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# BACTERIAL & FUNGAL BIOFILMS IN CHRONIC RHINOSINUSITIS

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF ADELAIDE



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*March 2011*

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त्वमेव माता च पिता त्वमेव  
त्वमेव बंधू च सखा त्वमेव  
त्वमेव विद्या च द्रविणं त्वमेव  
त्वमेव सर्वं मम देव देव ॥

*"O Lord, You Are My Mother, Father, Kinsman And Friend.*

*You Are My Wealth Of Knowledge, Strength, Velour And Power.*

*You Are My All God Of Gods".*

*Dedicated to the anchor of my life, my dear husband*  
**Nimit**

*And my adorable children*  
**Gunin & Niya**

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## ABSTRACT

### BACTERIAL-FUNGAL BIOFILMS IN CHRONIC RHINOSINUSITIS

Chronic Rhinosinusitis (CRS) is a recalcitrant disease, characterized by headache, nasal discharge / blockage, which substantially impairs daily functioning and negatively affect quality of life. Endoscopic Sinus Surgery (ESS) is an important treatment option for CRS, but has variable success rates. Biofilms are well organised heterogeneous communities of microbes embedded in a mosaic of extracellular matrix, adherent to biotic / abiotic surfaces. As they are resistant to host defences and medical treatments, they have been touted as possible pathogenic factors in CRS, which may perpetuate the recurrent and recalcitrant character of the disease and negatively affect treatment outcomes.

This thesis encompasses research undertaken to enhance our understanding about the effect that presence and types of biofilms have on the clinical profile and treatment outcomes of patients suffering with chronic rhinosinusitis. An in-vitro model of fungal biofilms and a potential tool to assay in-vivo mucosal biofilms on sinonasal tissues has also been described.

Chapter 1 of the thesis comprehensively reviews the scientific literature pertaining to biofilms and CRS, and exhaustively evaluates the evidence present in relation to bacterial and fungal biofilms in CRS.

Chapter 2 describes a study to investigate the effect of biofilms on outcomes following ESS in CRS patients using internationally accepted standardised symptom scores, quality of life measures and endoscopy scores to assess the disease. It showed that patients with biofilms presented with more severe disease before surgery, and after surgery had persistent symptoms, ongoing mucosal inflammation and infections necessitating extra post-operative visits and multiple antibiotic treatments. This study thus strengthened the evidence for the role that biofilms may play in recalcitrant CRS.

Chapter 3 describes a further subgroup analysis of the above patients in whom the specific organisms forming the biofilms were identified and how patients with specific biofilm types progressed after surgery was studied. Patients with polymicrobial biofilms suffered more severe disease and had worse post-surgery mucosal outcomes requiring more post-operative visits. *S.aureus* biofilms played a dominant role in negatively affecting outcomes of ESS with persisting post-operative symptoms, ongoing mucosal inflammation and infections.

Chapter 4 describes an in-vitro model characterizing *A. fumigatus* biofilm formation on primary human sinonasal epithelium cultures under different growth conditions. 3-dimensional biofilm structures with parallel-packed and cross-linked hyphae, channels/passages, extracellular matrix (ECM) encasing the hyphae, were formed. Biofilms formed under flow conditions displayed more robust and faster growth kinetics as compared to those under static conditions, with extensive ECM production.

Chapter 5 investigates application of an analysis program 'COMSTAT 2' for assaying & quantitatively describing the 3-dimensional in-vivo biofilm structures observed via confocal scanning microscopy on sino-nasal mucosal samples. This can be used for temporal analysis of biofilm development, comparison of different types of biofilms formed under controlled conditions, analysis of influence of varying environmental factors on biofilms and the efficacy of different antibiofilm treatments.

Chapter 6 summarises and discusses the salient features of the studies included in this thesis which has attempted to characterize fungal and bacterial biofilms and the impact they may have in CRS patients.

### **THESIS DECLARATION**

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Deepti Singhal 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.

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Singhal D, Psaltis A.J, Foreman A, Wormald P.J.  
American Journal of Rhinology & Allergy, 2010 May; **24** (3): p. 169-74.
  
- *Staphylococcus Aureus* Biofilms: Nemesis of Endoscopic Sinus Surgery  
Singhal D, Bardy J.J, Foreman A, Wormald PJ,  
Laryngoscope, 2011 (Under publication)
  
- *Aspergillus fumigatus* biofilm on primary human sinonasal epithelial culture  
Singhal D, Baker L, Wormald PJ, Tan LW  
American Journal of Rhinology & Allergy, 2011 (Under publication)
  
- Quantitative analysis of mucosal bacterial biofilms  
Singhal D, Field J, Boase S, Jardeleza C, Foreman A, Wormald PJ  
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## ACKNOWLEDGMENTS

*I am extremely honoured and thankful for the privilege of working under the excellent supervision of Prof PJ Wormald, whose guidance and support have been the strength behind this thesis right from its conception to its culmination. I will forever be grateful for the faith he showed in me when he accepted to supervise and guide an overseas trained medical graduate especially who came with plenty of domestic baggage. He has helped me fulfil a vital gap in my medical education by giving me the opportunity to delve into the nuances of scientific research methodologies.*

*I would like to express my sincere gratitude to my co-supervisor Dr LorWai Tan as without her scrupulous guidance, scientific suggestions and constructive help in the laboratory at the Institute, I would not have been able to progress with much of the work for the thesis. I am also grateful to The Queen Elizabeth Hospital Research Foundation for granting me the scholarship that has supported me during my PhD program.*

*Much of the effort in this endeavour has been of my colleagues, both past and present and I wish to extend a special word of thanks to Mr Alkis Psaltis for his guidance and encouragement right from the beginning of this study. I am especially grateful to my co-researcher Dr Andrew Foreman for his co-operation, encouragement and critical advice at every step of the work in the thesis. Co-researchers in the department - Dr Rowan Valentine, Dr Sam Boase, Dr Josh-Jervis Bardy, and Dr Camille Jardeleza have all supported me at odd times and helped with the nuts and bolts of work conducted for the thesis. Ms Leonie Baker has always gone out of the way in providing excellent technical support for the conduct of the laboratory work and I am very grateful for that.*

*Very special thanks goes to Ms Lyn Martin whose gentle guidance, valuable suggestions and friendship have been priceless over the six years that I have been in Adelaide. I am also indebted to Holly McLean and Paula Murray at the ENT clinic in North Adelaide who made the extensive task of collection of data not only feasible but also as smooth going as possible. I would also like to acknowledge the co-operation extended to me by the staff at Adelaide Microscopy, University of Adelaide especially Ms Lyn Waterhouse and Dr Peter Self for their technical guidance extended during the imaging studies required for the work in the thesis.*

*I would like to dedicate this thesis to my family for their trust and confidence in me. Ma and Pa have enduringly nurtured values and principles in me which have made me capable of taking on the responsibility of this PhD program. Big thanks to Anshul & Sonia for the prompt IT support extended across the Pacific for my research work. Last but not the least this thesis would not have been possible without the unfaltering and unconditional support of my husband Nimit. Inspiration and encouragement from him has been endless over these years especially when precious family moments were threatened. My pride rests in my son Gunin and my daughter Niya whose smiles and cuddles have always been with me even while pursuing my research and they have been my motivation to complete this work to the best of my ability.*

*Finally, this work has reached its present shape and presentation with the blessings of Almighty God.*