BACTERIAL & FUNGAL BIOFILMS IN CHRONIC RHINOSINUSITIS

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF ADELAIDE

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"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/passes, 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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- *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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