

PERIOCCULAR MALIGNANCY AND EYELID RECONSTRUCTION

A thesis submitted for the degree of Doctor of Philosophy

Dr Michelle Tian Sun MBBS

Discipline of Ophthalmology and Visual Sciences

South Australian Institute of Ophthalmology

The University of Adelaide and Royal Adelaide Hospital

August 2016

DEDICATION

To my parents, Kim and Eileen, and my husband Chris.

TABLE OF CONTENTS

DEDICATION	2
TABLE OF CONTENTS	3
ABSTRACT	10
DECLARATION	12
ACKNOWLEDGEMENTS.....	13
PUBLICATIONS AND PRESENTATIONS	15
CHAPTER ONE: LITERATURE REVIEW.....	18
1.1 Overview	18
1.2 Periocular Skin Cancer	20
1.2.1 Introduction to Periocular Skin Cancer	20
1.2.2 Basal Cell Carcinoma	23
1.2.2.1 Introduction	23
1.2.2.2 Basal Cell Carcinoma Histological Subtypes.....	23
1.2.2.3 Basal Cell Carcinoma with Orbital Invasion.....	26
1.2.2.4 Clinical Presentation and Investigation of Basal Cell Carcinoma with Orbital Invasion	28
1.2.2.5 Management of Basal Cell Carcinoma with Orbital Invasion	30
1.2.3 Squamous Cell Carcinoma	36
1.2.3.1 Introduction	36
1.2.3.2 Histological Features and Subtypes	36
1.2.3.3 Prognostic Factors.....	38
1.2.3.4 Surgical Management of Squamous Cell Carcinoma	40
1.2.3.5 Non-Surgical Management.....	42

1.3	The AJCC TNM Staging System	44
1.3.1	The TNM Staging System for Eyelid Carcinoma	44
1.3.2	Implementing the TNM Staging	46
1.3.3	TNM as a Predictor of Outcomes	47
Table 1: Definitions of TNM for Eyelid Carcinoma, AJCC Cancer Staging Manual, Seventh Edition.....		51
Table 2: Stage Grouping for Carcinoma of the Eyelid		53
1.4	Eyelid Reconstruction and Eyelid Tarsal Substitutes.....	54
1.4.1	Indications for Eyelid Reconstruction.....	54
1.4.2	Anatomic Considerations.....	54
1.4.3	Established Tarsal Substitutes in Eyelid Reconstruction	55
Figure 1: The Anatomy of the Eyelid		59
1.5	Principles of Bioengineering	60
1.5.2	Introduction	60
1.5.2	The Importance of Biomechanics	61
1.5.2.1	<i>In Vivo</i> Stress and/or Strain.....	62
1.5.2.2	Functional Demands: Sub-Failure and Failure Conditions	62
1.5.2.3	Prioritisation of Mechanical Properties as Design Parameters	62
1.5.2.4	Regulation and Interaction of Cells with an Extracellular Matrix <i>In Vivo</i>	63
1.5.2.5	Mechanical and Physical Factors Impacting Tissue Repair	63
1.5.2.6	Outcome Based Success Criteria and Methods to Model Tissue Growth.....	64

1.5.2.7	Biomechanical Studies within Ophthalmology.....	64
1.5.2.8	Specific Considerations for Eyelid Tarsus	66
1.5.3	The Role of the Scaffold	69
1.5.4	Tissue Engineering in Ophthalmology	72
1.5.4.1	Cornea	72
1.5.4.2	Glaucoma	74
1.5.4.3	Conjunctiva	75
1.5.4.4	Dry Eye.....	76
1.5.4.5	Orbital Fractures.....	77
1.5.5	Bioengineering Tarsus.....	80
1.5.6	Role of Cell Culture in Bioengineering.....	81
1.5.6.1	Fibroblast Culture	81
CHAPTER 2: BASAL CELL CARCINOMA.....		84
2.1	Introduction	84
2.2	Methods	86
2.3	Results	88
2.3.1	General Characteristics	88
2.3.2	Correlation between Biopsy and Excision	88
2.3.4	Biopsy and Excision Dimensions.....	89
2.4	Discussion.....	91
2.4.1	Summary of Findings.....	91
2.4.2	Characteristics of Basal Cell Carcinoma	91
2.4.2	Diagnostic Accuracy of Biopsy	92
2.4.3	Reporting of Margins	94
2.5	Study Limitations.....	97

2.6	Conclusion	98
	Table 3: Basal Cell Carcinoma Subtypes at Initial Biopsy and Excision.....	99
	Table 4: Biopsy Compared to Excision for Periorcular Basal Cell	
	Carcinoma of Mixed Histology	100
CHAPTER 3: SQUAMOUS CELL CARCINOMA		101
3.1	Introduction	101
3.2	Methods	103
3.2.1	Data Collection	103
3.2.2	Statistical Analyses	104
3.3	Results	105
3.3.1	Squamous Cell Carcinoma in Situ (Bowen Disease).....	105
3.3.2	Invasive Squamous Cell Carcinoma	106
3.3.3	Metastatic Disease	107
3.3.4	Perineural Invasion and Histological Data	107
3.3.5	Management.....	108
3.3.6	Follow-Up.....	109
3.4	Discussion.....	111
3.4.1	Summary of Findings	111
3.4.2	Previous Reports	111
3.4.3	Factors Associated with Recurrence and Metastases	112
3.4.4	Squamous Cell Carcinoma in Situ (Bowen Disease).....	114
3.5	Study Limitations.....	116
3.6	Conclusion	117
	Table 5: TNM Stages for Patients with Periorcular Squamous Cell	
	Carcinoma	118

Table 6: Recurrent Cases of Periocular Squamous Cell Carcinoma	119
CHAPTER 4: BIOMECHANICAL STUDIES OF THE EYELID TARSUS	123
4.1 Introduction	123
4.2 Methods	125
4.2.1 Sample Selection	125
4.2.2 CellScale BioTester	125
4.2.3 Biomechanical Parameters	126
4.3 Results	129
4.4 Discussion.....	130
4.4.1 Summary and Relevance	130
4.4.2 Alternate Tarsus Substitutes and Their Biomechanical Properties.....	130
4.4.3 Application in Tissue Engineering.....	133
4.5 Study Limitations.....	134
4.6 Conclusion	135
Table 7: Biomechanical Properties of Tarsus Tissue	136
Figure 2: The CellScale BioTester	137
Figure 3: Tarsus Biomechanical Testing	139
Figure 4: Stress-Strain Curve for Tarsus Tissue	140
CHAPTER 5: SCAFFOLD DESIGN FOR BIOENGINEERED TARSUS	141
5.1 Introduction	141
5.2 Methods	143
5.2.1 Scaffold Material	143
5.2.2 Scaffold Fabrication	144
5.2.3 Scaffold Characterisation and Preliminary Assessment.....	145

5.3	Results	146
5.3.1	Scaffold Characteristics and Biomechanics.....	146
5.3.2	Characterisation of Scaffolds	146
5.4	Discussion.....	147
5.4.1	Summary of Findings	147
5.4.2	Chitosan as Scaffold Material	147
5.4.3	Previous Studies of Bioengineered Tarsus.....	148
5.5	Study Limitations.....	150
5.6	Conclusion	151
	Figure 5: Formation of Scaffolds by Cryogelation.....	152
	Figure 6: Effect of Chitosan Concentration of Pore Size of Chitosan Scaffolds	153
	Figure 7: Tensile Elastic Modulus Scaffolds and Native Tarsus Tissue ...	154
	Figure 8: Pore Architecture of Chitosan Scaffolds.....	155
	Figure 9: Chitosan Scaffolds Support Fibroblast Culture.....	156
	CHAPTER 6: FIBROBLAST CULTURE FOR BIOENGINEERED TARSUS	157
6.1	Introduction	157
6.2	Methods	158
6.2.1	Orbital Skin Fibroblast Culture	158
6.2.2	Characterising Differentiation	159
6.2.3	Culture of Orbital Fibroblasts onto Bioengineered Scaffolds..	159
6.3	Results	161
6.3.1	Orbital Fibroblast Culture	161
6.3.2	Culture of Fibroblasts Over Scaffolds	161
6.4	Discussion.....	162

6.4.1	Summary of Findings	162
6.4.2	Previous Studies	162
6.4.3	Applicability in Clinical Practice	163
6.5	Limitations	165
6.6	Conclusions	166
Figure 10: Immunological and Gene Expression Analysis of Orbital Skin		
	Fibroblasts	167
Figure 11: Scanning Electron Microscopy of Fibroblast Culture of		
	Scaffolds	168
CHAPTER 7: FINAL DISCUSSION.....		169
CHAPTER 8: FUTURE DIRECTIONS.....		172
CHAPTER 9: REFERENCES.....		174

ABSTRACT

Non-melanoma skin cancer is the most common cancer in Australia. Basal cell carcinoma and squamous cell carcinoma are the two most frequently encountered types of non-melanoma skin cancer, and together they make up over 90% of all skin cancers. The periocular region is involved in 10% of cases and is associated with significantly more disease-related morbidity due to the local effect of both the disease and the surgical treatment on ocular adnexa. Therefore, it is imperative that high-risk tumours are correctly identified to ensure appropriate management and surveillance. Surgical excision remains the gold standard treatment but functional reconstruction of the eyelid represents an ongoing challenge. Despite the wide range of autologous and artificial eyelid substitutes, there is yet to be an ideal replacement for the specialised eyelid tissue called the tarsus. The tarsus is responsible for both structural support and physical form, making its adequate substitution fundamental to functional outcomes. Numerous uncertainties remain regarding the staging and management of periocular non-melanoma skin cancer which, combined with our lack of ideal eyelid tarsus substitutes, represents the basis for work undertaken as part of this thesis.

Previous studies contributing to our knowledge of periocular basal cell carcinoma histological subtypes and treatment of invasive disease are first reviewed in Chapter 2. Chapter 3 subsequently summarises our understanding of periocular squamous cell carcinoma with a particular focus on the utilisation and prognostic role of the most up-to-date American Joint Committee on Cancer (AJCC) staging system for the eyelid carcinoma.

In order to determine the required properties for the ideal tarsus tissue substitute, Chapter 4 analyses the normal biomechanical properties of the eyelid tarsus tissue. This study, the first of its kind for human tarsus tissue, provides a benchmark for bioengineering studies described in the following chapter. In Chapter 5, we describe the development of a novel bioengineered three-dimensional scaffold which is tailor-made to behave biomechanically like natural tarsus. In order to improve *in vivo* compatibility, we also successfully cultured fibroblasts from eyelid skin samples which were then seeded onto our bioengineered scaffolds, the results of which are described in Chapter 6.

Finally, insights into the presentation, staging and management of periocular basal cell carcinoma and squamous cell carcinoma, along with our novel bioengineered eyelid tarsus substitute are placed in the context of the previous literature in Chapter 7, before possible directions for future studies are discussed in Chapter 8.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Michelle T. Sun

August 2016

ACKNOWLEDGEMENTS

This doctoral thesis would not have been possible without the help, support and assistance of numerous people.

It has been my great privilege to have been taught by Professor Dinesh Selva. He has provided unwavering support and incredible mentorship throughout the past seven years. I continue to be inspired by his pursuit of excellence and attitude towards life and medicine. He has been instrumental in guiding me through my research development and I hope that this represents only the beginning of a long and fruitful collaboration. Similarly, I am extremely thankful to Professor Robert Casson, my co-supervisor. In addition to his academic talents and leadership, I will always admire Professor Casson's enthusiasm and commitment towards research and ophthalmology. I also look forward to continuing to work and collaborate with him in the years to come.

There are a number of other researchers and clinicians who I have learnt so much from and who I am also grateful to. Dr WengOnn Chan has been an immense support during the last few years and has been incredibly generous with his time, offering insightful advice on research, ophthalmology and life. I have also learned much from Dr John Wood, who has been an invaluable source of knowledge on laboratory-based research. Furthermore, Associate Professor Andrea O'Connor has helped me significantly in the area of bioengineering. Too many to mention are my many other colleagues and friends, whom I am thankful to for all their support and encouragement over the years.

Finally, a special thanks to my family. To my parents, Kim and Eileen – I can never be grateful enough for the sacrifices you have made to ensure I have had every opportunity in life. To my brother David – I am so glad to have your humour and companionship. Most importantly, I thank my husband Chris. Sometimes you meet someone truly exceptional, someone who believes and inspires you, encourages and pushes you to be better than you could imagine for yourself. I am lucky enough to have married that someone.

PUBLICATIONS AND PRESENTATIONS

Chapter One

1. Review: Sun MT, O'Connor AJ, Wood J, Casson R, Selva D. Tissue Engineering in Ophthalmology: Implications for Eyelid Reconstruction. Ophthalmic Plastic and Reconstructive Surgery 2016 [epub ahead of print]
2. Review: Sun MT, Wu A, Figueira E, Huilgol SC, Selva D. Management of periorbital basal cell carcinoma with orbital invasion. Future Oncology 2015;11:3003-10.
3. Manuscript: Wu A, Sun MT, Huilgol SC, Madge S, Selva D. Histological subtypes of periocular basal cell carcinoma. Clinical and Experimental Ophthalmology 2014;42:603-7.
4. Manuscript: Herbert HM, Sun MT, Selva D, Fernando B, Saleh G, Beaconsfield M, Collin R, Uddin J, Meligonis G, Leatherbarrow B, Atuallah S, Irion L, McLean C, Huilgol S, Davis G, Sullivan T. Merkel Cell Carcinoma of the Eyelid: Management and Prognosis. JAMA Ophthalmology 2014 Feb;132(2):197-204.
5. Manuscript: Watanabe A, Sun MT, Pirbhai A, Ueda K, Katori N, Selva D. Sebaceous Carcinoma in Japanese Patients: Clinical Presentation, Staging and Outcomes. British Journal of Ophthalmology 2013 Nov;97(11):1459-63
6. Presentation: Sun MT, Herbert HM, Selva D, Fernando B, Saleh G, Beaconsfield M, Collin R, Uddin J, Meligonis G, Leatherbarrow B, Atuallah S, Irion L, McLean C, Huilgol S, Davis G, Sullivan T. Merkel Cell Carcinoma of the Eyelid: Management and Prognosis. Annual

Royal Australian and New Zealand College of Ophthalmologists Annual Scientific Congress 2013

7. Presentation: Watanabe A, Sun MT, Pirbhai A, Ueda K, Katori N, Selva D. Sebaceous Carcinoma In Japanese Patients: Clinical Presentation, Staging and Outcomes. Annual Royal Australian and New Zealand College of Ophthalmologists Annual Scientific Congress, 2013

Chapter Two

8. Manuscript: Sun MT, Wu A, Huilgol SC, Selva D. Accuracy of Biopsy in Subtyping Periocular Basal Cell Carcinoma. Ophthalmic Plastic and Reconstructive Surgery 2015;31:449-51.
9. Research Letter: Sun MT, Figueira E, Huilgol SC, Selva D. Minimum Histological Safety Margins in Periocular Basal Cell Carcinoma. British Journal of Ophthalmology 2014;98:706.
10. Presentation: Sun MT, Wu A, Huilgol SC, Selva D. Accuracy of Biopsy in Subtyping Periocular Basal Cell Carcinoma. Annual Royal Australian and New Zealand College of Ophthalmologists Annual Scientific Congress 2014

Chapter Three

11. Manuscript: Sun MT, Andrew NH, O'Donnell B, McNab A, Huilgol S, Selva D. Periocular Squamous Cell Carcinoma: TNM Staging and Recurrence. Ophthalmology 2015;122:1512-6.
12. Presentation: Sun MT, Andrew N, O'Donnell B, McNab A, Huilgol S, Selva D. Periocular Squamous Cell Carcinoma: TNM Staging,

Management and Prognosis. European Society of Ophthalmology
Annual Congress, 2015

Chapter Four

13. Manuscript: Sun MT, Pham DT, O'Connor AJ, Wood J, Casson R, Selva D, Costi J. The Biomechanics of eyelid tarsus tissue. Journal of Biomechanics 2015;48:3455-9.
14. Presentation: Sun MT, Pham D, O'Connor A, Wood J, Casson R, Selva D, Costi J. The Biomechanics of Eyelid Tarsus Tissue. British Oculoplastic Surgical Society Annual Congress, 2015

Chapter Five and Six

15. Presentation: Sun MT, O'Connor A, Wood J, Casson R, Milne I, Biswa D, Selva D. Bioengineering Eyelids. Annual Royal Australian and New Zealand College of Ophthalmologists Annual Scientific Congress 2016