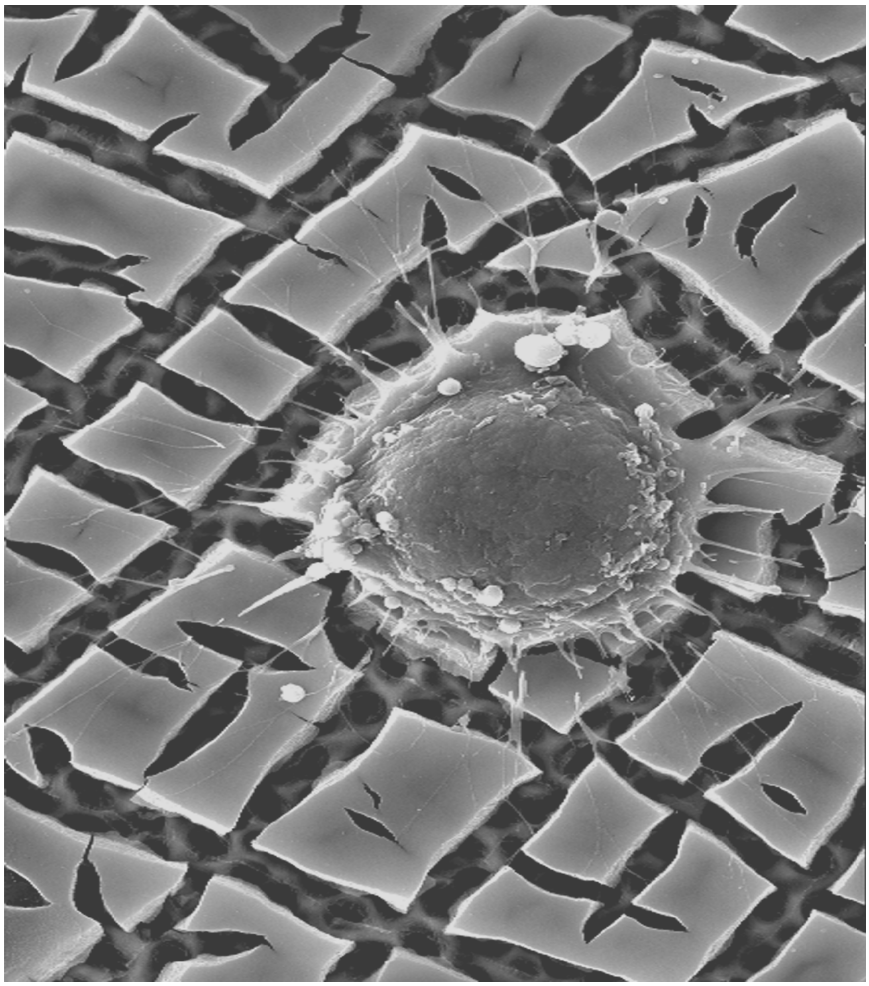
Development of porous silicon as a scaffold for the delivery of cells into ocular tissue



Scanning electron micrograph of a human lens epithelial cell cultured on macroporous silicon

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Scanning electron images presented on the title page and proceeding chapter title pages were all conducted on a Philips XL30 scanning electron microscope operating at 10 keV with a working distance of 10 mm. Samples shown on the title page and Chapter 1, 2 & 5 title pages, were all prepared as described on page 124. Samples shown on Chapter 3 & 4 title pages were prepared by coating with a thin layer of platinum.

Summary

Porous silicon has been shown to support the growth of cells and its capacity to fully degrade into harmless silicic acid, two properties that make porous silicon an appealing biomaterial. In this thesis, porous silicon was first tested in its suitability to support the growth of two different cell lines *in vitro*. The porous silicon surface was also surface-modified by oxidation, silanisation and by protein coatings to enhance its attachment properties. We found that silanisation with 3-aminopropyltrimethoxysilane (APTMS) was the simplest surface modification method that yielded the best cellular attachment characteristics and cellular morphology in comparison to the other surface modification methods tested. It was also discovered that surface modification was necessary to control the degradation rate of the porous silicon surface. APTMS-modified surfaces and thermally oxidised surfaces were both able to slow the degradation rate of the porous silicon surface and were thus used for subsequent experimentation.

Different forms of porous silicon were also tested, including membranes and particles. It was also discovered that certain colorimetric cell viability assays have the ability to interact with the redox-active porous silicon surface, thus yielding false positives. We focused upon assays such as Alamar Blue and the dye neutral red, both of which were able to generate a positive result with the porous silicon surface in the absence of cells.

We have shown that the porous silicon membranes were capable of supporting immortalised cells as well as primary cells isolated from human tissue. The biocompatibility of the porous silicon membranes was tested in a rat eye model, where the tissue response to the membrane could be observed macroscopically. It was noticed that there was a small inflammatory response around the membranes. Vascularisation and noticeable swelling was isolated to monofilament nylon sutures rather than the implanted membranes. The biocompatibility of porous silicon in the eye was also investigated through histological methods. The implanted porous silicon membranes only induced a small foreign body response which was noticeably smaller than the inflammatory response observed around commonly-used monofilament nylon sutures.

This is the first time that histological and microscopy evidence is given to show that porous silicon has good tissue biocompatibility. We offer evidence that the porous silicon membranes are able to degrade whilst implanted and the evidence also suggests that they are able to undergo full degradation.

Porous silicon was also investigated for its ability to act as a support scaffold for the delivery of cells into tissue. Primary cells were successfully cultured and implanted into eye of an animal. After one week, cells could be observed migrating away from the membrane into the surrounding tissue.

Therefore an enhanced porous silicon-based support has been developed that supports the attachment and growth of mammalian cells. This support is also biocompatible, biodegradable and can be used to deliver cells into tissue.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Suet Peng Low

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This work would not have been possible without the supervision and guidance of my principal supervisor Associate Professor Nicolas Voelcker and I would like to thank my co-supervisor Associate Professor Keryn Williams for encouragement and advice during my PhD.

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Publications Arising From This Thesis

Low, SP; Williams, KA; Canham, LT and Voelcker, NH (2006) Evaluation of mammalian cell adhesion on surface-modified porous silicon. *Biomaterials*, **27**:4538-4546.

Low, SP; Voelcker, NH; Canham, LT and Williams, KA. (2008) Porous silicon as a biomaterial for ophthalmic implants. *Biomaterials.* (Submitted October 2008)

Low, SP; Williams, KA; Canham, LT and Voelcker, NH (2008) Generation of reactive oxygen species from porous silicon particles in cell culture medium. (In preparation).

List of Abbreviations

°C	Degrees Celsius
µg	Microgram
µm	Micrometre
µM	Micromolar
2-D	2- Dimensional
3-D	3- Dimensional
3T3	Mouse Fibroblast Cells
AFM	Atomic Force Microscopy
AM	Amniotic Membrane
APTMS	3-aminopropyltrimethoxysilane *(modified surface)*
ARVO	Association for Research in Vision and Ophthalmology
ATF	Artificial Tear Fluid
CCD	Charge Coupled Device
CHO	Chinese Hamster Ovary cells
CK	Cytokeratin
C_{OX}	Concentration of Oxidised Form (Alamar Blue)
C_{RED}	Concentration of Reduced Form (Alamar Blue)
DCM	Dichloromethane
dH_2O	Distilled Water
DMEM	Dullbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
ECM	Extra Cellular Matrix
EDTA	Ethylenediaminetetraacetic Acid
EDX	Energy Dispersive X-Ray
EOT	Effective Optical Thickness
F12	Cell Culture Medium Formulation by Ham [1]
FBS	Foetal Bovine Serum
FDA	Fluorescein diacetate

FITC	Fluorescein isothiocyanate
FTIR	Fourier Transform Infra-Red
g	Grams
H&E	Haematoxylin and Eosin
HEMA	hydroxyethyl methacrylate
HF	Hydrofluoric Acid
HLE	Human Lens Epithelial Cells
HO	Hoechst 33342 *(cellular nuclear dye)*
IOL	Intraocular Lens
IU	International Units
kD	KiloDalton
keV	Kilo Electron Volt
kHz	KiloHertz
kN	Kilo Newton
m	Metre
M	Molar
mA	Milliamps
MicroCT	X-ray Micro Computerised Tomography
MilliQ	Purified Deionised Water *(with a resistivity of 18.2 MΩ·cm at 25 °C)*
mins	Minutes
ml	Millilitre
mm	Millimetre
mM	Millimolar
mmol	Millimoles
MOPS	4-Morpholinepropanesulfonic Acid
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide
MTS	3-(4,5-Dimethylthiazol-2-yl)-5-(3-Carboxymethoxyphenyl)-2-(4 Sulfophenyl)-2H-Tetrazolium Inner Salt
mV	Millivolts
NADH	Nicotinamide Adenine Dinucleotide Hydride

NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PBS	Phosphate Buffered Saline (pH 7.4)
	Containing: 8g/L NaCl + 0.2 g/L KCl + 2.68 g/L $Na_2HPO_4.2H_2O$ + 0.24 g/L KH_2PO_4
PC12	Rat Pheochromocytoma *(cell line)*
PEG	Polyethylene glycol
PEGS	N-(triethoxysilylpropyl)-O-polyethylene glycol urethane *(modified surface)*
PHEMA	poly(hydroxyethyl methacrylate)
PKH26	Cell Tracker Dye
PLGA	Poly(lactic-*co*-glycolic acid)
PMMA	Poly(methyl methacrylate)
ppm	Parts per Million
pSi	Porous Silicon *(abbreviation used in figure captions)*
PTFE	Polytetrafluoroethylene
rms	Root Mean Square
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
TAC	Transiently Amplifying Cells
TCPS	Tissue Culture Polystyrene
TRIS	Methacryloxypropyltris(trimethyl siloxy silane)
V	Volt
v/v	Volume per Volume
w/v	Weight per Volume
w/w	Weight per Weight
XTT	2,3-Bis(2-Methoxy-4-Nitro-5-Sulphophenyl)-5-Carboxanilide-2H-Tetrazolium, Monosodium Salt
Ω	Ohm