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Preparation of chemical gradients on porous silicon by a dip coating method

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ABSTRACT

Gradient surfaces have become invaluable tools for the high-throughput characterisation of biomolecule- and cell-material surface interactions as they allow for the screening and optimisation of surface parameters such as surface chemistry, topography and ligand density in a single experiment. Here, we have generated surface chemistry gradients on oxidised porous silicon (pSi) substrates using silane functionalisation. In these studies, pSi films with a pore size of 15-30 nm and a layer thickness of around 1.7 μm were utilised. The manufacture of gradient surface chemistries of silanes was performed using a simple dip coating method, whereby an increasing incubation time of the substrate in a solution of the silane led to increasing surface coverage of the silane. In this work, the hydrophobic n-octadecyldimethyl chlorosilane (ODCS) and pentafluorophenyldimethyl chlorosilane (PFPS) were used since they were expected to produce significant changes in wettability upon attachment. Chemical gradients were characterised using infrared (IR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and sessile drop water contact angle measurements. In addition, the surface chemistry of the gradient was mapped using synchrotron IR microscopy. The ODCS gradient displayed sessile drop water contact angles ranging from 12° to 71°, confirming the successful formation of a gradient. IR microscopy and an XPS line scan confirmed the formation of a chemical gradient on the porous substrate. Furthermore, the chemical gradients produced can be used for the high-throughput *in vitro* screening of protein and cell-surface interactions, leading to the definition of surface chemistry on nanostructured silicon which will afford improved control of biointerfacial interactions.

Keywords: Gradient surfaces, porous silicon, silanisation, surface analysis

1. INTRODUCTION

The ability to evaluate cell-material surface interactions effectively is one of the keys to the design of new and improved biomedical materials. However, the traditional *in vitro* cell culture methods for assessing the appropriateness of disparate surface properties in a specific application require large numbers of cells and amounts of materials, and have very limited sample throughput. While protein and cell microarrays are currently used to great effect for multiplexed processing and can be applied to the screening of cell-material surface interactions¹⁻³, it is anticipated that the introduction of gradient surfaces displaying spatial variation of physicochemical properties provide an alternative for the fast screening of biointerfacial phenomena^{4, 5}. This is of particular relevance for studying the attachment and proliferation of cells, which can be influenced by many different surface properties including topography^{6, 7}, surface chemistry⁸, wettability^{9, 10}, elastic modulus and the presence of chemical or biological signals^{11, 12}. Much attention has been directed towards surface modification of substrates to either enhance or minimise cellular attachment^{13, 14} as well as influence cell metabolism and function^{15, 16}. The analysis of individual or combined effects of these different properties on the cellular behaviour from discrete experiments is a tantalising task. The use of surface-bound gradients will greatly facilitate the determination and optimisation of the relevant variables and their limits. The use of a continuously graded

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surface can be both time- and material -efficient and this tool also offers advantages in regards to the minimisation of systematic errors normally associated with individual experiments. This approach may allow the tailoring of surfaces to deliver an optimum cell response in a wide range of applications including prosthetics and stem cell technologies¹⁷.

Gradient surfaces with variations in topography^{7, 18}, wettability^{9, 10}, chemical composition^{19, 20}, polymer thickness and cross linking density across one dimension have been investigated by a number of research groups using a variety of surface preparation techniques^{4, 5, 17}. Of greatest interest is the formation of surface chemistry gradients. Techniques including plasma polymerisation²¹, liquid²² and vapour diffusion²³, microcontact printing²⁴, and polymer grafting²⁵ have been utilised to prepare chemical gradients in the past, just to name a few.

It has been shown that wettability can influence the adsorption and attachment of proteins and cells²⁶⁻²⁸. There are two predominant approaches to modify the wettability of a surface: the introduction of chemical moieties onto the surface or altering the surface topography by the creation of patterned or textured surfaces with micro- or nanoscale features. Surface chemistry can be modified by many different methods including silanisation on silicon and silica²³, surface initiated polymerizations on a range of substrate surfaces²⁹ and alkanethiols on gold³⁰. Many of these techniques have been adapted to produce surface-bound chemical gradients. The first reported preparation of a chemical gradient involved the use of methylsilanes in 1987 by Elwing et al.²². Here, the methyl silane was bedded under a xylene solution containing the immersed silicon wafer. From here the methyl silane slowly diffused through the xylo region and was simultaneously bound to the surface. Since then, several researchers have utilised this method to create silane gradients^{23, 31, 32}. Silanes are attractive since the substrate attachment is fast and a wide range of silanes are commercially available, allowing for the incorporation of almost any functionality required to a surface. Monochlorosilanes are a popular choice in such modifications as they are highly reactive towards a Si-OH surface. Additionally, silane crosslinking and polymerisation does not occur for the monosubstituted silane, allowing for formation of a monolayer on the surface.

A key limitation of gradients is that several surface parameters may be altered at the same time³³. For example, the preparation of a surface chemical gradient by plasma polymerisation can also result in variations in surface topography and elastic modulus^{10, 34}. In such cases, it can be difficult to attribute the observed cell- material surface behaviour to a single surface property. Therefore, thorough characterisation of the deposited surface is essential to determine the surface characteristics that can vary along the gradient. Sessile drop water contact angle measurements are useful for obtaining quick information on the gradient profile. But those have a low spatial resolution. Other techniques such as X-ray photoelectron spectroscopy (XPS) and infrared (IR) microscopy, that have a higher spatial resolution, are required.

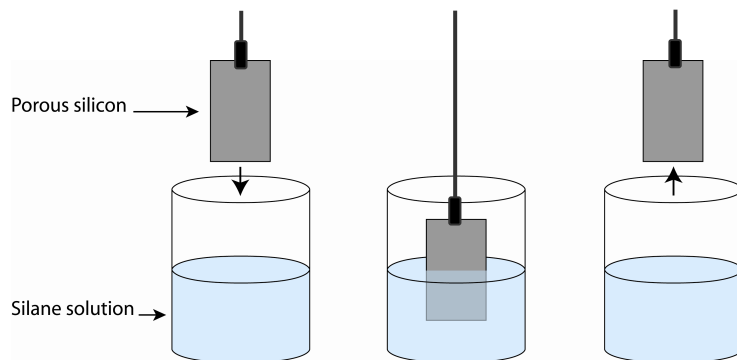
Porous silicon (pSi), a nanostructured version of semiconducting silicon, is increasingly being applied to biomedical applications biosensors, tissue engineering and drug delivery due to its biocompatibility, degradability and ease in tailoring its structural and optical properties^{7, 35-38}. pSi is formed through anodisation, with hydrofluoric acid (HF) as the electrolyte. The pore dimensions and the thickness of the porous layer can be controlled through varying etching current density and etching time, respectively.

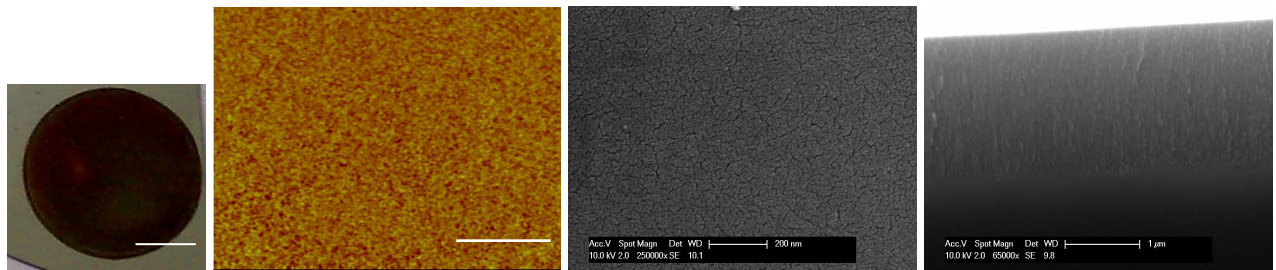
In this work, we have prepared wettability gradients on oxidised hydrophilic pSi substrates by dip coating with hydrophobic silanes. Synchrotron IR microscopy, contact angle measurements and X-ray photoelectron spectroscopy (XPS) was used to provide in depth characterisation of the prepared surface gradients.

2. EXPERIMENTAL

2.1 Porous silicon fabrication

pSi substrates were prepared by anodisation of p-type silicon (3-6 Ω .cm resistivity, <100> orientated, Boron doped, Virginia Semiconductors). Surfaces were etched by placing a platinum electrode parallel to and ~5mm from the silicon surface in a circular Teflon well. Hydrofluoric acid (HF) electrolyte solutions were prepared using 49% aqueous HF and 100% ethanol as a surfactant. A 1:1 HF/ethanol solution was used, applying a current density of 65 mA/cm² for 60 s. Following anodisation, samples were rinsed with ethanol, methanol, acetone and dichloromethane and subsequently dried with a gentle stream of nitrogen gas. Subsequently, the freshly etched pSi substrates were ozone oxidised using a Fischer OZON Ozon-Generator 500 for 40 min at a rate of 3.25 g/hr ozone. Following oxidation, surfaces were rinsed with ethanol and dried under a stream of nitrogen gas.





3.2 Silanisation of porous silicon substrates

pSi surfaces were ozone oxidized for 40 min to generate a surface that was stable when introduced into aqueous media. Oxidation of the pSi surface transforms the Si-H groups which are susceptible to oxidative hydrolysis into more stable Si-OH groups⁴⁰. Thermal oxidation of the surface prior to oxidation would further stabilize the surface; however, as the substrates are only in contact with aqueous media for short periods of time in these experiments, this step was not required. Ozone oxidized pSi was silanised with ODCS and PFPS for varying time periods to generate varying degrees of silane coverage on the surface.

Typical transmission IR spectra for each surface functionalisation can be seen in Figure 3. Following ozone oxidation of the freshly etched porous silicon surfaces, a characteristic peak at 1080 cm^{-1} corresponding to Si-O stretching vibrations is visible (Figure 3a). Following ODCS functionalisation, the strong peak at 1080 cm^{-1} remains, but new peaks at 2855 cm^{-1} and 2925 cm^{-1} appear, corresponding to C-H stretching. These peaks are indicative of successful attachment (Figure 3b). The PFPS surface (Figure 3c) also shows a peak at 1080 cm^{-1} attributed to Si-O stretching. A small characteristic peak at 1260 cm^{-1} was also observed which was attributed to C-F stretching vibrations as well as intense peaks at 1505 cm^{-1} and 1525 cm^{-1} attributed to the C=C stretching vibrations within the aromatic ring. This suggests that the surface chemistry of the pSi was successfully modified by silanisation.

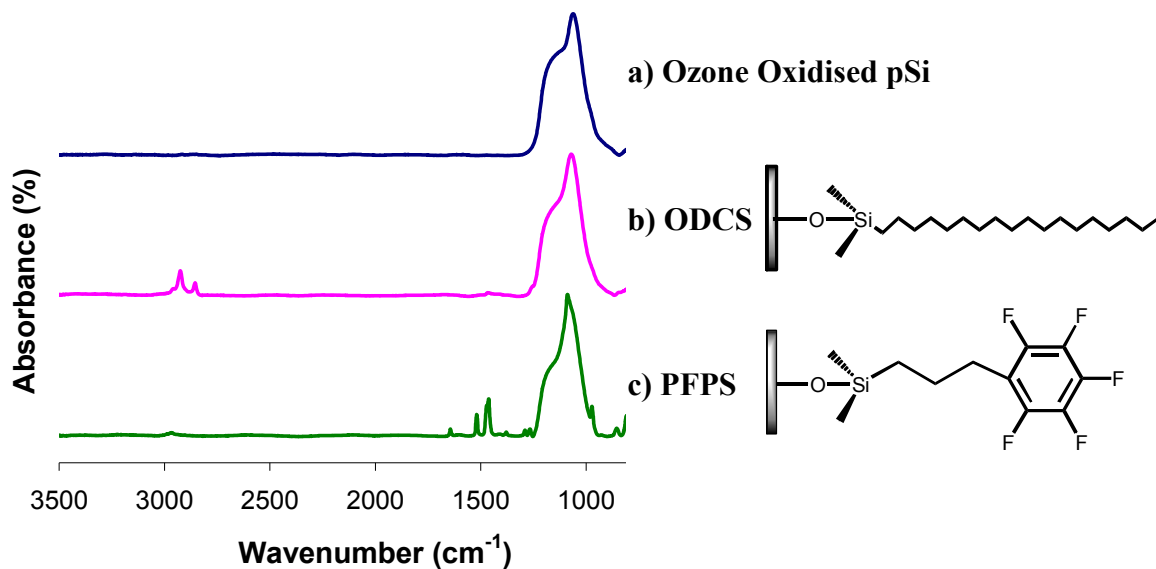


Figure 3: Transmission IR spectra of a) ozone oxidized pSi, b) ODCS silanised pSi and c) PFPS silanised pSi.

3.3 Optimisation of the kinetics of silanisation on pSi

The kinetics of silanisation on pSi were investigated in terms of the incubation time, the temperature of the solution and concentration of the silane as summarised in figure 4. For these experiments, discrete pSi samples were utilised. The incubation time of the pSi wafer in the silane solution was varied between 5 s and 1 h. For these initial studies, the static water contact angle measurements were used as the only characterisation method. In order to demonstrate the trend observed with varying conditions, results from 0 s to 60 s incubation are shown.

The incubation time of the substrate in the silane solution had an obvious influence on the extent of silanisation on the surface. As demonstrated in Figure 4, increasing the exposure time led to an increase in water contact angle which is indicative of increased surface coverage of the hydrophobic silanes. A plateau was observed after approximately 10 s incubation for experiments performed at 25°C , whilst at 2°C , a plateau was not observed until 30 s incubation.

The temperature at which the silanisation was conducted was also found to influence the extent of surface modification. As shown in Figure 4, the contact angle of the pSi increased rapidly from 9° to 47° after 10 s exposure to 100 mM

ODCS at 25°C, however, when the same reaction was conducted at 2°C an increase in contact angle from 9° to 26° was observed over the same time period. Following the silanisation reaction at 25°C over a time period of 60 s, a plateau in contact angle was observed saturate after 30 s. In contrast, a linear increase of contact angle up to 60 s was observed at 2°C. Longer incubations lead to an increase in contact angle with a maximum contact angle of 110° being achieved for overnight (16 h) incubation. The trend observed at 2°C is encouraging for the formation of a gradient, where a linear change in surface properties is desired.

The concentration of the silane solution appeared to alter the final contact angle achieved on the surface. It can be seen that, with the exception of the 2°C 100mM time series, for all silane concentrations, the initial rate of attachment was high for the first 30 s of exposure, however longer incubation times saw a plateau in adsorption of the silane. The 100mM series performed at 2°C however showed a linear trend over the full 60 s time period and hence was utilised for the preparation of gradient samples. Silanisation of the pSi at lower concentrations (<1 mM) produced little difference in the contact angle for different incubation times. For concentrations between 10 mM and 100 mM, an increase in contact angle was observed however a plateau was observed after 30 s. Increasing the concentration beyond 100mM led to solubility issues.

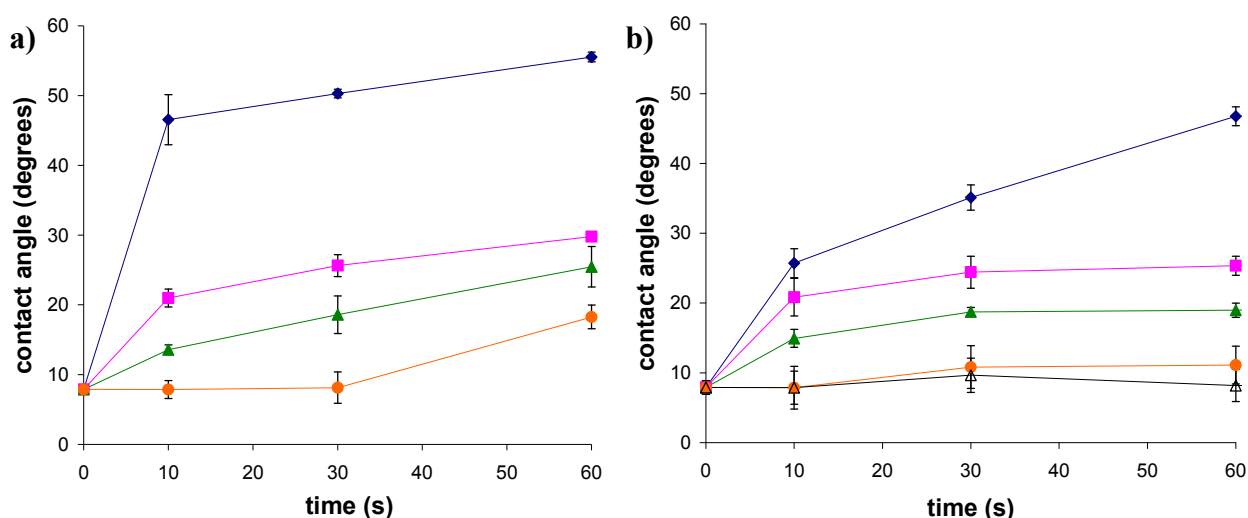


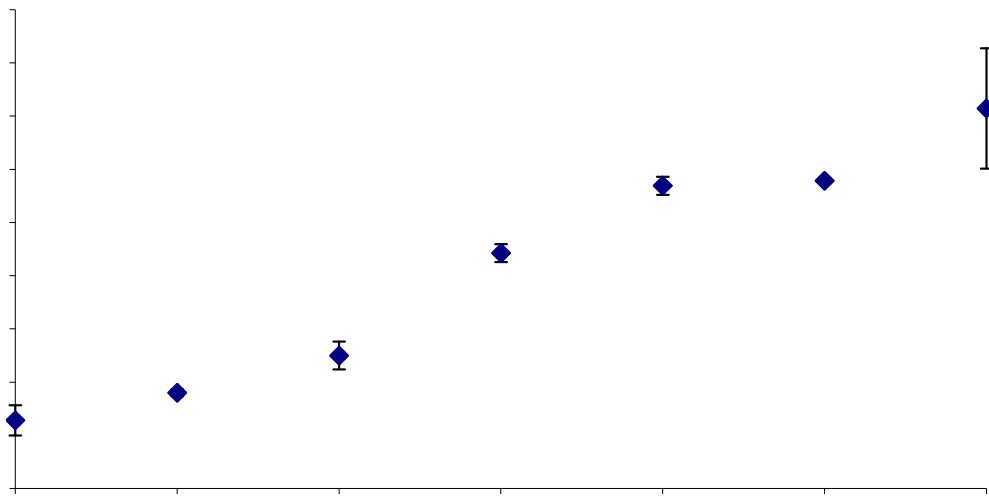
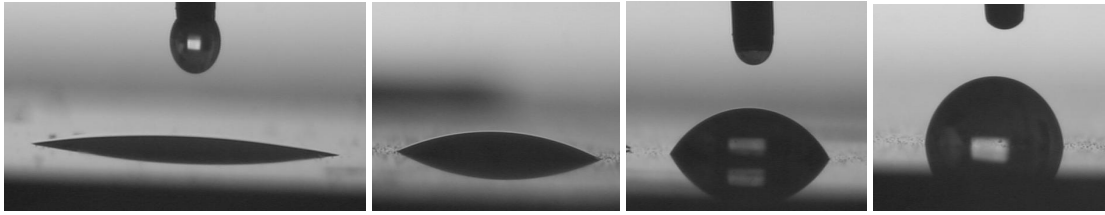
Figure 4: Static water contact angle measurements of discrete ODCS samples demonstrating the effect of concentration of silane on the kinetics of silane attachment for discrete samples at varying silane solution temperatures a) 25°C room temperature b) 2°C for concentrations spanning from 0.01mM (Δ), 0.1mM (\circ), 1mM (\blacktriangle), 10mM (\blacksquare), 100mM (\blacklozenge).

The substituents on the octadecylsilane were also investigated. The reactivity of ODCS and n-(octadecyldimethyl)methoxysilane (ODMS), a monomethoxy silane, were compared and found to be similar (results not shown). The reproducibility of the ODCS samples was high, with a maximum standard deviation of 2.8°. However the ODMS silanisations were not as reproducible with standard deviations of 5.8° (data not shown). Hence ODCS was chosen for subsequent experiments. Trimethoxysilanes and trichlorosilanes were not used due to their tendency to polymerise on the surface and affect surface topography.

Silanisation of PFPS was also optimised using methodology similar to that used to determine ODCS conditions (data not shown). For the PFPS, higher temperatures were required (60°C) to achieve adequate coverage for gradient formation. A time scale of 0 s to 2 min was also utilised for the PFPS, as was the 100 mM concentration.

3.4 Preparation of silane gradients

Silane gradients of ODCS and PFPS were prepared via a dip coating method using a geared stepper motor. pSi substrates were slowly dipped into a beaker containing the silane of interest over a period of 2 min followed by immediate washing with toluene. A 2 min incubation time was chosen in these experiments as the contact angles curve appeared relatively linear over this range as discussed in section 3.3.



corresponding to C-H stretching were utilised to create the IR map. The value obtained from each spectrum could be used to reconstruct a two-dimensional map of the surface, shown in Figure 7b whereupon the left hand side corresponds to the pSi with the shortest exposure time to the silane and the right hand side corresponds the longest exposure time. On the left hand side region of the gradient, the C-H stretching vibration is weak, which is confirmed by the representative spectra (Figure 7c). However towards the right hand region, the C-H vibrational peak was increased in intensity. A representative spectrum taken in the region on the right is shown in Figure 7d. The profile of the gradient (figure 7a) also shows that there is a gradual increase in the C-H stretching peaks from left to right, suggesting an increase in the silane density from left to right. This increase is approximately linear up to 1 cm, whereupon a much larger increase in the C-H stretching signal was measured up to the maximum signal at the far right hand side region of the gradient. This overall trend is in agreement with contact angle measurements. In addition to confirming the gradient distribution, the IR map of C-H stretching peaks shows evidence of heterogeneity within the coating.

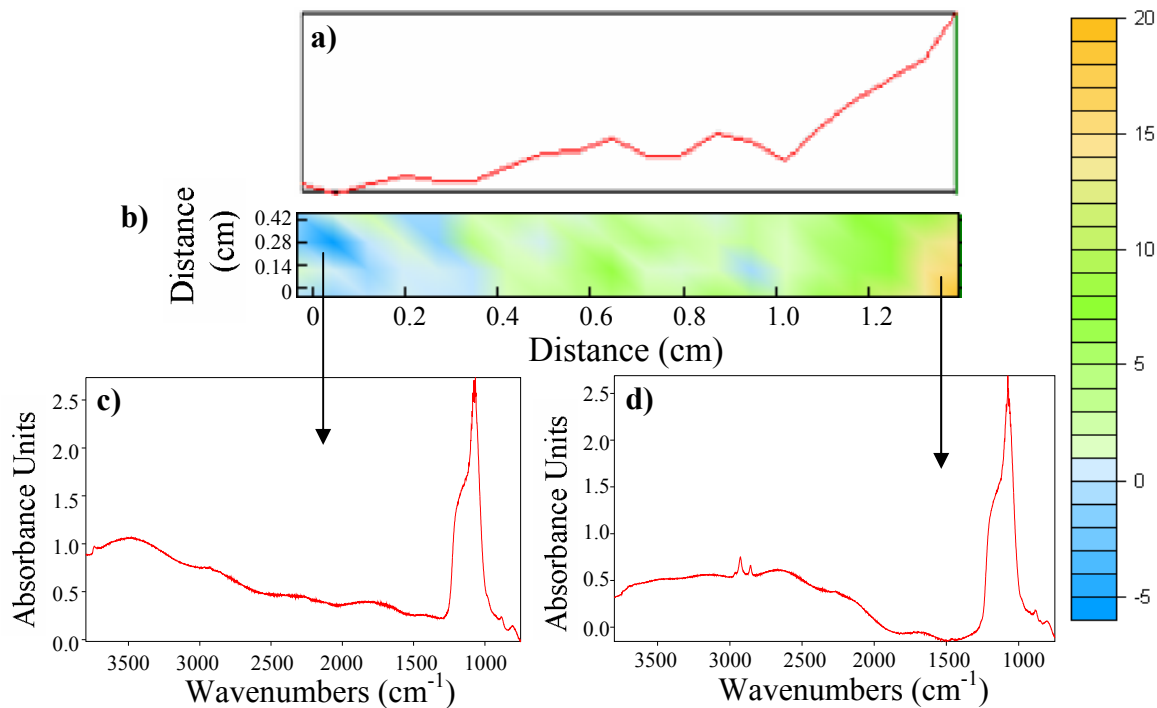


Figure 7: IR microscope image of ODCS treated surface. Gradient spans from left (shortest incubation time) to right (longest incubation time). a) gradient profile, b) Spectral map, integration under $3007\text{-}2820\text{cm}^{-1}$ c,d) representative spectra for the c) hydrophilic and d) hydrophobic ends of the gradient.

In the same manner as described for the ODCS gradient above, a map of the PFPS treated surface was created (Figure 8). For the PFPS map, C=C stretching peaks at 1505 cm^{-1} and 1525 cm^{-1} were utilised as they showed the highest intensity in the spectra. The left hand side of the map in Figure 8 corresponds to the pSi with the shortest exposure time to the silane and the right hand side corresponds to the longest exposure time. A weak C=C stretching peak is observed which was confirmed by the representative spectra (Figure 8c). In contrast, the right hand region the C=C stretching peak signal intensity was significantly higher, as can be seen at $1472\text{ - }1536\text{cm}^{-1}$ of Figure 8d. The gradient profile shown in Figure 8a indicates that there is a linear increase in C=C stretching peaks from left to right, suggesting an increase in silane density from left to right.

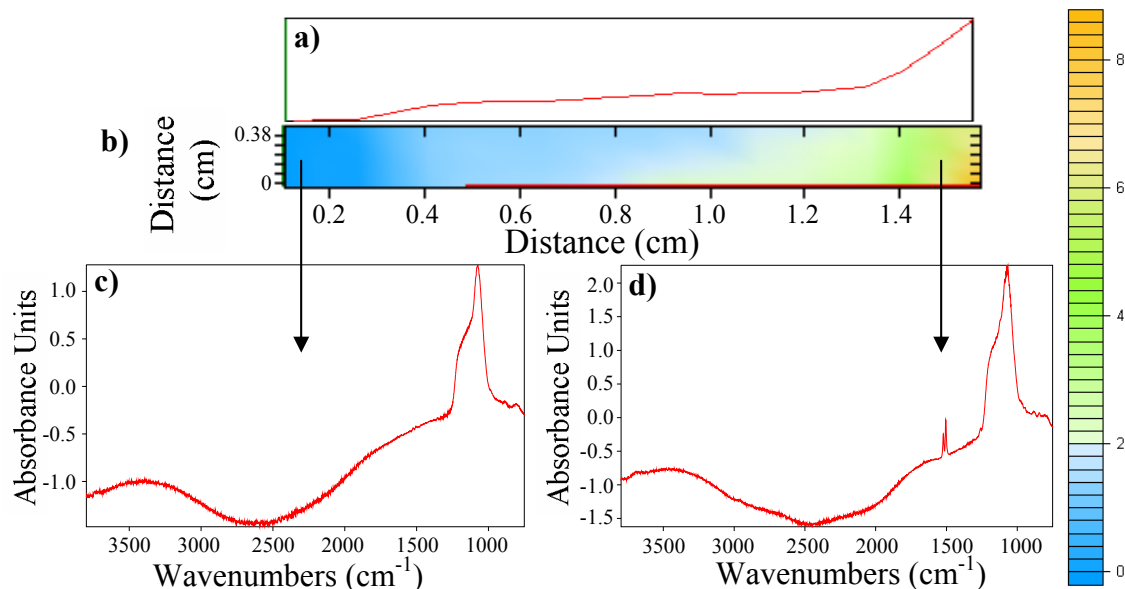


Figure 8: IR microscope image of PFPS treated surface. Gradient spans from left (shortest incubation time) to right (longest incubation time). a) gradient profile, b) Spectral map, integration under 1471.58 – 1536.24 cm⁻¹ c,d) representative spectra for the c) hydrophilic and d) hydrophobic ends of the gradient.

3.4.3 X-ray photoelectron spectroscopy (XPS) analysis

XPS was utilised in conjunction with contact angle and IR microscopy to determine the gradient's chemical profile. Survey spectra were acquired at intervals of 1.5 mm along the length of the prepared silane gradients. In order to investigate the presence of functional groups on the surface, characteristic peaks in the spectra were referenced to the silicon peak, which predominantly arises from the pSi substrate. For both the ODCS and PFPS surfaces, the carbon peak at 285.0 eV was utilised as an indicator of silane coverage due to the presence of the hydrocarbon chain and benzene ring respectively, which would give a distinct carbon signal as compared with pSi. The C/Si ratios across each of the silane gradients from the shortest exposure time (distance of 0.15 cm) to the longest exposure time (1.2 cm) are shown in Figure 9. An increase was seen moving from a distance of 0 to 0.45 cm, followed by a plateau after 0.45 cm was seen for both silanised gradients. This suggests that an increase in surface coverage of each of the silanes was achieved with increasing exposure time. This further confirms the successful formation of a chemical gradient.

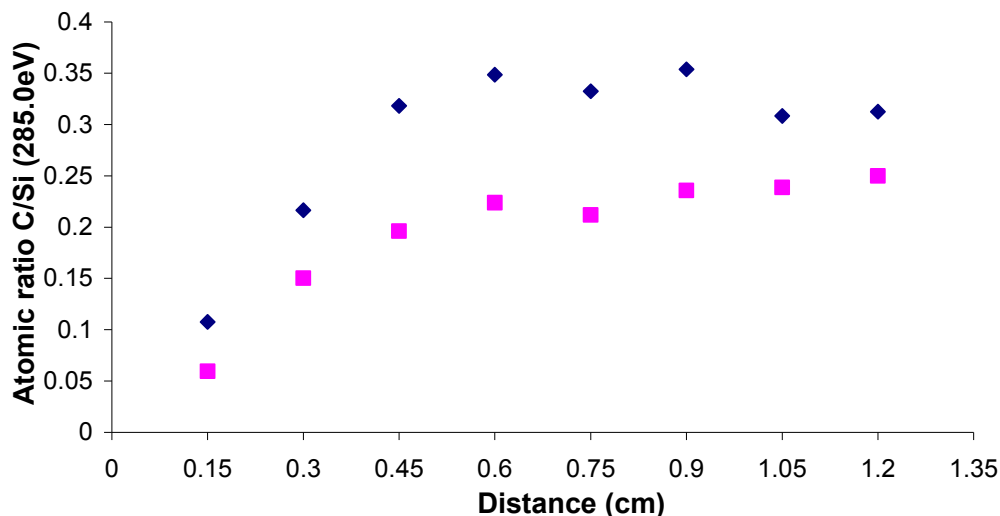


Figure 9: XPS analysis of silane gradients. C/Si ratios for ODCS (◆) and PFPS (■).

4. CONCLUSIONS

In this study, we have developed silanised chemical gradients via a dip coating method. ODCS gradients produced a range in wettability over the substrate, displaying a linear trend in sessile drop water contact angle measurements ranging from 12° to 71°. IR microscopy studies showed that the C-H stretching and C=C stretching peaks, characteristic to ODCS and PFPS respectively, increased with increasing incubation times, confirming gradient formation. Synchrotron IR mapping revealed evidence of heterogeneous distribution of the surface coating. Future experiments will exploit the highly focused synchrotron infrared beam to study this heterogeneity at length scales of the order of a few microns. The gradients were also studied by an XPS line scan along the gradient. All three analysis techniques strongly suggest the successful formation of a chemical gradient on pSi by silanisation. The IR microscopy and XPS trends were comparable in that a plateau was observed midway along the gradient. The contact angle measurements however displayed a linear increase in static water contact angle. This discrepancy between techniques may be due to the lower resolution analysis of water contact angle measurements.

Although in this study, uniform porous silicon was used as the substrate to generate a chemical gradient, it is possible to generate pore size gradients by asymmetric anodisation^{18,41}. An early study has shown that pore size alone can influence the behaviour of SK-N-SH cells⁷. Thus, the combination a pore size gradient with a surface chemistry gradient to determine the influence of both topography and wettability simultaneously is attractive and will be the subject of future studies. The method of dip coating to create silane gradients is a novel approach towards the preparation of controlled chemical gradients and can easily be adapted to include a range of functionalities which can then act as a template for biomolecule attachment. The use of chemical gradients in the analysis of protein and cell-surface interactions is a promising approach towards the identification and optimisation of surface conditions for biomolecular behaviour, and is an important method for the further development of biomedical devices including applications in stem cell technologies.

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