PERIOCULAR 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 In Vivo 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 In Vivo ..............................................................................................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
Table 3: Basal Cell Carcinoma Subtypes at Initial Biopsy and Excision

Table 4: Biopsy Compared to Excision for Periocular Basal Cell Carcinoma of Mixed Histology

CHAPTER 3: SQUAMOUS CELL CARCINOMA

3.1 Introduction

3.2 Methods

3.2.1 Data Collection

3.2.2 Statistical Analyses

3.3 Results

3.3.1 Squamous Cell Carcinoma in Situ (Bowen Disease)

3.3.2 Invasive Squamous Cell Carcinoma

3.3.3 Metastatic Disease

3.3.4 Perineural Invasion and Histological Data

3.3.5 Management

3.3.6 Follow-Up

3.4 Discussion

3.4.1 Summary of Findings

3.4.2 Previous Reports

3.4.3 Factors Associated with Recurrence and Metastases

3.4.4 Squamous Cell Carcinoma in Situ (Bowen Disease)

3.5 Study Limitations

3.6 Conclusion

Table 5: TNM Stages for Patients with Periocular Squamous Cell Carcinoma
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
  4.3.3 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. Numerable 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.
Chapter One


Chapter Two


Chapter Three


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

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