Chapter 5

Conclusions

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In this thesis, a series of different techniques for fabricating pSi for a range of different applications was described. We have shown that it is possible to rapidly modify certain surface attributes such as surface chemistry, hydrophobicity/hydrophilicity and porosity. These alterations to the surfaces, in turn, allowed the same material to be used to addressing a range of different questions. Firstly, surface modifications were found to improve the stability of the porous film under aqueous conditions. These modifications of the porous surface were studied in contact with adhering cells. A good example of this is as shown in Chapter 2 where we noticed that surface pre-modified with FBS prior to neuroblastoma (SK-N-SH) seeding can actually impair the development of neuronal processes. Cells cultured on surfaces coated with collagen showed good viability while, unsurprisingly, the adhesion of the cells was observed to be compromised over time on surfaces that had been functionalised with a PEG silane.

By controlling the anodisation current and duration, the size of the surface pores can be tuned and this permitted us to perform a direct correlation between surface chemistry and porosity and hepatocyte cell adhesion and behaviour. A long term (14 days) hepatocyte culture was performed on a range of different pore size and surface chemistries in order to evaluate their net influences on cell culture and also to assess the suitability of pSi as a biomaterial in Chapter 2. We found that both oxidised and APTMS silanised surfaces were not favourable in long term culture and had also identified day 6 as the onset for the extensive cell apoptosis on our pSi surface regardless of surface modification. We also noticed that hepatocyte growing on collagen coated surfaces with pore sizes of 23-38 nm formed multilayered cell sheets on the surface while those cultured on surfaces with pore sizes of 7-14 nm do not form these multilayered sheets. Urea and LDH analysis had also identified day 6 as onset for

hepatocyte apoptosis. However, these studies also shown that the presence of the multilayered hepatocytes found on 23-38 nm surfaces coated with collagen were more metabolically active compared with the single cell layer on the 7-14 nm pore surface. By documenting a long term hepatocyte culture on porous silicon films of different pore size and with different surface chemistries, we have clearly demonstrated the importance of both chemical and physical cues to cellular longevity and also reinforced the importance of surface topography in the nanometre range.

It is possible to selectively and rapidly remove chemically functionalised region on the pSi surface by means of laser ablation. This mode of surface patterning was discovered during the development of DIOS (desorption/ionisation on porous silicon) devices in our group and has been utilised to micropattern cell adhesion on the pSi surface. As described in chapter 3, when regions of a PEG functionalised pSi surface was interrogated using pulsed nitrogen laser from a commercial MALDI mass spectrometer, cells adhesion occurred almost exclusively on the ablated region while the non-ablated regions remain non-adhering[2]. Furthermore, it was possible to monitor the laser writing process in-situ by mass spectrometry. Deep laser ablation of a pSi film of up of 50 µm depth was also performed with a microdissection microscope laser in an attempt to reconstruct a 3D artificial hepatocyte cords *in vitro* in order to mimic the actual structure of liver tissue *in vivo*. As a result, a 4-layer hepatocyte stack was observed forming within these trenches and images obtained from confocal microscopy showed similarities with natural cords as reported in literature[3]. This actually represents an important step in reconstructing complex 3-dimensional liver structures which had previously proved difficult *in vitro*[4].

Tailoring a pore size gradient across a single pSi substrate was achieved by anodising the silicon in an asymmetrical fashion[5,6]. In this thesis, we have successfully produced lateral pore gradients with sizes spanning from 1-3 microns to as low as 5-20 nm. These gradients were used to study surface wettability behaviour in conjunction with different chemical modifications, as described in chapter 4. We found that it is possible to easily and rapidly identify desired wettability regimes relative to surface profile and chemical modifications on a single sample[7]. Contact angle measurement revealed that the attainable angle ranges from 17º for on pSi gradient surface functionalised with hydrophilic silanes to 127º on gradient surface functionalised with hydrophobic silanes. The same lateral surface gradient was also employed to study the influence of surface pores on the morphology and adhesion behaviour of neuroblastoma. We observed that cells displayed morphological characteristics that are influenced by their ability to adhere on the surface. On the large pores (1000-3000 nm), adhesion was not optimal and the cells had to rely on themselves through cell-cell contacts while reducing the reliance on cell-substratum contact. The cells were also found to adhere poorly on the surface pore of between 50-100 nm and this information might prove very useful for the design of low-fouling biomaterials in conjunction with chemical surface treatments to direct neuritic growth. Our results also showed that even < 20 nm, the surface can still exerts an influence on neuroblastoma morphology, once again highlighting the influence of nanotopography on the behaviour of adherent cells.

The same lateral anodisation approach was also employed to produce photonic gradients in Chapter 4. While this deviates slightly from the main biomaterial theme, we felt that the inclusion of this work can be interpreted as a portrayal of the very versatile nature of pSi. Optical reflectivity spectra for rugate- and Bragg-type gradients were evaluated from laterally anodised pSi photonic films. These photonic gradients were then overlayed in different

directions and the overall optical reflectivity spectra across the sample were recorded. We were able to distinguish photonic stop-bands from up to three gradients on a single substrate. Furthermore, we have also successful produced intact freestanding pSi films without the use of expensive equipment or tedious methodologies.

In summary, this thesis explored the use of pSi in a number of biological and photonic applications. Various fabrication approaches were chosen to tailor pSi structures specifically to address the different biological and photonic questions.

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