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

by

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Submitted in partial fulfilment of the requirements for the degree
*Philosophiae Doctor*

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# ACKNOWLEDGEMENTS

The completion of this dissertation was made possible by the following people whom I would like to thank:

My supervisors, Prof. Lyn-Marie Birkholtz, Dr Dalu Mancama, Prof. Heinrich Hoppe and Dr Sean Moolman for your guidance, support and encouragement. It was a privilege to work with you!

Avashnee Chetty, for stimulating discussions and for supplying the 3D scaffolds.

Kobus van Wyk, for technical assistance and help with the bioreactor experimental work.

Dr Jetsumon Sattabongkot (AFRIMS), for allowing me to train in your laboratory and for the long distance help via e-mail.

Megan Dowler (WRAIR), for your willingness to share your vast gametocyte culturing knowledge with me.

The CSIR and the 3R foundation in Switzerland for funding.

My fellow students, past and present: Dr Walter Campos and Dayaneethie Coopusamy for your help in tissue culture; Dr Tharina van Brummelen and Dr John Becker for teaching me to culture malaria parasites and Lia Rotherham for your friendship and encouragement.

My husband, Johann Rossouw, for all the weekends spent in the lab so I could feed my parasites, your unconditional love and unwavering support.

My parents, Gavin and Jane Munro and my family. I am so thankful for all the love, support and encouragement you have given me all my life.

# 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 *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.

# TABLE OF CONTENTS

Submission Declaration ..... ii
Plagiarism Statement ..... iii
Acknowledgements ..... iv
Summary ..... v
Table of Contents ..... vii
List of Figures ..... x
List of Tables ..... xiii
Abbreviations ..... xiv
Appendix A ..... xv

CHAPTER 1 ..... 1

Three dimensional cell culture systems and their applications: A review ..... 1

1.1. 2D vs 3D: Why do we need 3D? ..... 2
1.2. 3D Scaffolds ..... 5
1.2.1. Materials of choice ..... 5
1.2.2. Bulk modifications ..... 9
1.2.2. Surface modifications ..... 10
1.3. Applications of *in vitro* 3D cell culture ..... 11
1.3.1. *In vitro* anti-cancer drug screens: Tumour tissue models ..... 12
1.3.2. *In vitro* toxicology models ..... 13
1.3.3. *In vitro* host-pathogen interactions ..... 14
1.4. 3D cell culture: Future directions ..... 15
1.5. Objectives ..... 17

CHAPTER 2 ..... 19

Hepatocyte proliferation and thermally induced cell detachment on non-woven PP, PET and nylon three-dimensional scaffolds ..... 19

2.1. Introduction ..... 19
2.1.1. Scaffold selection ..... 21
2.1.2. Cell culture automation ..... 22
2.1.3. Objectives ..... 22
2.2. Materials and Methods ..... 24
2.2.1. Scaffold fabrication ..... 24

2.2.2. Grafting methods ..... 24
2.2.3. Cell-scaffold interaction ..... 25
2.2.4. Cell viability and proliferation ..... 27
2.2.4.1. AlamarBlue® assay ..... 27
2.2.4.2. DNA quantification using Hoechst 33258 ..... 28
2.2.5. Imaging cell-scaffold-interaction ..... 29
2.2.6. Hepatocyte metabolic activity measurement ..... 29
2.2.6.1. Bradford standard curve ..... 29
2.2.6.2. Albumin assay ..... 30
2.2.7. Cytochrome P450 mRNA expression ..... 30
2.2.7.1. RNA extractions ..... 31
2.2.7.2. cDNA synthesis and qRT-PCR ..... 32
2.2.8. Thermal release of cells from the various scaffolds ..... 32
2.2.9. Automated cell culture and thermal cell release ..... 33
2.3. Results ..... 36
2.3.1. Grafting methods ..... 36
2.3.2. Cell viability and proliferation ..... 37
2.3.3. Fluorescence microscopy and albumin quantification ..... 40
2.3.4. Cytochrome P450 mRNA expression ..... 43
2.3.5. Thermal release ..... 46
2.3.6. Automated cell culture device ..... 51
2.3.6.1. Prototype ..... 52
2.3.6.2. Final system design and testing ..... 54
2.4. Discussion ..... 58
CHAPTER 3 ..... 63
Application of A 3D scaffold: A sporozoite-hepatocyte model ..... 63
3.1. Introduction ..... 63
3.1.1. Malaria ..... 63
3.1.2. Sporozoite hepatocyte invasion ..... 65
3.1.3. *In vitro* culturing of hepatocytes for malaria sporozoite invasion ..... 65
3.2. Materials and Methods ..... 68
3.2.1. *In vitro* cultivation of asexual *P. falciparum* cultures ..... 68
3.2.2. *In vitro* cultivation of sexual stage *P. falciparum* parasites (gametocyte cultivation) ..... 70
3.2.2.1. Candle jar method ..... 70

3.2.2.2. Flask method ..... 71
3.2.2.3. Monitoring of exflagellation ..... 71
3.2.3. Mosquito rearing ..... 72
3.2.4. Mosquito feeding and dissections ..... 72
3.2.4.1. Mosquito preparation ..... 72
3.2.4.2. Mosquito mid-gut dissections and sporozoite isolation from salivary glands 73
3.2.5. Populating 3D scaffolds and 2D wells with hepatocytes ..... 75
3.2.6. Seeding sporozoites into 2D hepatocytes and 3D scaffolds ..... 76
3.2.7. gDNA isolation ..... 77
3.2.8. TaqMan® assay ..... 77
3.3. Results ..... 80
3.3.1. Gametocyte production and exflagellation ..... 80
3.3.2. Sporozoite invasion in 2D and 3D ..... 88
3.3.2.1. Mosquito feeding and dissections ..... 88
3.3.2.2. Sporozoite invasion of HC04 cells. ..... 89
3.3.2.3. Quantification of sporozoite invasion in HC04 cells ..... 90
3.4. Discussion ..... 93
CHAPTER 4 ..... 101
Concluding Discussion ..... 101
References ..... 106
Appendix A ..... 116

# LIST OF FIGURES

Figure 1.1. 3D cell culture publications per year (1977- June 2012)……..……..2

Figure 1.2. 2D and 3D cell culture morphology……3

Figure 1.3. Bridging the gap between 2D cell culture and natural tissue……..……4

Figure 1.4. The three criteria used to design 3D scaffolds…….……5

Figure 1.5. Polymer scaffolds for 3D cell culture…….……6

Figure 1.6. Selected *in vitro* applications of 3D cell culture…….……..…11

Figure 2.1. Poly(*N*-isopropyl acrylamide)……..……20

Figure 2.2. Temperature-responsive culture dishes……..……21

Figure 2.3. The prototype automated cell culture device……..……34

Figure 2.4. AlamarBlue® assay standard curves for HC04 and HepG2 hepatocytes…..……37

Figure 2.5. Cell proliferation on poly(propylene) (PP), poly(ethylene terephthalate) (PET) and nylon non-woven scaffolds, determined by the AB assay……..……38

Figure 2.6. Hoechst 33258 standard curve using crude cell lysates……39

Figure 2.7. Cell proliferation of hepatocytes growing on poly(propylene) (PP), poly(ethylene terephthalate) (PET) and nylon non-woven scaffolds, determined by DNA staining…….……40

Figure 2.8. Representative fluorescence micrographs of HC04 (top panel) and HepG2 (bottom panel) hepatocytes growing on the PP-*g*-PNIPAAm-B scaffold……41

Figure 2.9. Bradford assay and albumin standard curves…….……42

Figure 2.10. Relative albumin secretion in HC04 (A) and HepG2 (B) hepatocytes growing on a 3D non-woven control scaffold (pore size 200 µm)……43

Figure 2.11: Relative CYP gene expression……45

Figure 2.12. Thermal release of HC04 hepatocytes from grafted and control scaffolds 10 dpi.....47

Figure 2.13. Thermal release of HC04 and HepG2 hepatocytes……48

Figure 2.14. Additional controls used to assess if omitting any stages of the grafting process have any effect on thermal release……49

Figure 2.15. Effect of non-woven scaffolds' pore size on thermal release……50

Figure 2.16. The perfusion circuit layout and system components of the proposed automated cell culture system……52

Figure 2.17. HC04 hepatocytes growing on the three non-woven scaffolds housed in the bioreactor……54

Figure 2.18. The final automated cell culture system……56

Figure 2.19. HC04 hepatocytes released from grafted (PP-*g*-PNIPAAm-B1) and control (PP-Cont) non-woven scaffolds 10 dpi in the final automated system……57

Figure 3.1. The life cycle of *P. falciparum* parasites in the human host and the *Anopheles* mosquito vector……64

Figure 3.2. Mosquito mid-gut dissections……74

Figure 3.3. Mosquito salivary gland dissections……75

Figure 3.4. A qualitative comparison of the different gametocyte producing methods using three parasite strains after 10 days in culture……81

Figure 3.5. A comparison of culture health and gametocyte production for between the candle jar and flask method and the three parasite strains……..……83

Figure 3.6. Gametocyte staging and sexing..……85

Figure 3.7. *P. falciparum* gametocytogenesis..……86

Figure 3.8. Mature microgamete exflagellation..……..……88

Figure 3.9. Mosquito mid-gut and salivary gland dissections……89

Figure 3.10. HC04 hepatocytes populating the non-woven scaffolds..……90

Figure 3.11. TaqMan® expression data. ……..……91

Figure 4.1**.** An illustration of the **"**human-on-a-chip" concept……..104

# LIST OF TABLES

Table 2.1. Non-woven scaffolds used for this study……25

Table 2.2. A summary of successful methods used to achieve a grafted layer of PNIPAAm onto the surfaces of poly(propylene) (PP), poly(ethylene terephthalate) (PET) and nylon non-woven polymers……36

Table 2.3. Primer sequences for cytochrome qRT-PCR……43

Table 2.4. Hepatocyte cell release from scaffolds PP-*g*-PNIPAAm-B1, PP-*g*-PNIPAAm-B2 and PP-*g*-PNIPAAm-B3 via trypsin treatment or thermal release……51

Table 2.5. Experimental parameters assessed using the prototype automated cell culture device……53

Table 3.1. Mosquitoes fed for malaria infection studies……72

Table 3.2. The ring:trophozoite ratio……87

# ABBREVIATIONS

AB - AlamarBlue®

APS - Ammonium Persulphate

ATR-FTIR - Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR),

DMEM - Dulbecco's Modified Eagle Medium

DPI - Days Post Inoculation

DSC - Differential Scanning Calorimetry

ECM - Extracellular Matrix

EDTA - Ethylenediaminetetraacetic acid

EEF – Exoerythrocytic Form

FCS - Foetal Calf Serum

FDA - Fluorescein Diacetate

HPI - Hours Post Inoculation

HSPG – Heparin Sulphate Proteoglycan

LCST - Lower Critical Solution Temperature

MCTS - Multicellular Tumour Spheroid

MFP - Median Flow Pore

PBS – Phosphate Buffered Saline

PCL - Poly(ε-caprolactone)

PEG - Poly(ethylene glycol)

PET - Poly(ethylene terephthalate)

PNIPAAm - Poly(*N*-isopropylacrylamide)

PP - Poly(propylene)

SEM - Scanning Electron Microscopy

SFRP - Solution Free-Radical Polymerisation

XPS - X-Ray Photoelectron Spectroscopy

# APPENDIX A

Table A1. TaqMan® expression data……116

Table A2. TaqMan® expression data for increased primer and probe concentrations……...117

Table A3. TaqMan® expression data from invaded cells growing in 2D and nylon scaffolds...118