Characterisation of Biofilms in Chronic Rhinosinusitis and its Clinical and Immunological Consequences

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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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PUBLICATIONS ARISING FROM THIS THESIS

Characterization of Bacterial and Fungal Biofilms in Chronic Rhinosinusitis

Foreman, A., Psaltis, A. J., Tan, L.W., Wormald, P.J.

American Journal of Rhinology and Allergy 2009, 23 (6): 556-61.

Targeted Imaging Modality Selection for Bacterial Biofilms in Chronic Rhinosinusitis

Foreman, A., Psaltis, A. J., Singhal, D., Wormald, P.J.

Laryngoscope 2010, 120 (2): 427-31.

Different Biofilms, Different Disease? A Clinical Outcomes Study

Foreman, A., Wormald, P.J.

Laryngoscope 2010, 120 (8): 1701-6.

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

P. gingivalis	*Porphyromonas gingivalis*	RAST	Radioallergosorbent test
ORL	Otorhinolaryngology	DNA	Deoxyribonucleic acid
SEM	Scanning electron microscopy	EPS	Exopolysaccharide
		CSF	Cerebrospinal fluid
TEM	Transmission electron microscopy	SD	Standard Deviation
		L-M	Lund-MacKay
RNA	Ribonucleic acid	*P. oris*	*Prevotella oris*
CAZS	Citric acid zwitterionic surfactant	*E. aerogenes*	*Enterobacter aerogenes*
		K. pneumonia	*Klebsiella pneumoniae*
S. epidermidis	*Staphylococcus epidermidis*	IQR	Interquartile range
FBP	Fibronectin binding protein	Th_1	T-helper$_1$
PVL	Panton-Valentine Leukocidin	Th_2	T-helper$_2$
		ECP	Eosinophilic cationic protein
SaPI	Staphylococcal pathogenicity islands	MPO	Myeloperoxidase
agr	Accessory gene regulator		

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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-helper$_2$ 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-helper$_2$ 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.