment at different rates in a successive fashion[130].

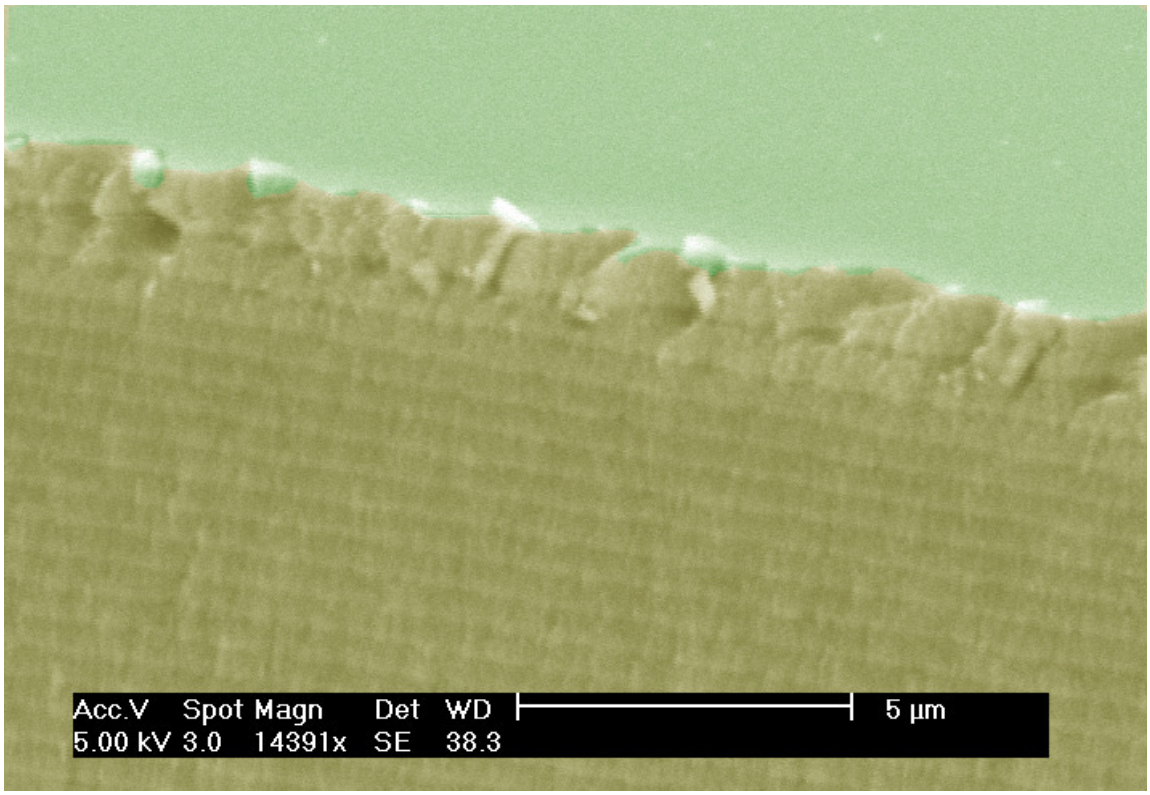


Figure 1.5 – SEM of a multilayered pSi film. The alternating layers within the film can be easily resolved.

1.4.5 Freestanding pSi films

The principle for producing freestanding pSi is rather simple. pSi films are anodised to the required thickness and a large electropolishing current is then introduced. This causes electropolishing at the bottom of the porous layer, which results in the film lifting off the bulk silicon.

Freestanding pSi films detached from the silicon support are extremely useful as bioimplants or drug delivery platform *in vivo* as these films can completely degraded over time[82]. Freestanding pSi film with very high porosity (>80%) can also be very useful in other applications such as thermal isolation[131], but one drawback when liberating these films from the silicon is that at such high porosity, they are very brittle and mechanically unstable during

drying[132]. This is primarily due to capillary forces exerted on the pores walls during the process of evaporation. It is very difficult to produce intact freestanding films from high porosity pSi since the structural integrity of these films is often compromised during drying. Furthermore, being detached from the silicon substrate also weakens the structural strength of the film. Fortunately, it is still possible to avoid these effects by using methods such as pentane evaporation and critical point drying[132,133]. This allows for the fabrication of intact freestanding pSi films[119,134,135] as shown in Figure 1.6.

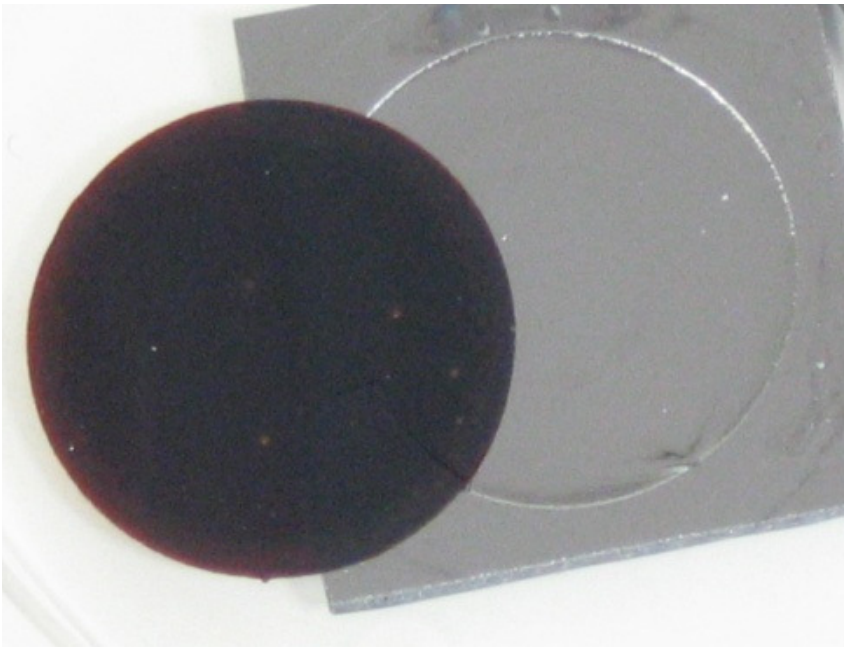


Figure 1.6 – An intact freestanding pSi film liberated from the silicon substrate by electropolishing.

1.4.6 Asymmetric anodisation of pSi

pSi films of homogenously distributed pore sizes are typically produced by helding the face of the platinum cathode parallel to the silicon suface (see figure 1.7 (a)). It is possible to fabricate a pSi film containing a wide distribution of pore sizes by anodising in an asymmetrically arrangement by which the face of the platinum cathode is held perpendicularly relative to the surface of the silicon (see figure 1.7 (b)). In the asymmetric setup, the current potential within the electrolyte solution varies as a function of distance from the electrode due to the resistance of the electrolyte, thus leading to a decrease in current density as the distance from the electrode increases. The end result is a surface displaying a wide gradient of pore sizes that can range from large (1 μm) to small (5 nm). The size range for these pores can be controlled by modifying the anodic current or by varying the HF concentration. Asymmetric anodisation of pSi (figure 1.7 (b)) offers an alternative way to grade the surface apart from the conventional orientation of the electrode which is parallel to the silicon as shown in Figure 1.7 (a).

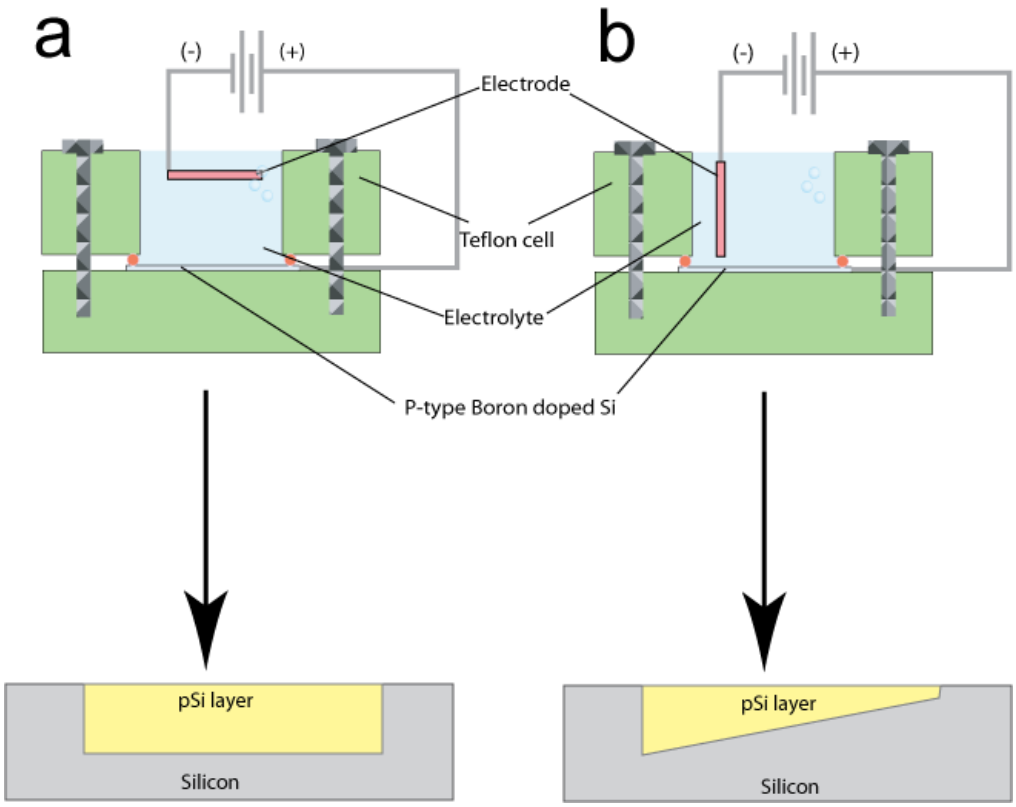


Figure 1.7 – (a) Conventional anodisation of pSi. The film is evenly anodised anisotropically, thus give an equal thickness throughout. (b) Asymmetric anodisation of pSi resulting in a thickness and pore size gradient.

1.4.7 Toxicity, biocompatibility and pSi degradation

Porous silicon on many occasions has previously been shown to demonstrate good biocompatibility[96, 98, 102, 103]. The by-product from pSi degradation is silicic acid which is easily excreted from the body system in urine[104]. Silicon can be found in daily food products (rice, cereal etc) and is often present at the same level with physiologically important elements such as iron, zinc and copper[104]. Thus, the degradation pSi is non-toxic and this can be a major advantage as compared to certain synthetic polymers as a biomaterial platform. The suitability of pSi as a growth support for cell culture has already been extensively investigated and results have shown that pSi is highly compatible in promoting cell adhesion and viability[50,120].

As with most biodegradable porous systems, the rate of degradation is dependent of level of porosity. Larger pore size will promote faster degradation in vivo. As the porosity and depth of the pSi layer is easily controlled by modulating the time and density of the anodizing current, it is therefore possible to generate a wide spectrum of pSi with degradation rates spanning from days to years. Furthermore, in conjunction with tuning porosity, improvements on the surface stability can also be achieved by functionalisation of the surface chemistry with a variety of physical and chemical stratagies[121-123]. These surface functionalisations permits for the tuning of different properties on the pSi surface such as wettability and terminal functional groups and are extremely useful towards tailoring a biomaterial surface. This attribute is especially important in biosensors and bioimplants.

1.5 Aims

pSi is an attractive biomaterial that can easily afford a large range of physical and chemical modifications without compromising the structural and mechanical integrity. There are only a few material candidates that match such flexibility and in many aspects, pSi will still be deemed superior. Engineering pores in silicon via electrochemical anodisation is a relatively easy process and the porosity and pore size can be altered by simple current density modulation. Furthermore, by asymmetrically anodizing the surface, it is even possible to achieve a nanoporous gradient on a single surface in just a one-step process. This inexpensive procedure is more cost/time effective compared lengthy and expensive methodologies such as coating and patterning techniques.

The study of cell-surface on nanostructured surfaces is of great significance with regards to developing the next generation biodevices. It is pivotal to establish a good understanding of the surface chemistry, topographical contours and bioactive molecules, and how these factors would collectively contribute to the desired cellular adhesion and long term maintenance of functions in-vitro. With a better perception regarding these external cues and their plausible effects, we can in turn to develop biomaterial surfaces with the reverse effects, i.e. discouragement of cell-surface interaction to produce low fouling biomaterials. These low fouling surfaces are well-suited for the intentions in bioimplant/drug delivery devices.

This dissertation aims to evaluate pSi as a potential biomaterial as well as producing multilayered photonics structures under four major themes. In particular, this dissertation aims to:

(1) evaluate cell culture on pSi surfaces functionalised by different chemical moieties and to maintain long term cell culture on these surfaces,

(2) micropattern pSi surface by direct laser writing in conjunction with surface chemistry in order to direct and control cell adhesion,

(3) produce porous gradients by asymmetrical anodisation and to use these gradients to investigate the effects on wettability and cell adhesion.

(4) fashioning photonic gradients to demonstrate the versatility and numerous potential of the asymmetrical anodisation technique.

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