Porous Silicon Structures for Biomaterial and Photonic Applications

by

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2 Evaluation of cell adhesion and growth on pSi surfaces

Abstract

The primary research aim in this thesis is to demonstrate the versatility of porous silicon based nanomaterials for biomaterial and photonic applications. In chapter 2 of this thesis, the suitability of porous silicon as a biomaterial was investigated by performing different surface modifications on the porous silicon films and evaluating biocompatibility of these surfaces *in vitro*. The porous silicon surfaces were characteriszed by means of atomic force microscopy (AFM), scanning electron microscopy (SEM), diffuse reflectance infrared spectroscopy (DRIFT) and interferometric reflectance spectroscopy (IRS). Cell attachment and growth was studied using fluorescence microscopy and cell viability assays. Both fabrication of the porous silicon films and subsequent surface modifications were demonstrated. Polyethylene glycol functionalised porous silicon prevented cell attachment, whilst collagen or fetal bovine serum coating encouraged cell attachment. Surface modifications were also performed on porous silicon films with different pore sizes and the influence of pore size and surface modification on primary hepatocyte growth was recorded over a course of 2 weeks by means of laser scanning confocal microscopy (LSCM), toxicity and metabolic assays. On collagen-coated surfaces with average pore sizes of 30 nm, multilayer cells stacks were formed. This stacking behaviour was not observed on samples with smaller pore sizes (10 nm), or in the absence of collagen. Hepatocytes remained viable and functional (judging by a metabolic assay) for 6 days, after which they generally underwent apoptosis. Collagen-coated porous silicon films showed later onset of apoptosis than porous silicon films not coated with collagen or collagen-coated flat silicon..

In chapter 3 of this thesis, the nitrogen laser of a laser desorption/ionization (LDI) mass spectrometer was used to selectively ablate regions on porous silicon films that had been functionalised with a non-fouling polyethylene oxide layer, affording a microscale patterning of

the surface. Surface characterization was performed by means of AFM, SEM, LDI mass spectrometry, DRIFT and IRS. This approach allowed the confinement of mammalian cell attachment exclusively on the laser-ablated regions. By using the more intense and focussed laser of a microdissection microscope, trenches in a porous silicon film were produced of up to 50 micron depth, which allowed the construction of cell multilayers within these trenches, mimicking the organization of liver cords *in vivo*. Fluorescent staining and LSCM was used to study cell multilayer organization.

To gain a better understanding of how surface topography influences cell attachment and behaviour, porous silicon films were fabricated containing a gradient of pore sizes by means of asymmetric anodisation (chapter 4). These gradients allowed the investigation of the effect of subtle changes of pore size on cell behaviour on a single sample. Analysis by means of LSCM and SEM showed that pore size can dictate cell size and area as well as cell density. In addition, a region of pore size where cell attachment and proliferation was strongly discouraged was also identified. This information can prove to be useful for designing non-biofouling surface topographies.

Using the same asymmetric anodisation setup, photonic mirrors gradients were produced and overlaid over one another to produce multidirectional lateral photonic mirror gradients that display a series of roving spectral features (photonic stop-bands) from each gradient layer (chapter 4). These multidirectional photonic gradients have the potential to serve as optical barcodes or contributing to the development of graded refractive index devices such as lenses for high quality image relay and graded-index optical fibers.

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Declaration

I declare that this thesis is my own original work, conducted under the supervision of Prof. Nicolas Hans Voelcker. It is submitted for the Doctor of Philosophy in the Physical Sciences at the School of Chemistry, Physics and Earth Sciences at Flinders University, South Australia. To my knowledge, no part of this research has ever been submitted in the past, or is being submitted, for a degree or examination at any other University.

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Publication list

The following is a list of peer-reviewed publications arising during my time as a PhD student at Flinders University from 2004-2008, of which paper 1, 3, 5 and 6 are used to present the main results in this thesis.

1. Y. L. Khung, S. D. Graney, and N. H. Voelcker, **Micropatterning of porous silicon films by direct laser writing**, Biotechnology Progress vol. 22, pp 1388-1393 (2006).

2. S. McInnes, S. Graney, Y.-l. Khung, and N. H. Voelcker, **Porous silicon microparticles as an alternative support for solid phase DNA synthesis**, Proceedings of SPIE-The International Society for Optical Engineering, vol. 6036 60361W/60361-60361W/60310 (2006).

3. Y. L Khung, M. A. Cole, S. McInnes and N. H. Voelcker, **Control over wettability via surface modification of porous gradients**, Proceedings of SPIE- vol. 6799, pp 679909 (2007)

4. Lauren R. Clements, Yit-Lung Khung, Helmut Thissen, Nicolas H. Voelcker, **2-directional gradient substrates for subsequent studies of cell-surface interaction**, Proceedings of SPIE- vol. 6799, pp 67990W (2007)

5. Y. L Khung, G. Barritt and N. H Voelcker, **The influence of surface topography on the behaviour of neuroblastoma cells investigated using continous porous silicon gradients**, Experiemental Cell Research, vol. 314, issue 4, pp 789-800 (2008)

6. Y. L Khung and N. H. Voelcker, **Multidirectional photonic mirror gradients**, Optic Materials, in preparation (2009)

Abbreviation list

AFM	Atomic force microscopy
APTES	3-aminopropyl triethoxysilane
APTMS	3-aminopropyltrimethoxysilane
DIOS	Desorption/ionisation on silicon
DMEM	Dulbecco's modified Eagle Medium
DRIFT	Reflectance infrared fourier transform spectroscopy
FBS	Fetal bovine serum
FDA	Fluorescein diacetate
H4IIE	Hepatoma cells
HDFS	Heptadecafluoro-1,1,2,2-tetrahydrodecyl dimethylchlorosilane
HF	Hydrofluoric acid
IRS	Interferometric reflectance spectroscopy
LSCM	Laser scanning confocal microscopy
MALDI	Matrix-assisted laser desorption/ionisation
PBS	Phosphate buffered saline
PC12	Rat pheochromocytoma cells
PDMS	Polydimethylsiloxane
PEG	N-(triethoxysilylpropyl)-O-polyethylene oxide urethane
PFPS	Pentafluorophenyl dimethylchlorosilane
pSi	Porous silicon
SEM	Scanning electron microscopy
SK-N-SH	Neuroblastoma cells