Development and applications of a novel, thermoresponsive scaffold for three-dimensional cell culture

by

Claire Louise Rossouw

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In the Faculty of Natural and Agricultural Sciences
Department of Biochemistry
University of Pretoria
Pretoria
South Africa

SUPERVISOR:
Prof. Lyn-Marie Birkholtz
Department of Biochemistry, University of Pretoria, South Africa

CO-SUPERVISORS:
Dr. Dalu Mancama
Molecular and Biomedical Technologies, Biosciences, CSIR, South Africa

Prof. Heinrich Hoppe
Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, South Africa

Dr. Sean Moolman
Licensing & Ventures, CSIR, South Africa

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SUMMARY

Although conventional two-dimensional (2D) cell culture is convenient for routine work, researchers are turning to three-dimensional (3D) cell culture for more accurate, physiologically representative information on the way their cells behave and respond to stimuli. Cells can now be routinely cultured in the many commercially available 3D formats.

In this study, we developed non-woven scaffolds for 3D cell culture and enhanced cell function. By making use of methods that measure the behaviour of liver cells in the 3D system we were able to demonstrate, compared to standard 2D systems, significantly higher expression of key liver enzymes involved in drug metabolism and albumin production (specifically cytochrome P450). Cell proliferation on the various scaffolds was comparable to that of a commercially available hydrogel 3D cell culture system, Algimatrix™.

When culturing cells in 3D, the means by which cells are harvested or extracted from the 3D scaffold for downstream applications is more challenging than in 2D. For this reason, many of the 3D scaffolds currently manufactured are either bio-degradable or require the use of salts to dissolve the scaffold which may negatively impact on the cells they contain. By grafting the non-woven scaffolds with the thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAAm), we demonstrated that cells growing on the scaffolds are able to be released from the scaffold in a 3D conformation, non-enzymatically, through temperature changes. Selected thermoresponsive non-woven fabrics were also tested in an automated cell culture device for cell proliferation and thermally induced harvesting.

One of the applications of a 3D cell culturing system would be in exploration of the many diseases plaguing mankind, in particular malaria which is still responsible for severe disease and mortality, especially in Africa. Most available antimalarials are designed to target the pathogenic blood stages in humans and to address the constant threat of drug resistance. However, to meet the objective of malaria eradication, medicines that block parasite transmission also need to be developed. Molecules that efficiently target the parasite stages in the liver would prevent pathogenesis, symptoms and transmission. Equipped with the knowledge that the infectious sporozoites traverse several hepatocytes prior to cell infection, it may be physiologically limiting to culture the exo-erythrocytic stage in vitro in a 2D cell culture system where the hepatocytes are in an unnatural flat conformation, distinctly different to their in vivo counterparts. Moreover, monolayer cell cultures lose their tissue-related functions rapidly, greatly impairing the predictive power of such assays. Thus, the second aim of this thesis was
to establish if hepatocytes that have been cultured on 3D non-woven scaffolds improve \textit{in vitro} sporozoite invasion compared to conventional 2D systems. Sporozoite invasion was detected in the conventional 2D monolayers using a TaqMan® assay but not in the hepatocytes growing in 3D. Future studies beyond the scope of this thesis will include modifications to the 3D scaffold to attempt achieving superior sporozoite invasion in this model system.
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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>AB</td>
<td>AlamarBlue®</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium Persulphate</td>
</tr>
<tr>
<td>ATR-FTIR</td>
<td>Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR)</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DPI</td>
<td>Days Post Inoculation</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EEF</td>
<td>Exoerythrocytic Form</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal Calf Serum</td>
</tr>
<tr>
<td>FDA</td>
<td>Fluorescein Diacetate</td>
</tr>
<tr>
<td>HPI</td>
<td>Hours Post Inoculation</td>
</tr>
<tr>
<td>HSPG</td>
<td>Heparin Sulphate Proteoglycan</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower Critical Solution Temperature</td>
</tr>
<tr>
<td>MCTS</td>
<td>Multicellular Tumour Spheroid</td>
</tr>
<tr>
<td>MFP</td>
<td>Median Flow Pore</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PET</td>
<td>Poly(ethylene terephthalate)</td>
</tr>
<tr>
<td>PNIPAAm</td>
<td>Poly(N-isopropylacrylamide)</td>
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<td>Poly(propylene)</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SFRP</td>
<td>Solution Free-Radical Polymerisation</td>
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<tr>
<td>XPS</td>
<td>X-Ray Photoelectron Spectroscopy</td>
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APPENDIX A

Table A1. TaqMan® expression data

Table A2. TaqMan® expression data for increased primer and probe concentrations

Table A3. TaqMan® expression data from invaded cells growing in 2D and nylon scaffolds