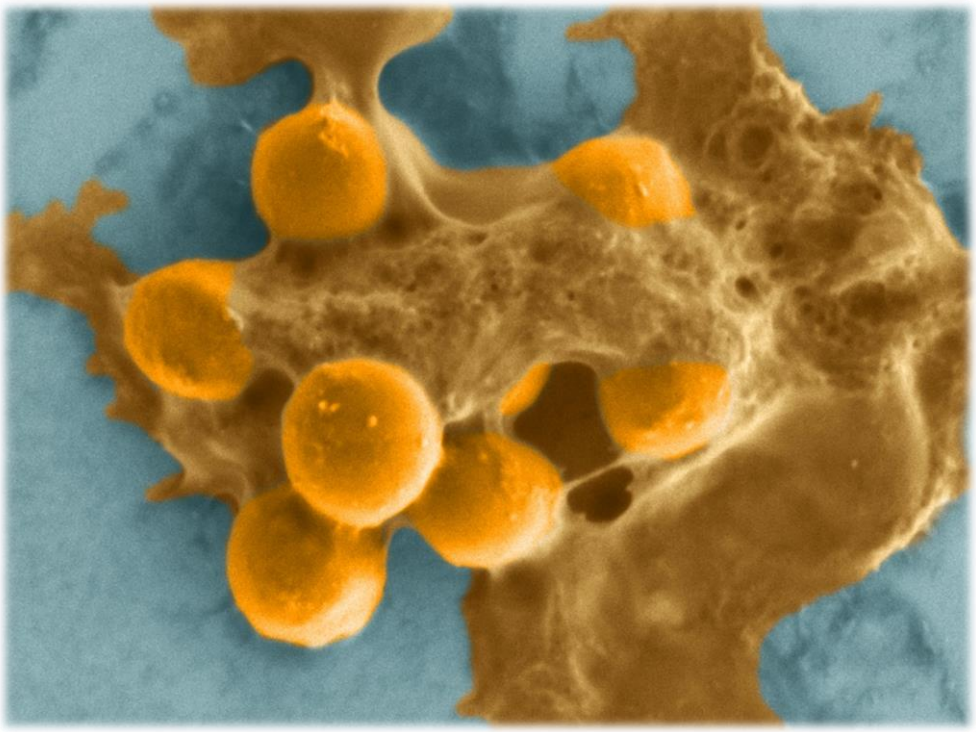
Towards Novel Antibiofilm Strategies



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THE UNIVERSITY of ADELAIDE

To my parents- who opened the world to me.

And to Nicky- who made my world complete.

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I. Abstract

The rise of multidrug resistant bacteria has global implications posing a threat to human health. Bacteria naturally reside in biofilms as complex communities of cells encased in a self-assembled matrix. The biofilm state renders bacteria up to 1000-fold less susceptible to antimicrobial treatments, while unarming the body's immune response and promoting antibiotic resistance. Biofilms are recognised as the origin of devastating, antibiotic-refractory diseases and are associated with 80% of infections in the body, including chronic rhinosinusitis. The capability of bacteria in biofilms to resist current antibiotic therapies emphasises the need for novel therapeutic strategies.

Whilst oral drug delivery is frequently ineffective to treat biofilm-related infections, topical treatments have the potential to deliver higher drug concentrations to the infection-site while reducing systemic side-effects. In this thesis, the development of two innovative topical strategies against antibiotic resistant bacteria and bacterial biofilms were explored, specifically: (i) colloidal silver nanoparticles (AgNPs) and (ii) a treatment combining the iron chelator deferiprone (Def) and the haem analogue gallium-protoporphyrin (GaPP).

(i) Whilst the antimicrobial activity of spherical AgNPs is well described in planktonic bacteria, little is known about their antibiofilm effects and the influence of particle shape. AgNP spheres, cubes and stars were synthesised and their cytotoxicity towards human macrophages and human bronchial epithelial cells, as well as their activities against *S. aureus*, MRSA and *P. aeruginosa* biofilms were evaluated. While non-desirable toxicity and stability limited the utilisation of AgNP cubes and stars, AgNP spheres showed significant antibiofilm activity against clinically relevant biofilms *in vitro* and in an *in vivo* infection model in *C. elegans*. Moreover, AgNP spheres were physically stable in suspension for over 6 months with no observed loss of antibiofilm activity. This research has led to a phase I human clinical trial that commenced in October 2016 at The Queen Elizabeth Hospital, Woodville, SA, Australia.

(ii) The antibiofilm activity of a novel treatment combining Def and GaPP was investigated. These compounds interfere with bacterial iron metabolism, which presents a unique alternative target vital for all human pathogens. Def-GaPP demonstrated synergistic antibiofilm effects against a series of bacteria, including reference strains and multidrug resistant clinical isolates of *S. aureus*, *S. aureus* small colony variants, MRSA, *S. epidermidis*, *P. aeruginosa* and *A. johnsonii*. Furthermore, Def-GaPP potentiated the activity of antibiotics. *In vitro* cell culture studies confirmed no toxicity of Def-GaPP in murine fibroblasts and human bronchial epithelial cells. Moreover, a clinically used chitosan-dextran hydrogel for wound healing was used as a delivery vehicle for Def-GaPP, thereby complementing wound healing effects with strong antibacterial properties. The Def-GaPP gel showed significant antibiofilm activity in an *in vitro* wound model and in an *in vivo* infection model in *C. elegans*. This work resulted in a patent approval.

Two innovative strategies (i.e. colloidal AgNPs and Def-GaPP gel) have arisen from this thesis that hold significant promise as topical antibiofilm treatments. Both strategies have potential as alternatives to antibiotics or as adjuvants for the treatment of multidrug resistant bacteria and biofilm-associated infections and are advancing for clinical use.

II. Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, 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.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

III. Acknowledgements

My PhD has been 3 years of excitement, frustration, happiness and chaos- the full rollercoaster of life. It has been quite a journey.

I met incredible people and inspiring characters, established networks all over the world and communicated with people of various backgrounds.

"It is the lives we encounter that make life worth living." -Guy de Maupassant-

My PhD has been an interdisciplinary project with a translational focus and is the result of successful teamwork and fruitful collaborations.

I am grateful for the supervision and guidance of Sarah Vreugde (ENT Surgery, Basil Hetzel Institute for Translational Health Research, University of Adelaide), Peter-John Wormald (ENT Surgery, The Queen Elizabeth Hospital, University of Adelaide) and Clive Prestidge (School of Pharmacy, University of South Australia).

Sarah's support as my principal supervisor was invaluable. Her guidance and encouragement have been an integral element of my PhD and I am grateful for all she has done. The on-going support and availability almost round the clock cannot be valued highly enough. I am happy to call you my "doctor mother".

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Seek light

Sub cruce lumen

IV. Presentations arising from this thesis

13 oral presentations at national and international scientific conferences, including

- *'Silver nanoparticles as a topical treatment for biofilm-related infections'*
  2017 Annual Meeting of the Australian Society for Microbiology, Hobart, TAS, Australia
- '*Bug Wars- Battlefront Biofilms*'
  Invited speaker at the 2017 Nurses and Midwives Research Symposium, Adelaide, SA, Australia
- '*A surgical hydrogel to improve wound healing and fight bacterial biofilms*'
  2017 Annual Meeting of the Australian Society for Otolaryngology Head and Neck Surgery, Adelaide, SA, Australia
- '*Silver nanoparticles as a topical chronic rhinosinusitis treatment*'
  2017 Annual Meeting of the Australian Society for Otolaryngology Head and Neck Surgery, Adelaide, SA, Australia
- *'A topical antibiotic-free treatment to fight bacterial biofilms'*
  2017 Antimicrobials and StaphPath Symposium, Adelaide, SA, Australia
- *'Nanoparticles to tackle clinically relevant biofilms'*
  2017 Australian Colloid and Interface Symposium, Coffs Harbour, NSW, Australia
- *'Silver nanoparticles to tackle clinically relevant biofilms'*
  2016 Annual The Queen Elizabeth Hospital Research Day, Woodville, SA, Australia
- *'A topical treatment not based on antibiotics to fight bacterial biofilms'*
  2016 Antimicrobial Resistance in Microbial Biofilms and Options for Treatment Conference, Gent, Belgium
- *'A surgical hydrogel to combat MSSA and MRSA biofilms'*
  2016 Annual Meeting of the American Rhinologic Society, San Diego, CAL, USA
- *'Bug Wars- Battlefront Biofilms'*
  2016 Annual Meeting of the Australian Society for Microbiology, Perth, WA, Australia
- *'To sneeze or not to sneeze- a novel approach to combat sinonasal biofilms'*
  2015 Annual The Queen Elizabeth Hospital Research Day, Woodville, SA, Australia
- *'Mind "De GaPP": in vitro efficacy of deferiprone and gallium-protoporphyrin against Staphylococcus aureus biofilms'*
  2015 Annual Meeting of American Rhinologic Society, Dallas, TX, USA
- *'A Novel strategy to fight Staphylococcus aureus biofilms'*
  2014 Annual The Queen Elizabeth Hospital Research Day, Woodville, SA, Australia

6 poster presentations at national and international scientific conferences, including

- *'Silver nanoparticles as topical antibiofilm approach'*
  5th European Congress on Microbial Biofilms (EUROBIOFILMS 2017), Amsterdam, The Netherlands
- *'A non-antibiotic approach to combat S. aureus biofilms using deferiprone and gallium-protoporphyrin'*
  2016 Biofilms7 conference, Porto, Portugal
- *'A non-antibiotic strategy to combat S. aureus biofilms by targeting iron metabolism'*
  2016 Annual Meeting of the Australian Society for Microbiology, Perth, WA, Australia
- *'A non-antibiotic approach to combat S. aureus biofilms'*
  2016 Annual Meeting of the Australian Society for Medical Research, Adelaide, SA, Australia
- *'It takes 2 to tango- in vitro efficacy of deferiprone and gallium-protoporphyrin against S. aureus biofilms'*
  2015 7th American Society for Microbiology Conference on Biofilms, Chicago, IL, USA
- *'A Novel treatment combination to combat Staphylococcus aureus biofilms'*
  2014 Annual Florey International Postgraduate Research Conference, Adelaide, SA, Australia

V. Awards and prizes arising from this thesis

2017

- Channel 9 **Young Achiever of the Year Award, Finalist** in "Science & Technology"
- **Conference Attendance Grant**, European Society of Clinical Microbiology and Infectious Diseases
- **Research Travel Award**, School of Medicine, University of Adelaide

2016

- **People's Choice Winner of the 3 Minute Thesis Competition** and University Finalist, University of Adelaide (youtube video: https://www.youtube.com/watch?v=ZE2q0L2fI8g)
- **Health Award**, Northern Communities Health Foundation
- **Trevor Prescott Memorial Scholarship**, Freemasons Foundation
- Channel 9 **Young Achiever of the Year Award, Finalist** in "Science & Technology"
- **Winner Best 3 Minute Thesis Presentation**, Australian Society for Microbiology
- **International Travel Award**, School of Medicine, University of Adelaide
- **Conference Attendance Grant**, European Society of Clinical Microbiology and Infectious Diseases
- **Student Award**, Australian Society for Microbiology, SA/NT Branch
- **Research Abroad Scholarship**, University of Adelaide

2015

- **D R Stranks Travel Fellowship**, University of Adelaide
- **Winner Best Lay Description**, The Queen Elizabeth Hospital Research Day
- **Bertha Sudholz Research Scholarship** for Excellence in ENT Research, Florey Medical Research Foundation
- **International Travel Award**, The Hospital Research Foundation
- **3 Minute Thesis Competition Faculty Finalist**, University of Adelaide

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- **Winner Best Oral Presentation**, The Queen Elizabeth Hospital Research Day

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VIII. Abbreviations

AgNPs	Silver nanoparticles
AJ	*Acinetobacter johnsonii*
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BK	Biofilm killing
CF	Cystic fibrosis
CFU	Colony forming units
CRS	Chronic rhinosinusitis
Def	Deferiprone
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
eDNA	Extracellular DNA
EPS	Extracellular polymeric substances
FDA	Food and Drug Administration (USA)
GaPP	Gallium-protoporphyrin
LDH	lactate dehydrogenase
MDR	Multidrug resistant
MIC	Minimal inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
MQ	Milly-Q (ultrapure) water
OD	Optical density
PA	*Pseudomonas aeruginosa*
PBS	Phosphate buffered saline

QS	Quorum sensing
QSI	Quorum sensing inhibitor
ROS	Reactive oxygen species
SA	*Staphylococcus aureus*
SCV	Small colony variant
SD	Standard deviation
SE	*Staphylococcus epidermidis*
SEM	Standard error of the mean
TEM	Transmission electron microscopy
UV-Vis	Ultraviolet-visible

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