Nitric Oxide and Staphylococcus aureus Biofilms: Defining their Intricate Relationship in Chronic Rhinosinusitis

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Dedicated to my parents Ted and Teret

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Statements of Authorship

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Thesis Abstract

This thesis aims to address the relationship of *Staphylococcus aureus (S. aureus)* biofilms to the endogenously produced gas nitric oxide (NO) in chronic rhinosinusitis (CRS). While *S. aureus* biofilms are associated with recalcitrance and high severity in CRS, the naturally elevated NO gas is significantly lower in sinuses of CRS patients. However, the relationship of these 3 important factors in CRS aetiopathogenesis is poorly defined. To further clarify this host-microbe-environment (NO) relationship, this thesis first looks into the history of each factor, the roles they play in other disease processes, and the most recent clinical findings and applications in current literature. Building on this foundation, the projects emanating from this thesis hoped to fill in some gaps in knowledge of these 3 components, identifying that all are linked to disease manifestation, and that each can mutually contribute to CRS pathogenesis.

The first project was designed to establish a clearer description of the relationship between NO and *S. aureus* biofilms. Utilizing *S. aureus* strains from CRS patients, these were grown as biofilms and exposed to various NO concentrations mimicking NO levels measured in healthy sinuses vs. CRS patients. We demonstrated the dualistic effects of NO on biofilm growth: increased at lower NO concentrations mimicking diseased sinuses, and anti-biofilm effects at higher concentrations similar to measurements in healthy sinuses. These findings became a stepping stone for the potential design of NO as a therapeutic agent in *S. aureus*-associated CRS.

But first, further characterization of NO's role on the host immune response was needed. The 2nd and 3rd projects aimed to define the host–NO relationship, focusing on the genes involved in NO regulation within the sinonasal mucosa. Because NO is

considered one of the reactive oxygen species (ROS), major players of the innate immune response, genes involved in ROS/innate immunity were investigated. CRS patients, with or without polyps, were sub-classified as either with or without *S. aureus* biofilms, allowing a separate analysis of the role *S. aureus* biofilms play in the alteration of gene expression. The results showed that *S. aureus* biofilm presence associates with a significant difference in the certain gene expressions which have specific roles in NO regulation. This indicates that the microorganism may alter or contribute to an impaired localized innate immune response in the sinuses, or alternatively favor growth in genetically susceptible individuals. Although the cause-effect timeline was not established, these results will serve as baseline for future gene and protein studies that will further increase our understanding of the NO-CRS pathophysiology.

Lastly, building on the therapeutic potential of NO as an anti-biofilm agent, we aimed to design a suitable NO-based topical agent against *S. aureus* biofilms. The 4th project tested a multitude of liposome-encapsulated NO formulations in-vitro with the best formulation tested for safety and efficacy in a sheep model of rhinosinusitis. These projects were designed with an aim for future clinical trials, to test a novel NO-based topical agent, which can be used as a safe and efficacious topical sinus rinse to benefit CRS patients.

Declarations

I certify that 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 awarded to Camille Jardeleza. To the best of my knowledge and belief, this work contains no material previously published or written by another person, except where due reference has been made in the text.

I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Dr. Camille Jardeleza

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Publications arising from this Thesis

The effects of nitric oxide on *Staphylococcus aureus* biofilm growth and its implications in chronic rhinosinusitis

Jardeleza C, Foreman A, Baker L, Paramasivan S, Field J, Tan LW, Wormald PJ.

International Forum of Allergy and Rhinology 2011, 1(6): 438-44.

Gene expression differences in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis

Jardeleza C, Jones D, Baker L, Miljkovic D, Boase S, Tan NC, Vreugde S, Tan LW, Wormald PJ.

International Forum of Allergy and Rhinology 2013, 3(3): 193-8.

Inflammasome gene expression alterations in *Staphylococcus aureus* biofilm-associated Chronic Rhinosinusitis

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Rhinology 2013, 51(4): 315-22.

Liposome-encapsulated ISMN: A novel nitric oxide-based therapeutic agent against *Staphylococcus aureus* biofilms

Jardeleza C, Rao S, Thierry B, Gajjar P, Vreugde S, Prestidge C, Wormald PJ.

Plos One 2014, 9(3): e92117.

Liposome-encapsulated nitric oxide donor: a novel topical treatment for *Staphylococcus aureus* biofilm- associated rhinosinusitis

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Prepared for submission

Awards arising from this Thesis

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Research Day, The Queen Elizabeth Hospital / Basil Hetzel Institute, Adelaide SA, Oct 2010

BASIC RESEARCH SCIENCE AWARD WINNER for best scientific paper

American Rhinologic Society, San Francisco Ca, USA, Sept 2011

RONALD GRISTWOOD MEDAL, Best South Australian ENT Registrar Presentation for Research, Adelaide, SA, Nov 2012

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Presentations arising from this Thesis

The role of nitric oxide in the pathophysiology of *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis
Basil Hetzel Institute Post Graduate Seminar, Adelaide, July 2010

The role of nitric oxide in the pathophysiology of *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis
The Queen Elizabeth Hospital / Basil Hetzel Institute Research day, Adelaide Oct 2010

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis
ASOHNS, Annual Scientific Meeting, Melbourne, April 2011

The role of nitric oxide on *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis
Basil Hetzel Institute Post Graduate Seminar, Adelaide, May 2011

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis
American Rhinologic Society Annual Scientific Meeting, San Francisco Ca, USA September 2011

Gene expression differences in nitric oxide regulation point to an altered innate immune response in chronic rhinosinusitis
The Australian Society for Medical Research SA Scientific Conference, Adelaide, June 2012

The role of nitric oxide on *Staphylococcus aureus* biofilm growth in chronic rhinosinusitis
Endoscopic Sinus and Skull Base Surgery Course August 2-4 2012, St. Vincent's Hospital, Sydney NSW, August 2012 - Invited speaker

The Efficacy of Liposome-encapsulated Nitric Oxide on *Staphylococcus aureus* biofilms in Chronic Rhinosinusitis
Basil Hetzel Institute Post Graduate Seminar, Adelaide, May 2012

Gene expression difference in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis
American Rhinologic Society Annual Scientific Meeting, Washington DC, USA September 2012

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis
Ronald Gristwood Medal, Adelaide, October 2012

Management of the recalcitrant sinus infection
With NC Tan, 15th Advanced FESS course, Adelaide, November 2012 – Invited speaker

Gene expression difference in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis
ASOHNS, Annual Scientific Meeting, Perth, March 2013

Liposome-encapsulated Nitric Oxide against *Staphylococcus aureus* biofilms in a rhinosinusitis sheep model
Basil Hetzel Institute Post Graduate Seminar, Adelaide, June 2013

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis
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Abbreviations Used in the Thesis

AdSA: Adenosine synthase A

AERD: Aspirin Exacerbated Respiratory Disease

AFS: Allergic Fungal Sinusitis

AIM2: Absent in Melanoma 2

ASC: Apoptosis-associated Speck-like protein with a CARD

bNOS: Bacterial Nitric Oxide Synthase

B– P-: Biofilm negative Polyp negative

B- P+: Biofilm negative Polyp positive

B+ P-: Biofilm positive Polyp negative

B+ P+: Biofilm positive Polyp positive

CARD: Caspase Recruitment Domain

CASP5: Caspase-5

CAT: Catalase

CCL2: Chemokine Ligand 2

CCL20: Chemokine Ligand 20

CF: Cystic Fibrosis

CFTR: Cystic Fibrosis Transmembrane Conductance Regulator

CLSM: Confocal Scanning Laser Microscopy

CRS: Chronic Rhinosinusitis

CRSsNP: Chronic Rhinosinusitis without Nasal Polyps

CRSwNP: Chronic Rhinosinusitis with Nasal Polyps

Cyclic GMP: Cyclic Guanosine Monophosphate

DAMP: Danger-Associated Molecular Patterns

DC: Dendritic Cells

DNA: Deoxyribonucleic Acid

DPPG: Dipalmitoylglycero-phosphoglycerol

dsDNA: Double stranded DNA

DUOX1: Dual Oxidase 1

EDRF: Endothelium Derived Relaxing Factor

EGFR: Epidermal growth factor

eNOS: Endothelial Nitric Oxide Synthase

EPS: Extracellular Polymeric Substance

ESS: Endoscopic Sinus Surgery

FISH: Fluorescence in situ hybridization

GTN: Glyceryl Trinitrate

H. influenzae: *Haemophilus influenzae*

HPLC: High Performance Liquid Chromatography

HSP90AA1: Heat shock Protein 90 KDα

IE: Infective Endocarditis

IgE: Immunoglobulin E

IgG: Immunoglobulin G

IL-1β: Interleukin 1-β

IL-18: Interleukin 18

IFN-γ: Interferon-γ

iNOS: Inducible Nitric Oxide Synthase

ISMN: Isosorbide Mononitrate

LPS: Lipopolysaccharide

LTA: Lipoteichoic acid

LFNO: Liposomal-formulated nitric oxide donor

MBEC: Minimum Biofilm Eradication Concentration

M. catarrhalis: *Moraxella catarrhalis*

MLV: Multilamellar vesicles

MRSA: Methicillin Resistant *Staphylococcus aureus*

N_2O: Nitrous Oxide

NFκβ: Nuclear factor kappa beta

NADPH: Nicotinamide adenine dinucleotide phosphate

NCF2: Neutrophil cytosoloic factor 2

NLR: NOD-like receptors

NLRP3: NOD-like receptor pyrin domain containing protein

NME5: Non-metastatic cell 5

NO2: Nitrite

NO3: Nitrate

NOD: Nucleotide Oligomerization Domain

nNOS: neuronal Nitric Oxide Synthase

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

OME: Otitis Media with Effusion

ONOO-: Peroxynitrite

OXR1: Oxidoreductase 1

P. aeruginosa: *Pseudomonas aeruginosa*

PALS: Phase analysis light scattering

PAMP: Pathogen-associated molecular patterns

PANX1: Pannexin 1

PDI: Polydispersity Index

PCD: Primary Ciliary Dyskinesia

PCR: Polymerase Chain Reaction

PRDX2: Peroxiredoxin 2

PRDX5: Peroxiredoxin 5

PRDX6: Peroxiredoxin 6

PRNP: Prion protein **PRR**: Pattern-recognition receptors **PSTPIP1**: proline-serine- threonine phosphatase interacting protein 1 **PYCARD**: PYD and CARD Domain containing gene **qRT-PCR**: Quantitative Real Time PCR **RANKL**: Receptor activator of nuclear factor kappa-β ligand **RAST**: Radioallergosorbent test **RLH**: RNA-sensing RIG-like helicases **RNS**: Reactive Nitrigen Species **ROS**: Reactive Oxygen Species ***S. aureus***: *Staphylococcus aureus* **SCV**: Small Colony Variants ***S. pneumoniae***: *Streptococcus pneumoniae* **TH1**: T-Helper 1 **TH2**: T-Helper 2 **TLR**: Toll-Like Receptor **TNFSF11**: Tumor necrosis factor member 11	**ULV**: Unilamellar vesicles **XIAP**: X-linked inhibitor of apoptosis protein **ZBP1**: Z-DNA-binding protein

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Figures