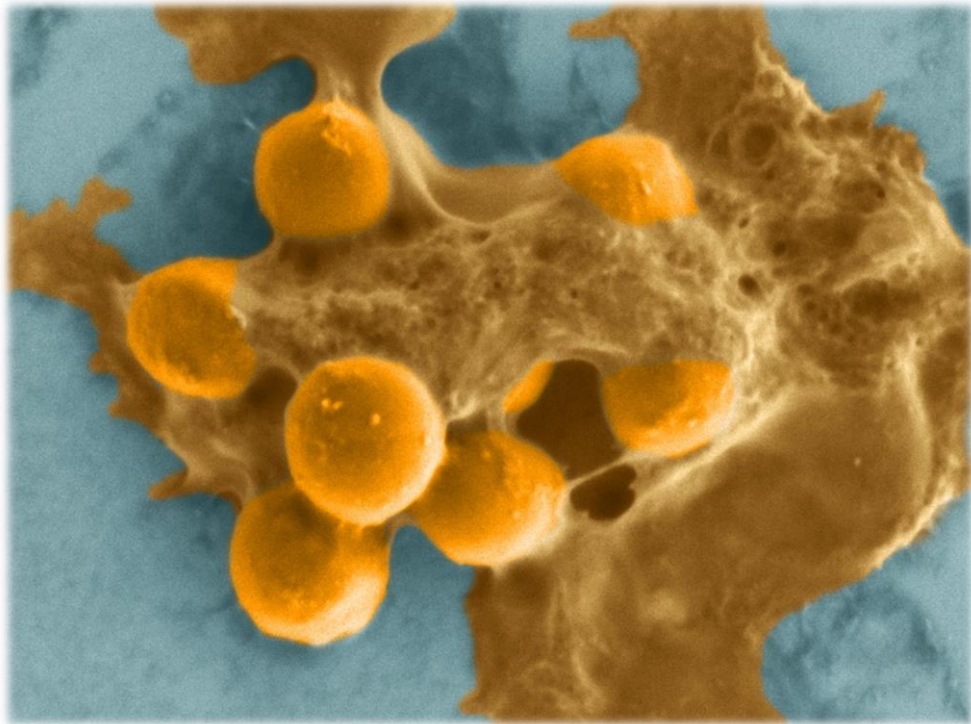


Towards Novel Antibiofilm Strategies



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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.

Table of contents

I. Abstract	i
II. Declaration	iii
III. Acknowledgements	iv
IV. Presentations arising from this thesis	vii
V. Awards and prizes arising from this thesis	ix
VI. List of tables	x
VII. List of figures	xi
VIII. Abbreviations	xviii
Chapter 1	1
Introduction	1
1.1 Biofilms- a historic view	2
1.2 Biofilm characteristics	4
1.3 Biofilm life cycle	5
1.3.1 Adhesion mechanisms exemplified by <i>S. aureus</i>	6
1.4 Biofilm matrix.....	7
1.4.1 Matrix components.....	10
1.5 Quorum sensing	14
1.6 Resistance, tolerance and persistence.....	15
1.6.1 Persister cells.....	19
1.6.2 Small colony variants	21
1.7 Biofilm infections	23
1.7.1 Chronic rhinosinusitis, a biofilm-associated condition	26

1.8	Current antimicrobial strategies	28
1.8.1	Treatment strategies for chronic rhinosinusitis.....	30
1.8.2	The antibiotic dilemma	34
1.9	Innovative antibiofilm strategies	38
1.10	The use of quorum sensing inhibitors to tackle <i>S. aureus</i> biofilms	39
1.11	Repurposing as a novel approach to find compounds active against <i>S. aureus</i> biofilms	41
1.11.1	What is drug repurposing?.....	41
1.11.2	Terfenadine	41
1.11.3	Antibacterial and antibiofilm activity of niclosamide and analogues	42
1.11.4	Antibacterial and antibiofilm activity of 5-fluorouracil and analogues	43
1.11.5	Statins.....	43
1.11.6	Repurposing candidates with potentiator activity.....	44
1.12	Other approaches	45
Chapter 2	48
Publication: “Taking the silver bullet- colloidal silver particles for the topical treatment of biofilm-related infections”	48
	Statement of authorship.....	48
2.1	Publication title page	51
2.2	Article	52
2.3	Abstract	52
2.4	Graphical abstract	53
2.5	Introduction	53
2.6	Materials and methods	55

2.6.1	Chemicals	55
2.6.2	Synthesis of colloidal silver nanoparticles	55
2.6.3	Characterisation of AgNPs.....	57
2.6.4	Bacterial strains and cell lines.....	58
2.6.5	Cytotoxicity studies	58
2.6.6	Antibiofilm activity studies.....	59
2.6.7	Statistical analysis	61
2.7	Results and discussion.....	61
2.7.1	Characterisation of AgNPs.....	61
2.7.2	Cytotoxicity studies	63
2.7.3	Antibiofilm activity studies.....	64
2.8	Conclusion	70
2.9	Acknowledgements.....	71
2.10	Funding information	71
2.11	Disclosures	71
2.12	Supplementary data.....	72
Chapter 3.....		74
The use of gallium based therapeutics against bacterial biofilms.....		74
3.1	Rationale	75
3.2	Iron metabolism	75
3.3	Iron acquisition and homoeostasis by pathogens	76
3.4	Bacterial iron metabolism as therapeutic target	79
3.5	Gallium	79

3.6	Resistance	81
3.7	Gallium therapeutics.....	81
3.7.1	Gallium salts	81
3.7.2	Gallium complexes	82
3.8	Pharmaceutical formulations.....	84
3.9	Combination therapies with gallium.....	85
3a.	Publication: “Mind “De GaPP”: <i>in vitro</i> efficacy of deferiprone and gallium-protoporphyrin against <i>Staphylococcus aureus</i> biofilms”	86
	Statement of authorship.....	86
3a.1	Publication title page	88
3a.2	Article	89
3a.3	Abstract	89
3a.4	Introduction	90
3a.5	Materials and methods	91
3a.5.1	Antibiofilm efficacy studies	91
3a.5.2	Minimal inhibitory concentration	93
3a.5.3	Cytotoxicity studies	94
3a.5.4	Statistics and software	94
3a.6	Results.....	95
3a.6.1	Antibiofilm efficacy studies.....	95
3a.6.2	Minimal inhibitory concentration	99
3a.6.3	Cytotoxicity studies	99
3a.7	Discussion.....	100

3a.8	Conclusion.....	103
3a.9	Acknowledgements.....	103
3a.10	Funding information	103
3a.11	Disclosures	103
3a.12	Supplementary data.....	104
3b.	Publication: “A topical hydrogel with deferiprone and gallium-protoporphyrin targets bacterial iron metabolism and has antibiofilm activity”	105
	Statement of authorship.....	105
3b.1	Publication title page	107
3b.2	Article	108
3b.3	Abstract	108
3b.4	Introduction	109
3b.5	Materials and methods	110
3b.5.1	Bacterial strains and culture media	110
3b.5.2	Preparation of hydrogels.....	110
3b.5.3	Determination of drug release kinetics.....	111
3b.5.4	Determination of the minimal inhibitory concentration	111
3b.5.5	Activity in the agar diffusion model	111
3b.5.6	Activity in the colony biofilm model	112
3b.5.7	Biofilm visualisation	112
3b.5.8	Activity in an artificial wound model	113
3b.5.9	Statistics and software	113
3b.6	Results.....	113

3b.6.1	Drug release	113
3b.6.2	Minimal inhibitory concentration	114
3b.6.3	Effect of loaded hydrogels on bacterial biofilms	115
3b.7	Discussion.....	121
3b.8	Conclusion	125
3b.9	Acknowledgements.....	125
3b.10	Funding information	125
3b.11	Disclosures	126
3c.	Publication: “Deferiprone and gallium-protoporphyrin have the capacity to potentiate the activity of antibiotics in <i>Staphylococcus aureus</i> small colony variants”	127
	Statement of authorship.....	127
3c.1	Publication title page	129
3c.2	Article	130
3c.3	Abstract	130
3c.4	Graphical abstract	131
3c.5	Introduction	131
3c.6	Materials and methods	132
3c.6.1	Bacterial strains.....	132
3c.6.2	Characteristics of bacterial strains	133
3c.6.3	Hydrogel preparation.....	134
3c.6.4	<i>In vitro</i> activity in the colony biofilm model	134
3c.6.5	<i>In vitro</i> activity in an artificial wound model.....	135
3c.6.6	<i>In vivo</i> activity in a <i>C. elegans</i> infection model	136

3c.6.7	Statistics and software	136
3c.7	Results	137
3c.7.1	Characteristics of bacterial strains	137
3c.7.2	Colony biofilm model	139
3c.7.3	Macroscopic biofilm analysis	141
3c.7.4	Artificial wound model.....	141
3c.7.5	<i>In vivo</i> infection model in <i>C. elegans</i>	142
3c.8	Discussion.....	144
3c.9	Conclusion	148
3c.10	Acknowledgements.....	148
3c.11	Funding information	148
3c.12	Disclosures	149
Chapter 4	150
Conclusion, future perspectives and translational prospects	150
	Colloidal silver nanoparticles	152
	Def-GaPP	153
	Multi-pronged strategies	156
Appendix	158
Publication: “Innovative approaches to treat <i>Staphylococcus aureus</i> biofilm-related infections”¹	158
	Statement of authorship.....	158
	Publication title page	160
References	161

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.

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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=ZE2qOL2fl8g>)
- **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

2014

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

VI. List of tables

Table 1. Functions of extracellular polymeric substances in bacterial biofilms. Adapted from ¹⁸	13
Table 2. The World Health Organization’s global priority list of antibiotic-resistant bacteria to guide research, discovery and development of new antibiotics ¹⁴²	25
Table 3. Standard therapeutic strategies (upper part, page 32) and alternative approaches (lower part, page 33) for chronic rhinosinusitis management.....	32
Table 4. Minimal inhibitory concentrations of deferiprone (Def), gallium-protoporphyrin (GaPP), the combination of both compounds and ciprofloxacin (Cip).	114
Table 5. Characteristics of small colony variant SCV1, SCV2 and parent strain P1, including catalase, coagulase and haemolytic activity, auxotrophy type, as well as MICs (in µg/ml) of deferiprone (Def), gallium-protoporphyrin (GaPP), the combination of both compounds, ciprofloxacin (Cip), gentamicin (Gent), mupirocin (Mup), doxycycline (Doxy), chloramphenicol (Chlor), cephalixin (Ceph), vancomycin (Van), amoxicillin (Amoxi) and streptomycin (Strep).	138

VII. List of figures

Figure 1. (a) Antony van Leeuwenhoek (1632–1723) who was the first to observe and describe microbial biofilms in his own mouth. (b) A replica of his "microscope". Reprinted with permission ³	2
Figure 2. Scanning electron micrograph of <i>Staphylococcus aureus</i> biofilm.	3
Figure 3. Dr. Sean D. Taverna's artistic interpretation of the four driving forces behind bacterial biofilm formation. Reprinted with permission ¹²	4
Figure 4. The biofilm life cycle exemplified by <i>Staphylococcus aureus</i> . 1: Adhesion. 2: Growth. 3: Detachment.	6
Figure 5. The extracellular polymeric substances matrix at different dimensions. (a) A model of a bacterial biofilm attached to a solid surface. (b) The major matrix components — polysaccharides, proteins and DNA — are distributed between the cells in a non-homogeneous pattern, setting up differences between regions of the matrix. (c) The classes of weak physico-chemical interactions and the entanglement of biopolymers that dominate the stability of the EPS matrix ⁵⁷ . (d) A molecular modelling simulation of the interaction between the exopolysaccharide alginate (right) and the extracellular enzyme lipase (left) of <i>Pseudomonas aeruginosa</i> in aqueous solution. The coloured spheres represent 1,2-dioctylcarbamoyl-glycero-3-O-octylphosphonate in the lipase active site, except for the green sphere, which represents a Ca ²⁺ ion. The aggregate is stabilised by the interaction of the positively charged amino acids arginine and histidine (indicated in blue) with the polyanionic alginate. Image courtesy of H. Kuhn, CAM-D Technologies, Essen, Germany. Reprinted with permission ¹⁸	9
Figure 6. Examples of quorum sensing molecules of the (a) acylhomoserine lactone system, (b) autoinducing peptide system and (c) autoinducer-2 system.	14
Figure 7. Major types of clinically relevant resistance mechanisms: target modification (of enzymes, ribosomes or cell-wall precursors, for example, mutation in the 30S ribosomal protein RpsL confers resistance to streptomycin), inactivation or modification of the antibiotic (for example, by beta	

lactamases), restricted penetration and/or increased efflux of the drug (for example, efflux of linezolid by the AcrAB–TolC multidrug pump), bypass of pathways inhibited by antibiotics and overproduction of targets ^{82,83} . Reprinted with permission ^{77,84}	17
Figure 8. Left: Characteristic minimum inhibition concentration (MIC) for a (a) drug susceptible, (b) resistant, (c) tolerant and (d) persistent bacterial strain. Coloured wells indicate bacterial growth, white wells indicate a drug concentration dependent growth inhibition. Right: Characteristic minimum duration for killing of 99% (MDK ₉₉) and 99.99% (MDK _{99.99}) of bacteria in the population for a susceptible (green), tolerant (blue) and persistent (grey) strain. Concentrations and timescales were chosen for demonstration purposes only. Adapted from ⁷⁵	18
Figure 9. <i>S. aureus</i> small colony variants (a) and parent strain (b).....	21
Figure 10. Examples of biofilm associated infections. Reprinted with permission ¹⁴¹	24
Figure 11. Contributing factors to chronic rhinosinusitis (CRS).	26
Figure 12. Scanning electron micrograph of <i>Pseudomonas aeruginosa</i> biofilm.	27
Figure 13. Targets of antibiotics. There are approximately 200 conserved essential proteins in bacteria, but the number of currently exploited targets is very small. The most successful antibiotics hit only three targets or pathways: the ribosome (which consists of 50S and 30S subunits), cell wall synthesis and DNA gyrase or DNA topoisomerase. Reprinted with permission ^{77,84}	30
Figure 14. Chronological order of antibiotics (year of discovery), clinical approval (underlined years, left) and emergence of resistance (underlined years, right).	36
Figure 15. Chemical structure of HAM (top) and two more active derivatives. Table shows some key properties of HAM. The EC ₅₀ values shown are the concentrations needed to double the effect of vancomycin in vitro.	40
Figure 16. Transmission electron microscopy images of (a) quasi-spherical, (b) cubic, and (c) star-shaped silver nanoparticles.	62
Figure 17. Cell viability (%) of Nuli-1 (black) and THP-1 cells (grey) after 1 hour exposure to quasi-spherical (●), cubic (■) and star-shaped (*) silver nanoparticles, compared to negative (-, untreated)	

and positive (+, Triton X-100) controls. Data represent the mean \pm SD of 3 biological replicates. Statistical comparison to untreated control. * $p < 0.05$	64
Figure 18. Biofilm killing (%) of (a) <i>S. aureus</i> , (b) MRSA and (c) <i>P. aeruginosa</i> biofilms after exposure to quasi-spherical (●, orange), cubic (■, red) and star-shaped (*, grey) silver nanoparticles. Antibiotic controls included 100 $\mu\text{g/ml}$ gentamicin (Gent, dark blue) and 5 $\mu\text{g/ml}$ ciprofloxacin (Cip, light blue). Data represent the mean \pm SD of 3 biological replicates. Statistical comparison to Cip. ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$	66
Figure 19. <i>C. elegans</i> survival (%) over 3 days of uninfected worms (grey bars); worms infected (black bars) with (a) <i>S. aureus</i> , (b) MRSA, or (c) <i>P. aeruginosa</i> ; and infected worms treated with quasi-spherical silver nanoparticles (orange bars). Data represent the mean \pm SEM of at least 12 biological replicates. ** $p < 0.01$ **** $p < 0.0001$	68
Figure 20. Colony forming units (CFU) per <i>C. elegans</i> worm after 3 days of infection (black bars: 1, 3, 5) with (a) <i>S. aureus</i> , (b) MRSA, or (c) <i>P. aeruginosa</i> and treatment with quasi-spherical silver nanoparticles (orange bars: 2, 4, 6). Data represent the mean \pm SEM of at least 12 biological replicates. * $p < 0.05$	68
Figure 21. X-ray tomography of <i>C. elegans</i> (blue) after exposure to silver nanoparticles, scale bar is 19.4 μm . Colours reflect various intensities of AgNPs (green = high density of AgNPs, orange = low density of AgNPs, blue = iodine staining, absence of AgNPs).	69
Figure 22. Red blood cells contain haemoglobin which comprises of alpha and beta chains (yellow and red) surrounding 4 haem molecules (blue).....	78
Figure 23. Gallium as iron analogue and gallium-protoporphyrin as haem analogue can be taken up by bacterial iron transporters (yellow) as (a) free gallium, (b) gallium-protoporphyrin and (c) siderophore-gallium. Inside bacteria gallium inhibits vital cellular pathways, ultimately leading to the generation of reactive oxygen species (ROS) that induce cell death.	80
Figure 24. Structural similarities between haem and gallium–protoporphyrin that is able to mimic haem as iron source.....	83

Figure 25. <i>S. aureus</i> biofilm killing (%) by (a) deferiprone (Def in mM) and (b) gallium-protoporphyrin (GaPP in $\mu\text{g}/\text{ml}$) relative to untreated control. Data are the mean of 3 biological repeats \pm SD. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$	95
Figure 26. Gallium-protoporphyrin (GaPP in $\mu\text{g}/\text{ml}$) and dual treatments compared to deferiprone (Def in mM) treatment. (a) Def 20, (b) GaPP 200, (c) concurrent Def 20 + GaPP 200, (d) consecutive Def 20 + GaPP 200. Data are the mean of 3 biological repeats \pm SD. ** $p < 0.01$ *** $p < 0.001$	96
Figure 27. Visualisation of treated <i>S. aureus</i> biofilms using LIVE/DEAD BacLight staining and confocal laser scanning microscopy. (A) untreated control, (B) deferiprone (Def) 20 mM, (C) gallium-protoporphyrin (GaPP) 200 $\mu\text{g}/\text{ml}$, (D) consecutive Def 20 mM + GaPP 200 $\mu\text{g}/\text{ml}$	97
Figure 28. Consecutive treatments of deferiprone (Def in mM) and gallium-protoporphyrin (GaPP in $\mu\text{g}/\text{ml}$) compared to single GaPP treatment (horizontal bars). Data are the mean of 3 biological repeats \pm SD. # potential synergistic effects.	98
Figure 29. Comparison of consecutive treatments with different deferiprone (Def in mM) exposure: 2h Def followed by 2h gallium-protoporphyrin (GaPP in $\mu\text{g}/\text{ml}$) (black) versus 8.5h Def followed by 2h GaPP (grey). Data are the mean of 3 biological repeats \pm SD. * $p < 0.05$	99
Figure 30. Cell viability (%) of L929 (black) and Nuli-1 (grey) cells compared to untreated controls after a consecutive deferiprone (Def) and gallium-protoporphyrin (GaPP) treatment. Data are the mean of 3 biological repeats \pm SD. * $p < 0.05$	100
Figure 31. Iron metabolism in <i>S. aureus</i> and interference by deferiprone (Def) and gallium-protoporphyrin (GaPP) treatment (red). Reactive oxygen species (ROS).....	101
Figure 32. Release profiles of gels loaded with 20 mM deferiprone (Def, green circles), 500 $\mu\text{g}/\text{ml}$ gallium-protoporphyrin (GaPP, red squares) or a combination of both (Def combi: purple circles; GaPP combi: blue squares, dotted lines). Data represent the mean \pm SD of 3 replicates.....	114
Figure 33. Inhibition zone diameter (cm) of (a) Gram-positive and (b) Gram-negative bacteria after exposure to loaded hydrogels. Strains used include <i>S. aureus</i> ATCC 25923 (SA), a clinical MRSA isolate (MRSA), <i>S. epidermidis</i> ATCC 12228 (SE), <i>P. aeruginosa</i> PA01 (PA01), a clinical <i>P. aeruginosa</i>	

isolate from a cystic fibrosis patient (PA (CF)) and *A. johnsonii* ATCC 17946 (AJ). Hydrogels include control: blank gel (black), Cip: ciprofloxacin 5 µg/ml (pink), Def: deferiprone 20 mM (light green), GaPP 100: gallium-protoporphyrin 100 µg/ml (dark green), Def-GaPP 100 (blue), GaPP 500 (orange), Def-GaPP 500 (red). Data represent the mean ± SD of 3 biological replicates. Statistical comparison to ciprofloxacin-loaded gel. * p<0.05 O p<0.01 ^ p<0.001 # p<0.0001..... 115

Figure 34. Log₁₀ reduction of (a) Gram-positive and (b) Gram-negative colony biofilms after exposure to loaded hydrogels. Strains used include *S. aureus* ATCC 25923 (SA), a clinical MRSA isolate (MRSA), *S. epidermidis* ATCC 12228 (SE), *P. aeruginosa* PA01 (PA01), a clinical *P. aeruginosa* isolate from a cystic fibrosis patient (PA (CF)) and *A. johnsonii* ATCC 17946 (AJ). Hydrogels include Cip: ciprofloxacin 5 µg/ml (pink), Def: deferiprone 20 mM (light green), GaPP 100: gallium-protoporphyrin 100 µg/ml (dark green), Def-GaPP 100 (blue), Def-GaPP 100-Cip (black), GaPP 500 (orange), Def-GaPP 500 (red). Data represent the mean ± SD of 3 biological replicates. Statistical comparison to ciprofloxacin-loaded gel. * p<0.05 O p<0.01 # p<0.0001 116

Figure 35. Bacterial biofilm growth over time after initial exposure to loaded hydrogels. Strains used include *S. aureus* ATCC 25923, a clinical MRSA isolate, *S. epidermidis* ATCC 12228, *P. aeruginosa* PA01, a clinical *P. aeruginosa* isolate from a cystic fibrosis patient and *A. johnsonii* ATCC 17946. Hydrogels include blank control gel (B), ciprofloxacin 5 µg/ml (C), deferiprone 20 mM (D), gallium-protoporphyrin 500 µg/ml (G), Def-GaPP 500 (DG)..... 118

Figure 36. Cross-section of *S. aureus* colony biofilm after exposure to Def-GaPP 500 gel. Visualisation by confocal laser scanning microscopy after Live/Dead staining. The green autofluorescent filter membrane is visible under the red stained *S. aureus* biofilm and gel. 119

Figure 37. Correlative light/electron microscopy image of *S. aureus* biofilm exposed to Def-GaPP 500 gel, stained for live/dead cells. Green filter membrane (top left, green autofluorescence), red stained *S. aureus* biofilm (center) and gel (bottom, yellow) are shown..... 120

Figure 38. Effects of loaded hydrogels in an artificial wound model. Log₁₀ reduction of *S. aureus* ATCC 25923 (SA), a clinical MRSA isolate (MRSA) and *P. aeruginosa* PA01 (PA01) after exposure to loaded hydrogels with Def: deferiprone 20 mM (light green), GaPP 500: gallium-protoporphyrin 500

µg/ml (orange) and Def-GaPP 500 (red). Data represent the mean ± SD of 3 biological replicates.	121
Figure 39. <i>S. aureus</i> small colony variant SCV1 (a), SCV2 (b) and parent strain P1 (c).	137
Figure 40. Growth curves of small colony variant SCV1 (green triangles), SCV2 (red dots) and parent strain P1 (blue diamonds).	137
Figure 41. Log ₁₀ reduction of small colony variant SCV1 (a), SCV2 (b) and parent strain P1 (c) colony biofilms after exposure to drug loaded hydrogels compared to untreated control. 1: Gentamicin (Gent) 100 µg/ml (light grey), 2: Ciprofloxacin (Cip) 5 µg/ml (purple), 3: Deferiprone (Def, 20 mM)-Gallium-protoporphyrin (GaPP) 100 µg/ml (blue), 4: Def-GaPP100-Cip (black), 5: Def-GaPP500 (red), 6: GaPP 500 (orange), 7: Def-GaPP100-Gent (dark grey). Data represent the mean ± SD of 3 biological replicates. O p<0.001 # p<0.0001 ns-not statistically significant.....	139
Figure 42. Synergy of treatment combinations against small colony variant SCV1, SCV2 and parent strain P1 colony biofilms. Deferiprone 20 mM (Def)-Gallium-protoporphyrin (GaPP) 100 µg/ml (blue circles), Def-GaPP500 µg/ml (red squares), Def-GaPP100-ciprofloxacin 5 µg/ml (black triangles), Def-GaPP100-gentamicin 100 µg/ml (grey diamonds). The higher the value the higher the degree of synergy.....	140
Figure 43. Inhibitory effect of drug loaded hydrogels on biofilms after 5 days exposure. Elevated biofilm inhibition was observed for gels containing deferiprone, gallium-protoporphyrin and ciprofloxacin or gentamicin (DGCip, DGGent). Strains used: Small colony variant SCV1, SCV2 and parent strain P1. Hydrogels- B, Blank control gel; Cip, Ciprofloxacin 5µg/ml; Gent, Gentamicin 100µg/ml; DG, Deferiprone 20 mM-Gallium-protoporphyrin 100µg/ml; DGGent, Def-GaPP100-Gent; DGCip, Def-GaPP100-Cip.....	141
Figure 44. Effects of hydrogels in an artificial wound model compared to untreated control. Log ₁₀ reduction of small colony variant SCV1, SCV2 and parent strain P1 after exposure to hydrogels loaded with ciprofloxacin 5 µg/ml (purple), deferiprone 20 mM (Def, grey), gallium-protoporphyrin 500 µg/ml (GaPP, orange) and Def-GaPP500 (red). Data represent the mean ± SD of 3 biological replicates. * p<0.05 ** p<0.01 # p<0.0001.....	142

Figure 45. *C. elegans* survival (%) over 3 days in uninfected controls (light grey) and after infection (black bars) with small colony variant SCV1 (a), SCV2 (b) or parent strain P1 (c) and treatment with loaded hydrogels: deferiprone 20 mM (Def, dark grey), gallium-protoporphyrin 500 µg/ml (GaPP, orange) and Def-GaPP500 (red). Data represent the mean ± SEM of at least 6 biological replicates. ** p<0.01 # p<0.0001..... 143

Figure 46. Log₁₀ of CFU per *C. elegans* worm after 3 days infection (black bars) with small colony variant SCV1 (a), SCV2 (b) or parent strain P1 (c) and treatment with drug loaded hydrogels- Def: deferiprone 20 mM (grey), GaPP: gallium-protoporphyrin 500 µg/ml (orange) and DG: Def-GaPP500 (red). Data represent the mean ± SD of at least 6 biological replicates. * p<0.05 144

Figure 47. Number of (a) intracellular and (b) extracellular SCVs in a human bronchial epithelial cell infection assay (1: untreated control) after treatment with 2: gentamicin 100 µg/ml, 3: deferiprone 20 mM + gallium-protoporphyrin 100 µg/ml, 4: combination of 2 and 3. Data represent the mean ± SD of 3 biological replicates. * p<0.05 O p<0.001 # p<0.0001 154

Figure 48. Log₁₀ reduction of *S. aureus* colony biofilms after exposure to loaded hydrogels compared to untreated controls. 1: ciprofloxacin 5 µg/ml (Cip), 2: deferiprone 20 mM (Def), 3: gallium-protoporphyrin 100 µg/ml (GaPP), 4: Def-GaPP 100, 5: hamamelitannin 250 µg/ml (HAM), 6: Def-GaPP-HAM, 7: Def-GaPP-Cip. Data represent the mean ± SD of 3 biological replicates. Statistical comparison to Cip-loaded gel. # p<0.0001 156

VIII. Abbreviations

AgNPs	Silver nanoparticles
AJ	<i>Acinetobacter johnsonii</i>
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 <i>Staphylococcus aureus</i>
MQ	Milly-Q (ultrapure) water
OD	Optical density
PA	<i>Pseudomonas aeruginosa</i>
PBS	Phosphate buffered saline

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

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