

# THE ROLE OF BACTERIAL BIOFILMS IN CHRONIC RHINOSINUSITIS

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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*Dedicated to my wonderful parents Jim and Lela  
& to my beautiful wife Angela*

*"To climb steep hills requires slow pace at first."*  
William Shakespeare

## 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 and, to the best of my knowledge and belief, contains no material published or written by another person, except where due reference has been made in the text.

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1st June 2008

# ABSTRACT

ALKIS J PSALTIS

PHD THESIS

## THE ROLE OF BACTERIAL BIOFILMS IN CHRONIC RHINOSINUSITIS

This thesis embodies research investigating the role that bacterial biofilms play in the pathogenesis of chronic rhinosinusitis (CRS). It focuses on their detection on the sinus mucosa of CRS patients and the implications of their presence. Finally, it addresses deficiencies in the innate immune system that may predispose to their development in this condition.

Bacterial biofilms are structural assemblages of microbial cells that encase themselves in a protective self-produced matrix and irreversibly attach to a surface. Their extreme resistance to both the immune system as well as medical therapies has implicated them as playing a potential role in the pathogenesis of many chronic diseases. Although their role in many diseases is now well established, their objective presence and importance in CRS remains largely unknown.

Chapter 1 of this thesis reviews the current literature pertaining to CRS and biofilms and critically evaluates the small body of research relating to this topic.

Chapter 2 describes the development of a sheep model to study the role of bacterial biofilms in rhinosinusitis. It compares the use of traditional electron microscopy (EM) and more recent confocal scanning laser microscopy (CSLM) in the detection of biofilms on the surface of sinus mucosa. The results of this study inferred a causal relationship between biofilms and the macroscopic changes that accompany rhinosinusitis. Furthermore it illustrated the superiority that CSLM has over EM in the imaging of biofilms on sinus mucosa

Chapter 3 and 4 outline the results of human studies utilizing the more objective CSLM to evaluate the prevalence of bacterial biofilms on the sinus mucosa of CRS patients and their effect on post-operative mucosal healing. The results of these studies demonstrated a biofilm prevalence of approximately 50% in the CRS population studied and suggested, that biofilm presence may predispose to adverse post-operative outcomes following sinus surgery.

Chapter 5 and 6 describe experiments examining the level of the innate immune system's anti-biofilm peptide lactoferrin, in patients with CRS. Lactoferrin was found to be down-regulated at both an mRNA and protein level in the majority of CRS patients, with biofilm positive patients demonstrating the most significant reduction.

In summary, this thesis provides further evidence that bacterial biofilms play a major role in the pathogenesis and disease persistence in a subset of CRS patients. Deficiencies in components of the innate immune system, such as lactoferrin, may play an important role in the predisposition of certain individuals to the initial development of bacterial biofilms.

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## ABBREVIATIONS USED IN THIS THESIS

CRS	- Chronic Rhinosinusitis	FISH	-Fluorescent In Situ Hybridization
EM	-Electron Microscopy	OSA	-Obstructive Sleep Apnoea
CSLM	-Confocal Scanning Laser Microscopy	TEM	-Transmission Electron Microscopy
mRNA	-Messenger Ribosomal Nucleic Acid	SEM	-Scanning Electron Microscopy
GP	-General Practitioner	PMN	-Polymorphonuclearcytes
RCT	-Randomized Control Trial	HSV	-Herpes Simplex Virus
FESS	-Functional Endoscopic Sinus Surgery	CMV	-Cytomegalovirus
ESS	-Endoscopic Sinus Surgery	HIV	-Human Immunodeficiency Virus
NA	-Not available	HBV	-Hepatitis B Virus
CNS	-Coagulase Negative Staphylococci	HCV	-Hepatitis C Virus
SA	- <i>Staphylococcus aureus</i>	RSV	-Respiratory Syncytial Virus
SP	- <i>Streptococcus pneumoniae</i>	LPS	-Lipopolysaccharide
GNR	-Gram Negative Rods	NO	-Nitric Oxide
SV	- <i>Streptococcus viridans</i>	TNF $\alpha$	-Tumour Necrosis Factor alpha
PA	- <i>Pseudomonas aeruginosa</i>	IL8	-Interleukin 8
H inf	- <i>Haemophilus influenzae</i>	NK	-Natural Killer Cells
DIC	-Differential Interference Contrast	cDNA	-Complementary strand DNA
PCR	-Polymerase Chain Reaction	CF	-Cystic Fibrosis
MHC	-Major Histocompatibility Complex	ATCC	-American Type Culture Collection
Th1	-T Helper Cell 1	MQ	-Milli-Q
Th2	-T Helper Cell 2	PBS	-Phosphate buffered solution
SEA	-Staphylococcal Enterotoxin A	RAST	-Radioallergosorbent testing
SEB	-Staphylococcal Enterotoxin B	CT	-Computerized tomography
IgG	-Immunoglobulin G	ICC	-Interobserver correlation coefficient
CRS/NP	-Chronic Rhinosinusitis with Nasal Polyposis	AFS	-Allergic Fungal Sinusitis
TSST-1	-Toxic Shock Syndrome Toxin 1	NAFES	-Non Allergic Fungal Eosinophilic sinusitis
EPS	-Exopolysaccharide matrix	NANFES	-Non allergic, Non Fungal Eosinophilic sinusitis
Bap	-Biofilm associated proteins	OR	-Odds Ratio
PIA	-Polysaccharide Intercellular Adhesin	C.I.	-Confidence Interval
MDR	-Multidrug efflux pumps	VAS	-Visual Analogue Scale
CAM	-Cationic Antimicrobial Peptides	CSS	-Chronic sinusitis survey
OME	-Otitis Media with Effusion	qRT-PCR	-Quantitative real-time reverse- transcriptase polymerase chain reaction
ELISA	-Enzyme linked immunosorbent assay	LF	-Lactoferrin
HPRT	-Hypoxanthine-guanine phosphoribosyltransferase		
Ct	-Cycle threshold		

## ACCOMPLISHMENTS

### AWARDS OBTAINED FOR RESEARCH ASSOCIATED WITH THIS THESIS

**The Ronald Gristwood Medal for Best Presentation by a South Australian Ear Nose and Throat Registrar**, for the presentation titled “Lactoferrin expression in chronic rhinosinusitis patients” Adelaide September 2005

**The Queen Elizabeth Hospital Research Day Presentation Award for the Best Clinical Presentation** titled “ Lactoferrin expression in Chronic Rhinosinusitis Patients” Adelaide October 2005

**The Maurice H Cottle Award for the best paper presented at the Annual Meeting of the American Rhinologic Society**, for paper titled “ A sheep model for the study of biofilms in rhinosinusitis” Toronto September, 2006

**The Sir Edwin Hughes Memorial Prize for Clinical Research in Surgery for the best presentation by an Australian or New Zealand Surgical Registrar**, for the presentation titled “A new technique for the study of biofilms in sinusitis” Monash University, Victoria, October 2006

**The Queen Elizabeth Hospital Research Day Presentation Award for Best Clinical Presentation in Higher Degree Category** for the presentation titled “ A sheep model to study the role of biofilms in rhinosinusitis” Adelaide, October 2006.

**The R.P Jepson Medal awarded for the best presentation at the Annual Scientific Meeting at the Royal Australian College of Surgeons, SA and NT Sub-committee**, for the presentation titled “The effect of biofilms on post-sinus surgical outcomes” Adelaide August 2007

**The Best Poster Award at Inaugural Post-Graduate Research Expo for the Faculty of Health Sciences of the University of Adelaide** for the poster titled “Development of a sheep model for the study of biofilms in rhinosinusitis” Adelaide, October 2007

**The Best Presentation and Overall Winner at the Inaugural Research Expo for the Faculty of Health Sciences of the University of Adelaide**, “Development of a sheep model for the study of biofilms in rhinosinusitis” Adelaide, October 2007



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Psaltis AJ and Ha KR (Co-first authors), Tan L, Wormald PJ.  
American Journal of Rhinology. 2007 May-Jun;21(3):339-45.  
Erratum in: American Journal of Rhinology. 2007 Jul-Aug;21(4):519.

**Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis.**

Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ.  
Laryngoscope. 2007 Jul;117(7):1302-6.

**Nasal mucosa expression of lactoferrin in patients with chronic rhinosinusitis.**

Psaltis AJ, Bruhn MA, Ooi EH, Tan LW, Wormald PJ.  
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**Reduced Levels of Lactoferrin in Biofilm-Associated Chronic Rhinosinusitis.**

Psaltis AJ, Wormald PJ, Ha KR, Tan LW.  
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**The effect of biofilms on post-sinus surgical outcomes.**

Psaltis AJ, Weitzel E, Wormald P-J  
American Journal of Rhinology. 2008 Jan-Feb;22(1):1-6.

**In Vitro Activity of Mupirocin on Clinical Isolates of Staphylococcus aureus and its Potential Implications in Chronic Rhinosinusitis.**

Ha KR, Psaltis AJ, Butcher AR, Wormald PJ, Tan LW.  
Laryngoscope. 2007 Dec 3; [Epub ahead of print]

## PRESENTATIONS

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Biofilm Roundtable Discussion, Sydney Australia, October 2005.

### **The Heterogeneity of Lactoferrin Expression in Patients with Chronic Rhinosinusitis**

Queen Elizabeth Hospital Research Day, Adelaide Australia, October 2005.

### **The Expression of Lactoferrin in Patients with Chronic Rhinosinusitis.**

International Rhinological Society Meeting, Sydney Australia, October 2005.

### **Biofilms and the nose**

Australasian Rhinological Society Annual Meeting, Barossa, South Australia, October 2006

### **A sheep model for the study of biofilms in Chronic Sinusitis**

Annual Meeting of the American Rhinologic Society, Toronto, Canada September 2006

### **A new technique for the study of biofilms in sinusitis**

The Cabrini Institute, Monash University, Melbourne Victoria October 2006

### **An animal model to study biofilms in chronic rhinosinusitis**

Queen Elizabeth Hospital Research Day, Adelaide, Australia, October 2006

### **CSLM study of biofilms in human CRS patients**

ASOHNS Scientific Meeting, Adelaide, Australia, March 2007

### **Biofilms and Chronic Rhinosinusitis**

Invited Speaker Divisions of Surgery Departmental Meeting, Adelaide, Australia June 2007

### **The effect of biofilms on post-sinus surgical outcomes.**

Annual Scientific Meeting at the Royal Australian College of Surgeons, Adelaide, Aug 2007

### **The effect of biofilms on post-sinus surgical outcomes.**

American Rhinologic Society Meeting, Washington DC, USA September 2007

### **The effect of biofilms on post-sinus surgical outcomes.**

Queen Elizabeth Hospital Research Day, Adelaide, Australia October 2007

### **A sheep model for study of biofilm in rhinosinusitis.**

Research Expo of the Faculty of Health Sciences, University of Adelaide, Australia Oct 2007

### **The role of biofilms in CRS.**

Centre for Genomic Studies , Allegheny General Hospital Pittsburgh, PA. USA April 2008

**A sheep model investigating several potential antibiofilm treatments.** The American Triological Society of Otorhinolaryngology (COSM), Spring Meeting, Orlando Florida, USA May 2008

**Biofilms and Chronic Rhinosinusitis** –American Academy of Head and Neck Surgeons and Otolaryngologists, Annual Scientific Meeting , Chicago, Illinois, USA September 2008

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