

CHARACTERISATION OF BIOFILMS IN CHRONIC RHINOSINUSITIS AND ITS CLINICAL AND IMMUNOLOGICAL CONSEQUENCES

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B. Physio, B.M.B.S. (Hons.)

Submitted for the title of Doctor of Philosophy
January 2011

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*Dedicated to my gorgeous girls,
wife Benita and daughter Macy*

DECLARATION

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Dr Andrew Foreman

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ACKNOWLEDGEMENTS

The completion of a PhD is never an individual effort and I formally recognise the following people who have provided significant assistance during my journey of the last three years.

Firstly, to Professor PJ Wormald, who has been a mentor, an inspiration and above all, a friend during my time in the department. His clinical and surgical excellence coupled with his commitment to academic surgery has truly inspired me. Prof has always believed in me and encouraged me to pursue my own research ideas, confident that we were leading the way in this field. His unwavering faith has given me strength in times when I have doubted the direction of this work.

To my co-researchers and friends Dr Alkis Psaltis and Dr Joshua Jarvis-Bardy. Alkis is a pioneer in this field who guided me in the early days. His commitment to excellence has been a motivating force for me to strive for the same. Josh re-energised me during the second year of my PhD with his incredible enthusiasm for research. Both have provided close friendships that I will value long after my research time ends.

My good friends Dr Rowan Valentine and Dr Sam Boase who have shared the highs and lows of research with me over the last three years, always receptive to my problems and always insightful in their responses. To my laboratory supervisor, Dr Lorwai Tan, for her invaluable scientific input as well as the laboratory staff, in particular Ms Leonie Baker. To Dr Deepti Singhal and Dr Marc Tewfik who have contributed intellectually. I also acknowledge the contribution of all members of the Queen Elizabeth Hospital ENT department, in particular Ms Lyn Martin, who has solved all of my small problems and many of the big ones too!

I need to acknowledge my co-collaborators at the University of Ghent, Belgium, Prof Claus Bachert and Ms Gabrielle Holtappels for their expertise in mucosal immunity, along with Dr John Field for his statistical assistance. To Dr Craig James and the staff of Adelaide Pathology Partners, Ms Lyn Waterhouse and the staff of Adelaide Microscopy and Dr Graham van Renen, Ms Cathy Jarman and the staff at Memorial Hospital thank you for facilitating my work in each of your departments.

Finally, I am indebted to the Garnett Passe and Rodney Williams Memorial Foundation for their financial support of Otolaryngology research in this country. Their assistance has ensured our research remains on the cutting edge in this field as well as fostering its presentation on the international stage.

On a personal note, to my wife Benita, undoubtedly this PhD could not have been finished without her unconditional support. She has been incredibly understanding of the haphazard hours I have sometimes kept and supported me to put this work ahead of other things in our life on innumerable occasions. She has been a sounding board after the tough days and shared the spoils when this research has achieved success. It is impossible to thank her for everything with the brevity required here, but she knows how grateful I've been for her sacrifices.

To my sisters, Sarah and Catherine, and brother-in-law, Shaundeeep, whose excellence in their chosen fields has always inspired me to be the best I can be. Lastly to my parents, John and Carole, who have taught me the rewards of hard work and the satisfaction that comes from following my dreams. Their support of my aspirations has been unwavering despite my occasional changes of direction.

Thankyou.

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Adaptive Immune Responses in *Staphylococcus aureus* Biofilm Associated Chronic Rhinosinusitis

Foreman, A., Holtappels, G., Psaltis, A., Jervis-Bardy, J., Field, J., Wormald, P.J., Bachert, C.

Prepared for submission

Non-invasive *Staphylococcus aureus* Biofilm Determination in Chronic Rhinosinusitis by Detecting the Exopolysaccharide Matrix Component Poly-N-Acetylglucosamine

Foreman, A., Jervis-Bardy, J., Boase, S., Tan, L.W., Wormald, P.J.

Prepared for submission

AWARDS ARISING FROM THIS THESIS

Travel Grant

Surgical Research Society of Australasia, ASM, Adelaide, November 2009

Best Presentation, Clinical Higher Degree Students

TQEH Research Foundation Research Day, Adelaide, October 2009

Ronald Gristwood Medal

ASOHNS SA Annual Registrar's Presentation Meeting, Adelaide, August 2009

Best Poster

TQEH Research Foundation Research Day, Adelaide, October 2008

Best Oral Presentation

Australasian Rhinologic Society Meeting, Auckland, New Zealand, September 2008

Highly Commended Poster

University of Adelaide Postgraduate Research Day, July 2008

PRESENTATIONS ARISING FROM THIS THESIS

The Role of Biofilms in Chronic Rhinosinusitis

13th Advanced FESS Course, Adelaide, November 2010, invited speaker

Characterisation of biofilms in Chronic Rhinosinusitis and its clinical and immunologic significance

Basil Hetzel Institute Post-Graduate Seminar, Adelaide, November 2010

Biofilms in Chronic Sinusitis

AAO-HNS Annual Scientific Meeting, Boston, MA, USA, September 2010

S. aureus biofilms in Chronic Rhinosinusitis

5th Australasian Rhinologic Society, Sydney, September 2010, invited speaker

Biofilms and Chronic Rhinosinusitis

2nd Endoscopic and Skull Base Dissection Course, Sydney, August 2010, invited speaker

Characterisation of biofilms in Chronic Rhinosinusitis and its clinical and immunologic significance

Frontiers in Otolaryngology, Melbourne, July 2010

Do S. aureus biofilms contribute to the pathogenesis of Chronic Rhinosinusitis?

ASOHNS ASM, Sydney, March 2010

Blocks and Bugs: Failing Endoscopic Sinus Surgery

Otolaryngology Head & Neck Nurses Group, Sydney, Australia, March 2010

Mechanisms of Recalcitrance in Chronic Rhinosinusitis

Advanced FESS Course, Adelaide, November 2009, invited speaker

Characterization of Bacterial and Fungal Biofilms in Chronic Rhinosinusitis

American Rhinologic Society Annual Scientific Meeting, San Diego, USA, October 2009

Do S. aureus Biofilms play a role in Chronic Rhinosinusitis?

Surgical Research Society of Australasia, ASM, Adelaide, November 2009

Do S. aureus Biofilms play a role in Chronic Rhinosinusitis?

TQEH Research Foundation Research Day, Adelaide, October 2009

Do S. aureus Biofilms play a role in Chronic Rhinosinusitis?

RP Jepson Medal, Adelaide, October 2009

'Closing the Loop' on Biofilms in Chronic Rhinosinusitis

ASOHNS SA Annual Registrar's Presentation Meeting, Adelaide, August 2009

Biofilms in CRS

Roundtable discussion, University of Ghent, Ghent, Belgium, invited speaker, May 2009

Biofilms in CRS- A FISH Study

ASOHNS ASM, Gold Coast, May 2009

Why do patients fail endoscopic sinus surgery?

Otolaryngology Head & Neck Nurses Group, Gold Coast, Australia, May 2009

The Role of Biofilms in CRS

11th Advanced FESS Course, Adelaide, November 2008, invited speaker

Biofilms in CRS- A FISH Study

Australasian Rhinologic Society Meeting, Auckland, New Zealand, September 2008

Biofilms in CRS- A FISH Study

ASOHNS SA CME Meeting, August 2008

ABBREVIATIONS USED IN THIS THESIS

CRS	Chronic Rhinosinusitis	IFN	Interferon
FISH	Fluorescence <i>in situ</i>	IL	Interleukin
	Hybridisation	COPD	Chronic obstructive
CSLM	Confocal Scanning Laser		pulmonary disease
	Microscopy	TGF	Transforming growth factor
PNAG	Poly- <i>N</i> -acetylglucosamine	<i>S. pneumoniae</i>	<i>Streptococcus pneumoniae</i>
ICD	International Classification	<i>H. influenzae</i>	<i>Haemophilus influenzae</i>
	of Diseases	<i>M. catarrhalis</i>	<i>Moraxella catarrhalis</i>
ABRS	Acute Bacterial	<i>S. aureus</i>	<i>Staphylococcus aureus</i>
	Rhinosinusitis	CNS	Coagulase negative
CT	Computed Tomography		staphylococci
US	United States	<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
CRSwNP	Chronic rhinosinusitis with	ESS	Endoscopic sinus surgery
	nasal polyps	SCV	Small colony variant
CRSsNP	Chronic rhinosinusitis	APC	Antigen presenting cell
	without nasal polyps	TCR	T cell receptor
EM	Eosinophilic mucus	MHC	Major histocompatibility
AFS	Allergic fungal sinusitis		complex
NAFES	Non-allergic fungal	V- β	Variable- β
	eosinophilic sinusitis	SE	Staphylococcal enterotoxin
NANFES	Non-allergic, non-fungal	TSST	Toxic shock syndrome
	eosinophilic sinusitis		toxin
IgE	Immunoglobulin E	ABPA	Allergic bronchopulmonary
TLR	Toll-like receptor		aspergillosis
PAMP	Pathogen associated	PCR	Polymerase chain reaction
	molecular pattern	DGH	Distributed gene hypothesis

<i>P. gingivalis</i>	<i>Porphyromonas gingivalis</i>	RAST	Radioallergosorbent test
ORL	Otorhinolaryngology	DNA	Deoxyribonucleic acid
SEM	Scanning electron microscopy	EPS	Exopolysaccharide
TEM	Transmission electron microscopy	CSF	Cerebrospinal fluid
		SD	Standard Deviation
		L-M	Lund-MacKay
RNA	Ribonucleic acid	<i>P. oris</i>	<i>Prevotella oris</i>
CAZS	Citric acid zwitterionic surfactant	<i>E. aerogenes</i>	<i>Enterobacter aerogenes</i>
		<i>K. pneumonia</i>	<i>Klebsiella pneumoniae</i>
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>	IQR	Interquartile range
FBP	Fibronectin binding protein	Th ₁	T-helper ₁
PVL	Panton-Valentine Leukocidin	Th ₂	T-helper ₂
SaPI	Staphylococcal pathogenicity islands	ECP	Eosinophilic cationic protein
<i>agr</i>	Accessory gene regulator	MPO	Myeloperoxidase

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THESIS SUMMARY

The research contained within the PhD thesis investigates the role of biofilms in chronic rhinosinusitis (CRS). Following an exhaustive literature review of CRS aetiology, pathogenesis and microbiology, the key deficiencies in our understanding of this disease were highlighted; with particular attention paid to potential role biofilms might play in this disease. It was clear from the literature that the issue of the common biofilm-forming organisms in CRS was incompletely understood and that if this was clarified, it could then be used as a basis for more species-specific investigation of biofilms in CRS. Adopting a species-specific approach may facilitate the development of targeted, novel anti-biofilm strategies that could be employed to improve the outcomes of our most recalcitrant patients.

This research investigation commenced with a study to directly correlate a newly developed Fluorescence *in situ* Hybridisation (FISH) protocol with the current gold standard- BacLight staining imaged on the confocal scanning laser microscope (CSLM). Not only did this project validate FISH as a tool for biofilm identification in CRS but also it also clearly elucidated the research scenarios in which each of these complementary techniques could be used. This will assist to guide future research. A larger cohort study then identified *S. aureus* as the most common biofilm-forming organism in an often polymicrobial mix that may contain fungal biofilms. The clinical relevance and immunological consequences of biofilm characterisation were then explored.

A retrospective clinical investigation found that patients with unimicrobial *H. influenzae* biofilms had mild disease that was highly responsive to current treatment strategies. In contrast, patients with *S. aureus* biofilms whether alone or in association with other species had severe disease and poor evolution after surgery. These results suggest that in CRS, not all biofilms are the same. Investigating the immunological consequences of biofilm

characterisation in CRS also separated out the two common biofilm-forming organisms *H. influenzae* and *S. aureus*. *H. influenzae* biofilms were not associated with a particular skewing of the T-helper adaptive immune response or a release of superantigens. However *S. aureus* biofilm interact with the immune system both directly, with a skewing of the T cell response towards the T-helper₂ cascade and a subsequent eosinophilic inflammation, and indirectly via dispersing planktonic clones that may release superantigens into the sinuses. Importantly this study was also able to differentiate the effect of superantigens and *S. aureus* biofilms by discovering that superantigens act via IgE induction whereas *S. aureus* biofilms skew the T-helper response towards the T-helper₂ pathway with elevated IL-5, ECP and TGF- β_1 being characteristic of their presence. This novel discovery highlights a potential independent role for *S. aureus* biofilms in CRS pathogenesis, providing a link between biofilms and disease for the first time.

Finally, with an eye to the future, we developed a novel, non-invasive diagnostic test for *S. aureus* biofilms. We were able to achieve this via detection of the exopolysaccharide matrix component poly-*N*-acetylglucosamine (PNAG). PNAG is essential for biofilm formation by *S. aureus* and its detection allows us to differentiate between purely planktonic cultures of this organism and the more quiescent biofilm form, which may escape detection by routine microbiological testing, but has been demonstrated already to be associated with severe, surgically recalcitrant disease. The development of this test requires further testing but will ultimately be able to diagnose *S. aureus* biofilms without the need for mucosal biopsies, allowing pre- and post-operative biofilm detection to guide directed and aggressive anti-biofilm treatment strategies.