Final Scientific Program

Scientifically sponsored by the following organizations

ESCMID | European Society of Clinical Microbiology and Infectious Diseases
ESGB | ESGB Study Group for Biofilms
ESGARS | European Society of Genitourinary and Reconstructive Surgery

To note: the views expressed by the authors do not necessarily reflect the official position of the organizations above.

Sponsors

@EBF2022
eurobiofilms2022.com
Local Organising Committee (LOC):

**President:** Antonio Oliver  
**Vice-President:** María Dolores Macià

Estrella Rojo-Molinero  
Elena Jordana-Lluch  
María Fernández-Billón  
and ESGB Executive Committee**

International Scientific Advisory Board:

Thomas Bjarnsholt (DK)  
Tom Coenye (BE)  
Oscar Murillo (ES)  
Sussanne Haussler (DE)  
Oana Ciofu (DK)  
Kendra Rumbaugh (USA)  
Frank Schreiber (DE)  
Eduard Torrents (ES)  
and ESGB Executive Committee**

*ESGB Executive Committee is composed by:

**Chairperson:** Prof. Gordon Ramge (UK)  
**Secretary:** Dr Claus Moser (DK)  
**Treasurer:** Prof. Joana Azeredo (PT)  
**Scientific Officer:** Dr Maria Dolores Macià (ES)  
**Educational Officer:** Prof. Elisa Borghi (IT)
<table>
<thead>
<tr>
<th>AUGUST 31st</th>
<th>SEPTEMBER 1st</th>
<th>SEPTEMBER 2nd</th>
<th>SEPTEMBER 3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 - 10:00</td>
<td>Key Note Lecture</td>
<td>Key Note Lecture</td>
<td>Parallel session 9</td>
</tr>
<tr>
<td>10:00 - 10:30</td>
<td>Coffee break</td>
<td>Coffee break</td>
<td>Parallel session 10</td>
</tr>
<tr>
<td>10:30 - 12:30</td>
<td>Parallel session 1</td>
<td>Parallel session 2</td>
<td>Coffee break</td>
</tr>
<tr>
<td>12:30 - 13:30</td>
<td>Lunch break</td>
<td>Lunch break</td>
<td>FINAL PLENARY SESSION</td>
</tr>
<tr>
<td>13:30 - 15:00</td>
<td>Poster viewing</td>
<td>Poster viewing</td>
<td>CLOSING CEREMONY</td>
</tr>
<tr>
<td>15:00 - 17:00</td>
<td>Parallel session 3</td>
<td>Parallel session 4</td>
<td>Best communication awards</td>
</tr>
<tr>
<td>17:15 - 17:45</td>
<td>Coffee break</td>
<td>Coffee break</td>
<td>Last remarks and farewell</td>
</tr>
<tr>
<td>18:00 - 18:30</td>
<td>Opening and welcome addresses</td>
<td>Poster viewing</td>
<td>14:00</td>
</tr>
<tr>
<td>18:30 - 19:30</td>
<td>Opening lecture</td>
<td>Poster viewing</td>
<td>Picnic to carry on</td>
</tr>
<tr>
<td>19:30</td>
<td>Opening reception (Wines &amp; tapas)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td>Congress dinner</td>
<td></td>
</tr>
</tbody>
</table>
**WORKSHOPS***

Antibiotic treatment strategies, PK/PD parameters and resistance development in biofilm models  
Estrella Rojo-Molinero (ES) and María Fernández Billón (ES)

In Vivo Biofilm models - establishment, endpoints and treatment testings  
Claus Moser (DK) and Christian Lerche (DK)

9:00 - 13:00

Check in and registration

15:00

Opening and welcome addresses (ESGB, LOC presidents and local authorities)

MM1 Room

**OPENING LECTURE**

José María Miró (ES)

Clinical management of biofilm-driven infective endocarditis.

18:30 – 19:30

Opening reception (Wines & tapas)

*Workshops will take place on the Medicine classrooms and IdisBa laboratories from Son Espases Hospital (-1 H F). Ctra. Valldemossa, 79. 07120 Palma de Mallorca
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>KEY NOTE LECTURE</td>
<td>Sussanne Haussler (DE)</td>
<td>Bacterial adaptation to biofilm growth.</td>
</tr>
<tr>
<td>10:00</td>
<td>Coffee break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>Parallel session 1</td>
<td></td>
<td>Clinical Management of chronic osteoarticular and wound infections.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chaired by Oscar Murillo and Kendra Rumbaugh</td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>Oscar Murillo (ES)</td>
<td></td>
<td>Therapeutic strategies based on anti-biofilm efficacy of antibiotics: improving the prognosis of difficult-to-treat osteoarticular infections.</td>
</tr>
<tr>
<td>11:10</td>
<td>Trine Rolighed Thomsen (DK)</td>
<td></td>
<td>It is not the hole in the patient- but the whole patient. Biofilm is a key factor in wounds.</td>
</tr>
<tr>
<td>11:25</td>
<td>Christopher Doherty (UK)</td>
<td></td>
<td>An in vivo assessment of the antibiofilm effects of wound dressings</td>
</tr>
<tr>
<td>11:40</td>
<td>Franziska A. Schwartz (DK)</td>
<td></td>
<td>Wound-healing promoting 3C patches display clinically relevant broad-range antibacterial activity.</td>
</tr>
<tr>
<td>11:55</td>
<td>Erin Magee (IE)</td>
<td></td>
<td>Non-invasive, 3D printed sensors for pathogen detection and therapeutic monitoring of wound biofilms.</td>
</tr>
<tr>
<td>12:10</td>
<td>Claus Moser (DK)</td>
<td></td>
<td>Hyperbaric oxygen therapy augments ciprofloxacin effect against <em>Pseudomonas aeruginosa</em> biofilm infected chronic wounds in a mouse model.</td>
</tr>
</tbody>
</table>
# Parallel session 2

**Mechanisms of antibiotic resistance in biofilms.**

Chaired by Frank Schreiber and Mariló Macià

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30</td>
<td>Frank Schreiber (DE)</td>
<td>Resistance evolution towards biocides and antimicrobial surfaces.</td>
</tr>
<tr>
<td>11:10</td>
<td>Kasper Nørskov Kragh (DK)</td>
<td>TnSeq-guided identification of genes that play a role in antibiotic tolerance of <em>Pseudomonas aeruginosa</em> biofilms.</td>
</tr>
<tr>
<td>11:25</td>
<td>Fauve Vergauwe (BE)</td>
<td>Experimental evolution of <em>Pseudomonas aeruginosa</em> biofilms as a tool to identify adaptive mechanisms leading to reduced antibiotic susceptibility.</td>
</tr>
<tr>
<td>11:40</td>
<td>María Antonia Gomis Font (ES)</td>
<td>Resistance genomics of a new siderophore cephalosporin against <em>Pseudomonas aeruginosa</em> chronic respiratory isolates.</td>
</tr>
<tr>
<td>11:55</td>
<td>Jenny Littler (UK)</td>
<td>The effect of growth environment on the antibiotic resistance of <em>P. aeruginosa</em>.</td>
</tr>
<tr>
<td>12:10</td>
<td>Dean Walsh (UK)</td>
<td>Biocides trigger multiple regulatory systems in <em>Staphylococcus aureus</em> to induce antibiotic tolerance.</td>
</tr>
</tbody>
</table>

**Lunch break**

12:30 –13:30

**Poster viewing**

13:30 –15:00
**Parallel session 3**

**Heterogeneity and metabolic adaptations at the special biofilm microenvironment.**

Chaired by Thomas Bjarnsholt and Susanne Häußler

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00</td>
<td>Thomas Bjarnsholt (DK)</td>
<td>The infectious microenvironment of chronic infections, implications for treatment</td>
</tr>
<tr>
<td>16:10</td>
<td>Tim Tolker-Nielsen (DK)</td>
<td>The small molecule Disperazol interferes with c-di-GMP signaling and induces dispersal of <em>Pseudomonas aeruginosa</em> biofilms</td>
</tr>
<tr>
<td>16:40</td>
<td>Begoña Heras (AU)</td>
<td>Breakthroughs into understanding how bacteria form biofilms</td>
</tr>
<tr>
<td>16:55</td>
<td>Beatrice Bottura (UK)</td>
<td>Intra-colony channel morphology in biofilms is governed by nutrient availability and substrate stiffness</td>
</tr>
</tbody>
</table>
## Alternative therapeutic strategies to fight biofilms.

Chaired by Joana Azeredo and Elisa Borghi

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00</td>
<td><strong>Joana Azeredo (PT)</strong></td>
<td>Strategies to improve phage efficacy against infectious biofilms</td>
</tr>
<tr>
<td>15:00</td>
<td>Adline Princy Solomon (IN)</td>
<td>Design synthesis and therapeutic validation of a Quorum Quencher, UTIQ to combat gestational UTI</td>
</tr>
<tr>
<td>15:55</td>
<td>Olivier Lesouhaitier (FR)</td>
<td>The human Atrial Natriuretic Peptide as a powerful weapon against Pseudomonas aeruginosa biofilm</td>
</tr>
<tr>
<td>16:10</td>
<td>Helena Bujdáková (SK)</td>
<td>Photodynamic inactivation – an efficient approach for eradication of microbial biofilms</td>
</tr>
<tr>
<td>16:25</td>
<td>Sixuan Zhang (CH)</td>
<td>Probiotics for treatment of skin wound infection: fighting biofilms and promoting tissue regeneration</td>
</tr>
<tr>
<td>16:40</td>
<td>Fabien Lamret (FR)</td>
<td>The promising antibiofilm effects of decellularized Wharton’s Jelly</td>
</tr>
<tr>
<td>16:55</td>
<td>Makrina Totsika (AU)</td>
<td>Signal to kill: Nitroxide-induced biofilm dispersal for enhanced antibiotic eradication</td>
</tr>
</tbody>
</table>

### 17:15 – 17:45

Coffee break

### 17:45 – 18:45

Poster viewing
### Keynote Lecture

**Kendra Rumbaugh (USA)**  
**The pathogenicity of biofilms: the paradigm of chronic wounds.**

### Parallel Session 5

**Novel technologies to study biofilms.**  
Chaired by Eduard Torrents and Elena Jordana-Lluch

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker/Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30</td>
<td><strong>Eduard Torrents (ES)</strong> Application of nanotechnology in diagnosis and treatment of biofilm infections.</td>
</tr>
<tr>
<td>11:10</td>
<td><strong>Lukas Kriem (DE)</strong> The Use of Raman Technologies for Biofilm Mapping.</td>
</tr>
<tr>
<td>11:25</td>
<td><strong>Marius Colin (FR)</strong> Development of in vitro biofilm model representative of prosthesis infection.</td>
</tr>
</tbody>
</table>
Parallel session 6

Management of endovascular and catheter-related infections.

Chaired by David Lebeaux and Claus Moser

10:30 - 12:30

10:30 David Lebeaux (FR)

Biofilm and catheter-related infections: can we improve prevention or treatment?

11:10 Luisa Jordao (PT)

Catheter related bloodstream infection caused by E. cloacae and Candida parapsilosis: Are biofilms guilty?

11:25 Martijn Riool (NE)

Novel Synthetic Antimicrobial and Anti-biofilm Peptides (SAAPs)-containing coatings to prevent biomaterial-associated infection.

11:40 Jontana Allkja (PT)

Interactions of microorganisms within a urinary catheter four species biofilm model.

11:55 J. Scott VanEpps (US)

Graphene quantum dots for in situ treatment of vascular catheter biofilms.

12:10 Jiapeng Hou (DK)

Enhanced antibiotic and probiotic tolerance of an in vitro multi-species uropathogen biofilm model, useful for studies of catheter associated urinary tract infections.

Lunch break

12:30 - 13:30

Poster viewing

13:30 - 15:00
Parallel session 7

Optimizing antimicrobial efficacy to treat biofilm-related infection.

Chaired by Oana Ciofu and Antonio Oliver

15:00 - 17:00

15:00 Oana Ciofu (DK) | Strategies to potentiate antibiotics and avoid resistance

Therapeutic strategies based on antagonistic resistance mechanisms between novel B-lactam and carbapenem antibiotics to combat XDR Pseudomonas aeruginosa biofilms.

15:40 María Fernández-Billón (ES)

15:55 Eva Benavent Palomares (ES) | Comparative efficacy of meropenem in extended infusion and intermittent bolus alone and with colistin against Pseudomonas aeruginosa biofilm: a pharmacodynamic study.

16:10 Valentina Lember (DE) | Understanding the killing dynamics of levofloxacin treated Pseudomonas aeruginosa biofilms.

16:25 Cornelia Landersdorfer (AU) | Ceftolozane/tazobactam plus tobramycin against planktonic and biofilm states of hypermutable Pseudomonas aeruginosa isolates from paediatric patients with cystic fibrosis.

16:40 Ramon Garcia Maset (UK) | Synergistic effects of synthetic nano-engineered antimicrobial-peptide polymers and antibiotics against S. aureus and P. aeruginosa biofilms in in vitro and ex vivo models.
**Parallel session 8**

**Fungal biofilms in 2022 – how far we come?**
Chaired by Gordon Ramage and José Luis López-Ribot

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00</td>
<td>Gordon Ramage (UK)</td>
<td>From the ABC’s to the XTT’s of fungal biofilm research: two decades of progress</td>
</tr>
<tr>
<td>16:10</td>
<td>Emerenziana Ottaviano (IT)</td>
<td>Pilocarpine interferes with sphingolipids metabolism in <em>Candida albicans</em>.</td>
</tr>
<tr>
<td>16:25</td>
<td>Christopher Delaney (UK)</td>
<td>Understanding biofilm heterogeneity and adaptation in <em>Candida</em>.</td>
</tr>
<tr>
<td>16:40</td>
<td>Isabel Valsecchi (FR)</td>
<td>Mixed biofilm of <em>Aspergillus fumigatus</em> and <em>Stenotrophomonas maltophilia</em>: microscopic visualization of galactosaminogalactan and galactomannan polysaccharides in the extracellular matrix.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:00</td>
<td>Coffee break</td>
</tr>
<tr>
<td>17:30</td>
<td>Poster viewing</td>
</tr>
<tr>
<td>20:00</td>
<td>Congress dinner</td>
</tr>
</tbody>
</table>
## Cystic fibrosis and chronic respiratory infections

Chaired by Jaime Esteban and Antonio Oliver

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>Jaime Esteban (ES)</td>
<td>Mycobacterium biofilms and chronic infections</td>
<td>Impact of the sex steroid hormone estradiol on biofilm formation and phenotype of <em>Pseudomonas aeruginosa</em> isolates from cystic fibrosis patients.</td>
</tr>
<tr>
<td>9:40</td>
<td>Mareike Müller (DE)</td>
<td></td>
<td>Bacterial biofilms predominate in both acute and chronic human lung infections.</td>
</tr>
<tr>
<td>9:55</td>
<td>Mette Kolpen (DK)</td>
<td></td>
<td>Use of phages adapted to <em>Pseudomonas aeruginosa</em> biofilms to tackle respiratory tract infections in Cystic Fibrosis (CF).</td>
</tr>
<tr>
<td>10:10</td>
<td>Luciana Meneses (PT)</td>
<td></td>
<td>Deciphering genetic requirements for <em>Streptococcus pneumoniae</em> biofilm formation and maintenance.</td>
</tr>
<tr>
<td>10:25</td>
<td>Suyen Espinoza Miranda (US)</td>
<td></td>
<td>Modulation of <em>Pseudomonas aeruginosa</em> biofilm by corticosteroids in Chronic Obstructive Pulmonary Disease context.</td>
</tr>
<tr>
<td>10:40</td>
<td>Elena Jordana-Lluch (ES)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Cooperation, competition or quite the opposite: The complex interactions in biofilm communities

Chaired by Niamh Harrington and Estrella Rojo-Molinero

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00</td>
<td>Niamh Harrington (UK)</td>
<td>Polymicrobial interactions in cystic fibrosis biofilms.</td>
</tr>
<tr>
<td>9:40</td>
<td>Jean-Paul Motta (FR)</td>
<td>Epithelial Thrombin Modifies Gut Microbiota Biofilms And Fuels Dysbiosis.</td>
</tr>
<tr>
<td>10:25</td>
<td>Karishma S Kaushik (IN)</td>
<td>Multi-level structure and organization of mixed-species <em>Pseudomonas aeruginosa</em> and <em>Staphylococcus aureus</em> biofilms in a 4-D wound microenvironment.</td>
</tr>
<tr>
<td>10:40</td>
<td>Janette Harro (US)</td>
<td>Methicillin-Resistant Staphylococcus aureus is Protective against Infection with Cystic Fibrosis-Adapted <em>Pseudomonas aeruginosa</em> in the CF-like Airway of Scnn1b Transgenic Mice.</td>
</tr>
</tbody>
</table>

### Coffee break
FINAL PLENARY SESSION

Time to update the ESCMID guidelines for the diagnosis and treatment of biofilm infections?

Niels Høiby (DK)

The future of research in biofilms: Towards which horizon should we walk?

Tom Coenye (BE)

CLOSING CEREMONY

Best communication awards
Last remarks and farewell

Picnic to carry on
ABSTRACT BOOK

EBF
EuroBioFilms2022

Palma de Mallorca, 31st August- 3rd September
### Contents

**KEYNOTE LECTURES**

<table>
<thead>
<tr>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening Lecture</td>
</tr>
<tr>
<td>Parallel session 1: Clinical Management of chronic osteoarticular and wound infections</td>
</tr>
<tr>
<td>Parallel session 2: Mechanisms of antibiotic resistance in biofilms</td>
</tr>
<tr>
<td>Parallel session 3: Heterogeneity and metabolic adaptations at the special biofilm microenvironment</td>
</tr>
<tr>
<td>Parallel session 4: Alternative therapeutic strategies to fight biofilms</td>
</tr>
<tr>
<td>Parallel session 5: Novel technologies to study biofilms</td>
</tr>
<tr>
<td>Parallel session 6: Management of endovascular and catheter-related infections</td>
</tr>
<tr>
<td>Parallel session 7: Optimizing antimicrobial efficacy to treat biofilm-related infection</td>
</tr>
<tr>
<td>Parallel session 8: Fungal biofilms in 2022 – how far we come?</td>
</tr>
<tr>
<td>Parallel session 9: Cystic fibrosis and chronic respiratory infections</td>
</tr>
<tr>
<td>Parallel session 10: Cooperation, competition or quite the opposite: The complex interactions in biofilm communities</td>
</tr>
<tr>
<td>Poster session September 1st</td>
</tr>
<tr>
<td>Poster session September 2nd</td>
</tr>
</tbody>
</table>
Clinical management of biofilm-driven infective endocarditis.

Dr. Jose M. Miro

Infectious Service. Hospital Clinic-IDIBAPS. University of Barcelona. Barcelona, Spain. CIBERINFEC. Instituto de Salud Carlos III, Madrid, Spain.

Infective endocarditis is a constantly changing disease with an overall mortality of around 20% in most series. Clinical manifestations have evolved in response to significant epidemiological shifts in industrialized nations, with a move toward a nosocomial or health-care-related pattern, in older patients, with more episodes associated with prostheses and/or intravascular electronic devices and a predominance of staphylococcal and enterococcal etiology. Diagnosis is often challenging and is based on the conjunction of clinical, microbiological, and imaging information, with notable progress in recent years in the accuracy of echocardiographic data, coupled with the recent emergence of other useful imaging techniques such as cardiac computed tomography (CT) and nuclear medicine tools, particularly 18F-fluorodeoxyglucose positron emission/CT. The choice of an appropriate treatment for each specific case is complex, both in terms of the selection of the appropriate agent and doses and durations of therapy as well as the possibility of using combined bactericidal antibiotic regimens in the initial phase and finalizing treatment at home in patients with good evolution with outpatient oral or parenteral antimicrobial therapies programs. A relevant proportion of patients will also require valve surgery during the active phase of treatment, the timing of which is extremely difficult to define. Relapses can occur and are related to biofilm formation in the heart valve vegetations. Bacterial biofilms are highly resistant to the action of antibiotics and resting bacteria can contribute to these relapses. There is a need to improve of knowledge on biofilm formation, prevention and treatment in infective endocarditis. Rifampin-based antimicrobial combinations are currently the treatment of choice. Lysins represent a novel class of anti-infectives derived from bacteriophage that in vitro and in vivo can eliminate bacteria from biofilms. The management of infective endocarditis is very complex and requires a close collaboration of multidisciplinary endocarditis teams.
Bacterial adaptation to biofilm growth

Susanne Häussler

Janne G. Thöming\textsuperscript{1,2}, Monique Donnert\textsuperscript{2,3}, Matthias Preuße\textsuperscript{2,3}, Alejandro Arce-Rodriguez\textsuperscript{2,3}, Jürgen Tomasz\textsuperscript{2,3} and Susanne Häussler\textsuperscript{1,2,3,4}

\textsuperscript{1}Department of Clinical Microbiology, Copenhagen University Hospital – Rigshospitalet, 2100 Copenhagen, Denmark
\textsuperscript{2}Institute for Molecular Bacteriology, TWINCORE, Centre for Experimental and Clinical Infection Research, 30265 Hannover, Germany.
\textsuperscript{3}Department of Molecular Bacteriology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany.
\textsuperscript{4}Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, 30265 Hannover, Germany

Despite improvements in technology and healthcare services, morbidity and mortality due to chronic infections have remained unchanged over the past few decades. The emergence of a chronic infection disease burden calls for the development of modern diagnostics for biofilm resistance profiling and radically new therapeutic strategies to eradicate biofilm-associated infections. We have established a large genomic sequence variation and gene expression dataset of a plethora of clinical \textit{P. aeruginosa} isolates within the last years. Those genomic variations and transcriptional patterns are currently correlated with the expression of distinct biofilm tolerance phenotypes with the aim to develop new molecular diagnostics and innovative treatment strategies that target biofilm resistance mechanisms. Our data-driven science promises not only to provide a prediction of biofilm resistance based on the bacteria’s genotype, but also targeting the bacterial biofilm tolerance mechanisms holds promise to enhance the efficacy of current antibiotics for the management of chronic infections.
September 2\textsuperscript{nd}

**The pathogenicity of biofilms: the paradigm of chronic wounds**

Kendra Rumbaugh

PhD. Professor at Department of Surgery. Texas Tech University Health Sciences Center, Texas, (USA)

Since the ancient Egyptians, humans have tried different strategies to care for wounds. Some of these strategies have been successful and remain in practice today, others have been utter failures. However, the massive problem of chronic wounds is a modern-day dilemma that requires new and innovative solutions. Understanding the strategies microbes use to persist in wounds and cause chronic infection is paramount for developing effective treatments. This talk will cover what we know about biofilms in chronic wounds, the major gaps in knowledge and how we can refine experimental models to fill these research gaps and develop better treatments for chronic wound infections.
Time to update the ESCMID guidelines for the diagnosis and treatment of biofilm infections?

Niels Høiby

Professor, MD, DMSc, senior registrar, Dpt. Clin. Microbiology, Rigshospitalet & Costerton Biofilm Center, University of Copenhagen, Denmark

The ESCMID Biofilm Study Group was founded during the ECCMID Congress in Copenhagen 2005. The Guidelines was funded by a grant from ESCMID and developed during a meeting at the University of Copenhagen in 2012 with representatives from several countries (Høiby, Bjarnsholt, Moser, Bassi, Coenye, Donelli, Hall-Stoodley, Holá, Imbert, Kirketerp-Møller, Lebeaux, Ullmann, Williams & Zimmerli. The 14 representatives used a modified Delphi method as working method. The guidelines were accepted by ESCMID October 2014 and published online May 2015. Since then it has been cited 604 times in other publications without any signs of decline:

However, new knowledge about diagnosis and treatment possibilities of biofilm infections has of course been published since 2014. The most important new knowledge and also clinical relevant areas where new results are still lacking will be the topic of the presentation and likewise the possibility of including the most clinical relevant new results in either revised new guidelines or as supplementary information to the 2014 guidelines.
The future of research in biofilms: towards which horizon should we walk?

Tom Coenye

Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

Contact: Tom.Coenye@UGent.be

In this talk I will present a broad overview of the state of the biofilm research field, from a fundamental and biomedical perspective. I will highlight some initiatives that were taken in recent years and will subsequently take a look at the future, focusing on two important questions:

(i) How far along are we in our fundamental understanding of (lack of) antimicrobial susceptibility in microbial biofilms?

(ii) How can we help clinical microbiology labs to deal with biofilms?

To answer the first question, I will rely heavily on results obtained during experimental evolution studies. For the second question I will start by highlighting the work that has been done correlating biofilms susceptibility with clinical outcome. I will try to explain why this has often failed and will propose alternative approaches.
Parallel session 1: Clinical Management of chronic osteoarticular and wound infections

Invited speaker:

**Therapeutic strategies based on antibiofilm efficacy of antibiotics: improving the prognosis of difficult-to-treat osteoarticular infections**

Óscar Murillo

Dr. Med. Department of Infectious Diseases, Hospital Universitari de Bellvitge, IDIBELL. Barcelona, (ES)

Osteoarticular infections are a paradigm of difficult-to-treat biofilm-related infections. They are very common, and their prevalence has increased mainly due to the progressive aging of the population and the wide use of novel and sophisticated technologies and medical devices. In order to achieve their eradication, a therapeutic strategy combining surgery and selected antibiotics is mostly needed. However, the knowledge about the most effective antibiotics to be used in this setting as well as the best way to use them needs to be improved. The pharmacodynamic profiles of antimicrobials provide information about their activity; unfortunately, they were defined by using planktonic bacteria and have shown not to be useful against biofilm-related infections. Moreover, a significant rise in the proportion of osteoarticular infections caused by multidrug-resistant microorganisms has been recently noted and this poses more limitations to the therapeutic choices. Overall, physicians deal with the urgent need to treat these patients properly and demand useful information to be applied in their clinical practice. In last years, results from experimental works focused on the evaluation of the anti-biofilm efficacy of antibiotics have been translated into clinical experiences, this leading to improve the prognosis of these difficult-to-treat infections. In this session, which includes a translational perspective, we will talk about several pathogen-specific antibiotic therapies based on their anti-biofilm efficacy that are used against most difficult-to-treat osteoarticular infections.
S01-It is not the hole in the patient- but the whole patient. Biofilm is a key factor in wounds.

Trine Rolighed Thomsen1, 2, Yijuan Xu1, 2, Camilla Jessen7, Kim Tanja Hejselbak Nørgaard1, Jan Lorenzen1, Ida Clement Thaarup3, Thomas Bjarnsholt3, 4, Klaus Kirketerp-Møller5

1: Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark.
2: The Danish Technological Institute, Aarhus C, Denmark.
3: Costerton Biofilm Center, Institute of Immunology and Microbiology, University of Copenhagen, Denmark.
4: Department of Clinical Microbiology, Copenhagen University Hospital, Copenhagen, Denmark
5: Copenhagen Wound Healing Center, Bispebjerg University Hospital, Copenhagen, Denmark.

Introduction: Chronic wounds are a burden to society, the health care system and to the individuals affected. Microbes are known to exist as biofilms in the wounds contributing to the development of ‘chronicity’. They are generally difficult to eradicate and prevent despite treatments. Many wound care products and strategies are available today, however, only very few wound care products have been evaluated for their antibiofilm effect. Additionally, it must be time to combine local treatments with more holistic approaches looking at the whole patient.

Hypothesis: The aim of this study was to explore whether new in vitro- and ex vivo models can be used to evaluate amicrobial products and can they ultimately be combined with digital tools for citizens having diabetic wounds.

Methodology: Two different methodologies were utilized. Novel in vitro- and ex vivo models employing Staphylococcus aureus, Pseudomonas aeruginosa and Candida were applied to test antimicrobial products. A WoundApp developed, co-designed and tested by citizens in collaboration with healthcare professionals, and researchers in the project HealthD360 was applied as well. The citizen registers a wound, take photos, and report the wound size, pain, inflammation and wound fluid daily, which can be related to the biofilm status. Numbers of steps and other 24/7 data are collected from Apple Health and Google Fit.

Results: Stable co-existence of P. aeruginosa and S. aureus and Candida was confirmed in the models and test results will be presented. Inflammation and pain level registered on a daily/weekly basis was positively correlated with wound size of patients using the WoundApp and negatively correlated with activity level. Selected citizens using the WoundApp will be asked which antimicrobial bandages they use.

Conclusion: The in vitro- and ex vivo models combined with a citizen app has a large potential for evaluating antibiofilm products and other interventions in the future.
S02-An in vivo assessment of the antibiofilm effects of wound dressings

Doherty C., Thomason H.A. and McBain A.J. Christopher Doherty, MRC and 3M funded Doctoral Training Partnership Student, School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Manchester, United Kingdom

Professor Andrew McBain, School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Manchester, United Kingdom

Dr Helen Thomason, 3M Health Care, Medical Solutions Division, Knutsford, Cheshire, United Kingdom

Biofilms play a key role in the failure of chronic wound healing. Whilst dressings containing antimicrobial silver are commonly used to treat wound infections, their effectiveness against biofilms can vary. Here we compared the efficacy of dressings containing higher oxidative states of silver (Ag2+ and Ag3+), to dressings containing singly ionic silver (Ag1+) against biofilm infected wounds. Additionally, the ability of silver dressings to prevent the formation of biofilms within wounds was also assessed.

*S. aureus* or *P. aeruginosa* were applied to murine wounds as established biofilms or 1 x 102 planktonic bacteria. Wounds were immediately treated for 72 hours with silver or non-antimicrobial control carboxymethylcellulose absorbent dressings. Following treatment, wound biopsies were harvested and wound area and re-epithelialisation quantified using hematoxylin and eosin stained transverse sections. Viable bacteria were quantified by viable counting and biofilms were visualized by scanning electron microscopy (SEM). Extracellular polymeric substances were visualized by staining intracellular and extracellular DNA with Syto 60 and Toto 1 using confocal laser scanning microscopy (CLSM).

Dressings containing higher oxidative states of silver reduced wound area and increased re-epithelialisation in biofilm infected murine wounds compared to the non-antimicrobial control and other assessed silver dressings. SEM showed visibly less bacteria and EPS within Ag2+ and Ag3+ treated biofilm infected wounds. In addition, a reduction in extracellular DNA was observed in all wounds treated with silver dressings when observed using CLSM. Ag2+ and Ag3+ containing dressings also demonstrated the ability to prevent biofilm infection when compared to non-antimicrobial absorbent dressings. Dressings containing higher oxidative states of silver promoted healing of biofilm infected wounds, effectively reducing bioburden and the extracellular DNA associated with the biofilm matrix.
**S03-Wound-healing promoting 3C patches display clinically relevant broad-range antibacterial activity**

Franziska A. Schwartz (1), Magnus N. Bock (1), Luna Nielsen (1), Jessica Struve Andersen (1), Lars Christophersen (1), Rasmus Lundquist (2), Niels Høiby (1, 3), Claus Moser (1, 3)

(1) Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark  
(2) Reapplix, Birkerød, Denmark  
(3) Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, Denmark

**Introduction**: Infection of chronic wounds is a major problem. Bacterial biofilms are present in the majority of chronic ulcers. Autologous 3C patches® produced from whole blood consist of fibrin, platelets and leukocytes, promote the healing of chronic wounds and have antimicrobial activity against *Pseudomonas aeruginosa*. Prior to 3C Patch® application, wounds are debrided and disinfected, however, bacteria might remain in the wounds.

**Objective**: To further clarify the potential antimicrobial properties of the 3C patches®.

**Methodology**: 3C patches® (Reapplix, Birkerød, DK) were produced from blood of healthy volunteers and placed in a suspension of *Staphylococcus aureus* and/or *P. aeruginosa* (5·10^7 CFU/mL (0.5 mL)). After 0, 30, 60 or 90 minutes, patches were removed, washed, homogenized and plated for CFU count. Visualization was achieved by plating 1·10^5 CFU of *S. aureus*, *P. aeruginosa* or *Enterococcus faecalis* on 5% blood agar plates and placing 3C patches on top. Patches were removed after up to 90 minutes and imprinted on fresh agar plates for 10 seconds for CFU determination.

**Results**: The 3C patches reduced *P. aeruginosa* from 1.2·10^7 CFU/well to 3.6·10^6 after 30 (p=0.085), 5.4·10^5 after 60 (p=0.003) and 7.5·10^5 after 90 minutes (p=0.004). *S. aureus* numbers were reduced from 1.6·10^7 CFU/well to 1.4·10^7 (30 min, p=0.6), 1.1·10^7 (60 min, p=0.01) and 1.5·10^7 (90 min, p=0.98). When the patches were co-inoculated with both bacteria, *P. aeruginosa* was reduced from 7.1·10^6 CFU/well to 4.3·10^6 (30 min, p=0.35), 1.7·10^6 (60 min, p=0.99) and 1.2·10^6 (90 min, p=0.06), while *S. aureus* CFU remained relatively stable from 7.5·10^6 CFU/well at the start to 6.7·10^6 at 90 min (p=0.97). On the agar plates, CFU reduction was observed for all three tested bacterial strains.

**Conclusion**: The 3C patch® may be beneficial in controlling residual wound bacteria after standard debridement procedures.
**S04-Non-invasive, 3D printed sensors for pathogen detection and therapeutic monitoring of wound biofilms**

Erin Magee (a), Dilidaer Yusufu (b), Andrew Mills (b), Brendan Gilmore (a)

(a) School of Pharmacy, Queen's University Belfast, UK
(b) School of Chemistry, Queen's University Belfast, UK

The treatment and management of chronic wounds presents a significant challenge to modern healthcare. Evidence now supports microbial biofilms as a key deterrent to healing, with their presence occurring in up to 80% of non-healing wounds.

For a dressed wound, a small headspace exists between the skin and dressing film that differs in composition from ambient air and reflects the condition of the wound regarding bioburden. If a wound becomes colonised and infected, it stands to reason that the headspace composition will change due to bacterial metabolism, and hence, an opportunity exists to monitor this change as a marker of infection and biofilm development. A formulation containing LDPE, xylenol blue dye, and tetrabutylammonium hydroxide was 3D printed to form a CO2-sensitive colourimetric indicator film. Porcine skin explants were inoculated with *P. aeruginosa* and sealed in a wound dressing model for headspace monitoring. A photograph was taken every hour to monitor sensor colour change to developing infection. The ability of the sensor for monitoring treatment efficacy was also assessed by treating 24 h biofilms with a range of antibiotic concentrations and packaging in the wound dressing model for sensor monitoring.

The film changed colour from blue to yellow in response to wound infection. The time taken to reach a halfway colour change was found to be directly proportional to the time taken for the microbial load to exceed 10^6 CFU/mL, regardless of initial inoculum. In addition, this colour change occurred before a measurable increase in biofilm biomass. Sensors monitoring biofilms exposed to suboptimal antibiotic concentrations changed colour, whilst successfully treated biofilms elicited no colour change.

This sensor has the potential to aid wound care by allowing successfully treated wounds to heal undisturbed, prompting further intervention for failing treatment, and reducing costs and time dedicated to dressing changes.
S05-Hyperbaric oxygen therapy augments ciprofloxacin effect against *Pseudomonas aeruginosa* biofilm infected chronic wounds in a mouse model

Anne Sofie Laulund*, Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet, Henrik Harpstrøns Vej 4A, 2100 Copenhagen, Denmark, phone +4593999557.
Lars Christophersen, Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet.
Franziska Angelika Schwartz, Department of Clinical Microbiology, Copenhagen University; Rigshospitalet.
Niels Høiby, Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet and Department of Immunology and Microbiology (ISIM), University of Copenhagen.
*Claus Moser*, Department of Clinical Microbiology, Copenhagen University Hospital, Rigshospitalet.

**Introduction:** Chronic wounds have a microcirculatory dysfunction which leads to obstructed gas exchange in addition to a hindered delivery of nutrients, antimicrobial compounds and an accumulation of waste products. The presence of biofilm compromises the perfusion of antibiotics to the wound bed and the antibiotic effect is reduced due to the lowered oxygen levels.

**Hypothesis:** We speculate whether this pathologic process can be countered by hyperbaric oxygen therapy (HBOT).

**Methodology:** Third degree burns were inflicted on 28 BALB/c mice. *P. aeruginosa* embedded in seaweed alginate was injected under an eschar created by hot air to mimic a biofilm infected wound. Mice (n=10) were randomized to receive ciprofloxacin (500µL, 2 mg/mL, MIC 0.75-1.00 µg/mL) combined with 2x90min sessions daily of HBOT in general anesthesia with gradual acclimatization/de-acclimatization to the chamber, ciprofloxacin as monotherapy (n=11) or placebo (n=7) for 5 consecutive days. Wound sizes were estimated by planimetry at day 6 followed by euthanasia. Wounds were surgically removed in toto, homogenized and plated for quantitative bacteriology. Homogenates were kept for cytokine analysis.

**Results:** *P. aeruginosa* was present in all wounds at day 6. However, a lower bacterial load was seen in the HBOT treated group (mean±SD 3.33*10^6±2.39*10^6) when compared to both the monotherapy ciprofloxacin group (3.32*10^7±3.25*10^7), p=0.0008, and the placebo group (1.12*10^9±6.53*10^8), p=0.0001. Wound size estimated by planimetry did not yield any statistically significant differences between the groups. Wound cytokine levels are currently being measured.

**Conclusion:** In this experimental in vivo biofilm wound model, we found wounds with lowered bacterial load when mice were treated with ciprofloxacin and adjunctive HBOT compared to ciprofloxacin as a monotherapy. The results indicate a potential for clinical use of adjunctive HBOT against non-healing biofilm infected wounds.
Parallel session 2: Mechanisms of antibiotic resistance in biofilms.

Invited speaker:

**Resistance evolution towards biocides and antimicrobial surfaces**

Frank Schreiber

PhD. Senior Researcher. Federal Institute for Materials Research and Testing (BAM). Berlin (DE)

Biocides, including disinfectants and antimicrobial surfaces (AMCs), are important to prevent the spread of pathogens and antimicrobial resistant bacteria via surfaces. However, concerns have been raised about the evolution and selection of resistance against disinfectants and AMCs. In turn, resistance against disinfectants and AMCs can be associated to antibiotic resistance due to cross-resistance and co-resistance. We need to understand the mechanisms and risks of disinfectants and AMCs for resistance and cross-resistance evolution to optimize their application and safeguard their long-term efficacy. We used adaptive laboratory evolution (ALE) experiments based on repeated exposure of bacteria to disinfectants. Our results show that repeated disinfection of *E. coli* with benzalkonium chloride in suspension results in a 2000-fold increase in survival within 5 exposure cycles. Adaptation is linked to the initial presence of persister cells highly tolerant to benzalkonium chloride. We used the same approach to develop standardizable ALE experiments to determine resistance evolution to AMCs. The results highlight rapid adaptation of *E. coli* and *P. aeruginosa* towards copper surfaces. Moreover, there are multiple situations in the clinic or in the environment in which biocides and antibiotics co-occur and in which combination effects can shape their antimicrobial activity or their selective effects. Our work with *P. aeruginosa* shows prevalent combination effects of biocides and antibiotics, ranging from synergy to antagonism and resulting in the selection for or against antibiotic resistant strains. The combination effects are dependent on the biofilm mode-of-growth, manifesting in apparent differences in the structural arrangement of antibiotic sensitive and resistant strains in biofilms exposed to combinations. Furthermore, biocides affect rates of mutation and horizontal gene transfer, thereby having a potential facilitating effect on resistance evolution. Taken together, our work shows that the role of biocides as potential drivers of resistance evolution and selection deserves further study and regulative action.
**S06-TnSeq-guided identification of genes that play a role in antibiotic tolerance of *Pseudomonas aeruginosa* biofilms**

**Kasper Nørskov Kragh¹, Stefano Gualdi², Liang Ziwei¹, Leo Eberl² and Tim Tolker-Nielsen¹**

1Costerton Biofilm Center, University of Copenhagen, Denmark.
2Department of Plant and Microbial Biology, University of Zürich, Switzerland.

Bacterial infections can become chronic when bacteria form biofilm. In this mode of life, the bacteria display a remarkable tolerance to antibiotics and immune system responses. Although we know of some factors that play a role in biofilm-associated antibiotic tolerance, our overall knowledge of how the bacteria achieve it is still scarce.

Using a transposon sequencing (TnSeq) procedure and a newly developed biofilm aggregate model, we have conducted a whole-genome screening of the biofilm model-organism *Pseudomonas aeruginosa* for genes involved in the development of biofilm-associated antibiotic tolerance.

We generated a comprehensive data set mapping out genes essential for antibiotic tolerance when treated with several commonly used classes of antibiotics. We have validated the role of selected genes in biofilm-associated antibiotic tolerance with knock-out mutants in both the biofilm aggregate model and in traditional biofilm flow cell assays. Among our findings are a number of efflux pumps, possible biofilm-associated genes as well as a large number of genes with unknown functions.

TnSeq-guided genome-wide mapping of antibiotic tolerance genes may form the basis for a new understanding of the recalcitrance to antibiotic treatment displayed by microbial biofilms. Such knowledge is crucial for the development of novel efficient treatments against biofilm-based infections.
S07-Experimental evolution of *Pseudomonas aeruginosa* biofilms as a tool to identify adaptive mechanisms leading to reduced antibiotic susceptibility

**Fauve Vergauwe**¹, Andrea Sass¹, Tom Coenye¹

1 Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

The opportunistic pathogen *Pseudomonas aeruginosa* often causes chronic respiratory tract infections in people with cystic fibrosis (CF). *P. aeruginosa* has the capacity to form biofilms, i.e. structured communities embedded in an extracellular matrix that are highly tolerant to antibiotics, which complicates treatment. Repeated antibiotic treatments and the complex microenvironment within a biofilm allow the adaptation of the bacterium towards a resistant phenotype. In the present study, experimental evolution is used to identify adaptive mechanisms that lead to reduced antibiotic susceptibility in *P. aeruginosa* biofilms. *P. aeruginosa AA2-1* was allowed to form surface-attached biofilms on Microbank beads. After 24h of biofilm formation, bacteria were treated with tobramycin, meropenem or ciprofloxacin at a concentration of 3x MIC. After 24h of antibiotic treatment, bacteria were allowed to regrow before inoculating fresh beads with the bacterial suspension to start a new cycle. The evolution experiment was continued until a reduced susceptibility towards the antibiotic was observed. Subsequently, MIC- and whole-genome sequencing data were collected from samples taken at the beginning, in the middle and at the end of the evolution experiment.

Susceptibility testing showed that the MIC quickly increased for *P. aeruginosa* biofilms exposed to antibiotics during evolution, whereas no increase in MIC could be observed in evolved untreated control samples. Whole-genome sequencing indicated that biofilms exposed to antibiotics during evolution acquired mutations that have been previously reported in clinical CF isolates, including mutations in oprD, gyrA/gyrB, and fusA1, following exposure to meropenem, ciprofloxacin or tobramycin, respectively. Our results confirm that experimental evolution in biofilm models can be a useful tool to study the development of reduced susceptibility in biofilms.
S08-Resistance genomics of a new siderophore cephalosporin against *Pseudomonas aeruginosa* chronic respiratory isolates

Carla López-Causapé1,2, Aihnize Maruri-Aransolo2,3, María A. Gomis-Font1,2, Iván Penev1,2, María García Castillo2,3, Xavier Mulet1,2, Juan de Dios Caballero2,3, Rosa del Campo2,3, Rafael Cantón2,3, Antonio Oliver1,2

1 Hospital Universitario Son Espases-IdISBa, Palma de Mallorca, Spain. 2 CIBER Enfermedades Infecciosas, Madrid, Spain. 3 Hospital Ramón y Cajal-IRYCIS, Madrid, Spain.

**INTRODUCTION/OBJECTIVES.** Cefiderocol is a new siderophore cephalosporin approved for the treatment of infections by MDR Gram-negative bacteria, but data on its activity and mechanisms involved in resistance in chronic-respiratory *P. aeruginosa* isolates is still limited. Thus, the objective was to evaluate the activity of cefiderocol and comparators against *P. aeruginosa* isolates from chronically-colonized CF patients, as well as to investigate the potential mechanisms involved in resistance.

**MATERIAL AND METHODS.** Three sequential *P. aeruginosa* isolates from 50 chronically-colonized CF patients were studied. MICs for cefiderocol and comparators were determined by broth microdilution using EUCAST-2021 breakpoints and guidelines. Whole genome sequences were obtained (Miseq, Illumina) to assess clonality and the presence of mutations within a set of chromosomal genes involved in *P. aeruginosa* antibiotic resistance (n=40) and iron uptake (n=120).

**RESULTS.** Cefiderocol showed the lowest MIC50/90 values (0.25/2 mg/L). Susceptibility rates (94%) were comparable to the other novel antipseudomonal agents showing higher resistance breakpoints (range 90-97.3%) and higher than classical antipseudomonals (46.7-88.7%) except colistin (96%). Cefiderocol resistance was observed in 9 isolates (6 patients). Most patients (39/50) were colonized by a single Sequence type. Of note, 132 of the 160 genes investigated were mutated in at least 1 patient. Resistance genes associated with a statistically significant (p0.05) increase in cefiderocol MICs (1-3 two-fold dilutions) included mexA, galU, pmrA, pmrB, ftsI (PBP3) and ampC. Likewise, iron uptake genes mutated in cefiderocol resistant isolates and associated with a statistically significant increase in cefiderocol MICs included PA0434, cirA, fepG, optI, pchD, pchF, phuR, prpL, pvdE, pvdQ and sppR, but most of them were also mutated in susceptible isolates.

**CONCLUSIONS.** (i) Cefiderocol shows a potent activity against *P. aeruginosa* chronic respiratory isolates. (ii) Cefiderocol MICs are modulated by a complex mutational resistome that needs to be monitored upon introduction of this novel option into clinical practice.
S09-The effect of growth environment on the antibiotic resistance of *P. aeruginosa*

Jenny Littler and Dr Freya Harrison
School of Life Sciences, University of Warwick, Coventry, UK

Increases in antimicrobial resistance (AMR) rates are an increasing concern for both health and the economy. With very few new drugs being produced, research into the mechanisms of AMR is an important area of investigation. Biofilm formation has been implicated in higher levels of antibiotic resistance due to potentially blocking antibiotics from their targets. My work focuses on the opportunistic pathogen Pseudomonas aeruginosa, a key nosocomial pathogen which is greatly implicated in the lung infections of those with cystic fibrosis. *P. aeruginosa* is also a well-regarded model organism for studying biofilms. I have already established that antibiotic susceptibility in planktonic environment varies depending on the media that *P. aeruginosa* is grown in, and that the growth media in some cases changes whether the pathogen is classed as resistant or sensitive to an antibiotic. My next steps were to test antimicrobial resistance in biofilm models such as the ex vivo pig lung (EVPL) model of cystic fibrosis infection. *P. aeruginosa* strain PA14 produces a mucoid biofilm. A PA14 transposon mutant in pelA lacks the ability to make the main pellicle polysaccharide which PA14 strain uses to construct the biofilm matrix. Previous research has shown that this mutant cannot produce a mature biofilm, as such we used this mutant to look at differences between antimicrobial resistance levels in aggregates of PA14 with/without a mature matrix in the EVPL model. We found high levels of resistance to colistin and meropenem in both the PA14 WT and pelA mutant. This was an interesting result as it suggests that mechanisms beyond biofilm matrix production aid in the increase in antibiotic resistance observed in vivo. Our next steps are to discover what possible other mechanisms might be at play by looking at transcriptomics and metabolomics data.
S10-Biocides trigger multiple regulatory systems in *Staphylococcus aureus* to induce antibiotic tolerance.

D. Walsh¹, J. Aylott², C. Wolz³ and K. R. Hardie¹

1 School of Life Sciences, Biodiscovery Institute, University of Nottingham, University Park, NG7 2RD,
2 School of Pharmacy, University of Nottingham, University Park, NG7 2RD,
3 Institut für Med. Mikrobiologie und Hygiene, University Hospital Tübingen, University of Tübingen

**Hypothesis.** The biocide triclosan is used extensively in both household and hospital settings, resulting in chronic exposure to the biocide in individuals that use triclosan-containing products. Triclosan is thought to induce antibiotic tolerance and alter biofilm formation. We hypothesise that the widely used biocide alters biofilm architecture which contributes to antibiotic treatment failures of *Staphylococcus aureus*.

**Methodology.** To determine how triclosan induces antibiotic tolerance, *S. aureus* was pre-treated with triclosan prior to treatment with clinically relevant antibiotics. Viability, biofilm structure and gene expression were characterized.

**Results.** Planktonic *S. aureus* cultures pre-treated with triclosan had 1,000 fold higher viable counts compared to non triclosan pre treated cultures. Live/dead staining of biofilms revealed that triclosan pre-treatment protected *S. aureus* biofilms from treatment with otherwise lethal doses of antibiotics. Biocide induced antibiotic tolerance was not only limited to triclosan as subinhibitory concentrations of the biocides benzalkonium chloride and chlorhexidine digluconate were also able to induce antibiotic tolerance. Interestingly, biofilms of mutants with a defective stringent response were not protected from antibiotic treatment by exposure to triclosan, but they were by the other biocides, indicating alternate mechanisms were occurring. Confocal laser scanning microscopy revealed that incubation of *S. aureus* with triclosan altered biofilm structure, resulting in increased proportions of polysaccharide in the biofilm matrix that could potentially mediate protection against antibiotics. The stringent response mutant was also triggered to alter its biofilm by triclosan, suggesting another underlying response. Interrogation of the molecular mechanisms collectively delivering triclosan induced changes was carried out via RNA-sequencing. Gene expression changes point towards not only protection against antibiotics, but also protection against the host immune system.

**Conclusions.** We suggest that triclosan triggers multiple global regulatory systems in *S. aureus*, that subsequently induce tolerance to multiple antibiotic classes, alter biofilm structure, and potentially facilitate long-term persistence within the host.
Parallel session 3: Heterogeneity and metabolic adaptations at the special biofilm microenvironment.

Invited speaker:

The infectious microenvironment of chronic infections, implications for treatment

Thomas Bjarnsholt, Professor, PhD & DMSc

Department of Immunology and Microbiology, Costerton Biofilm Centre, University of Copenhagen, Denmark and Department of Clinical Microbiology, Copenhagen University Hospital, Denmark

Biofilms are increasingly associated with many chronic infections across the health field. The main problem with chronic infections is that biofilms are difficult to treat as bacteria in biofilms are tolerant to antimicrobials and the immune system. However, we have recently shown that the chronic infections also contain a lot of single cells too and acute infections also contain biofilms. The single cells are probably not comparable to in vitro planktonic bacteria but we do not know the role of the single bacteria. In addition, increasing evidence suggest that the microenvironment within especially chronic infections is special and possibly governs the disease progression and to some extend the treatment recalcitrance.

In this presentation, I will highlight the challenge that biofilms pose in implant-related infections, wounds and CF lungs, both in relation to treatment but also to the immune system. I will discuss the idea behind the infectious microenvironment and different hypothesis as to how chronic infections initiate. Lastly, I will present novel data on the transcriptomic response of the human body towards the presence of infecting bacteria and how this can be used for diagnostics.
Structured aggregates of bacterial cells within biofilms offer an increased tolerance to antimicrobials, providing either a physical barrier to antimicrobial penetration or leading to physiological adaptations among biofilm bacteria that may impact on antibiotic efficacy. Consequently, biofilms are causative of a range of chronic infections where the use of antimicrobials rarely eradicates the underlying infection. One feature of the development of microbial biofilm communities is that they often undergo lifecycle changes between aggregated and planktonic modes of growth. This transition between sessile and motile growth modes is referred to as biofilm dispersal and much of the biology and gene expression associated with dispersal is under the control of the intracellular second messenger c-di-GMP. Understanding and controlling the dispersal process is leading to novel adjunctive strategies to disrupt clinically important biofilms. Exogenous nitric oxide (NO) has been shown to regulate, in a dose-dependent manner, c-di-GMP and biofilm lifecycle dynamics and can induce the disruption of biofilm aggregates. The use of NO therefore offers a potential therapeutic approach to address the challenge of biofilm-associated antimicrobial tolerance. This presentation will cover the underpinning mechanisms of NO signalling and reception in biofilm regulation as well as novel NO-releasing chemistries, prodrugs, and strategies for clinical use.
S12-The small molecule Disperazol interferes with c-di-GMP signaling and induces dispersal of Pseudomonas aeruginosa biofilms

Jens Bo Andersen¹, Louise Dahl Hultqvist1, Charlotte Uldahl Jansen², Tim Holm Jakobsen¹, Martin Nilsson¹, Morten Rybtke¹, Jesper Uhd², Blaine Gabriel Fritz¹, Roland Seifert³, Jens Berthelsen¹, Thomas Eiland Nielsen¹, Katrine Kvortrup², Michael Givskov¹,⁴, Tim Tølker-Nielsen¹

¹ Costerton Biofilm Center. Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark.
² Department of Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark.
³ Institute of Pharmacology and Research Core Unit Metabolomics, Hannover Medical School Carl-Neuberg-Straße 1, D-30625 Hannover, Germany.
⁴ Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore.

Microbial biofilms are involved in a number of infections that cannot be resolved, as microbes in biofilms resist host immune defenses and current antibiotic therapies. Drug candidates that enable the current antibiotics to efficiently deal with biofilm bacteria are therefore urgently needed. We employed a high throughput screening approach to identify chemical compounds that reduce the intracellular c-di-GMP content in Pseudomonas aeruginosa. This led to the identification of a small molecule, termed Disperazol, that efficiently depletes P. aeruginosa for c-di-GMP, inhibits biofilm formation and disperses established biofilms in vitro as well as in animal models of biofilm infection. In addition to the PAO1 laboratory strain, Disperazol was capable of dispersing biofilms of both mucoid, non-mucoid, and small colony variants of clinical P. aeruginosa isolates from cystic fibrosis patients. A combination of Disperazol with standard of care antibiotics resulted in improved eradication of biofilms in vitro, as well as in animal biofilm infection models. Genetic analyses provided evidence that Disperazol specifically stimulates the activity of the c-di-GMP phosphodiesterase BifA in P. aeruginosa. Our work constitutes proof of concept for c-di-GMP phosphodiesterase-activating drugs administered in combination with antibiotics as a viable treatment strategy for otherwise recalcitrant biofilm infections.
S13-Evolution of biofilm-adapted gene expression profiles in clinical *Pseudomonas aeruginosa* isolates

Janne G. Thöming 1,2; Alexander Jeske 2,3; Matthias Preusse 2,3; Alejandro Arce-Rodriguez 2,3; Jürgen Tomash 2,3 and Susanne Häussler 1,2,3,4

1 Department of Clinical Microbiology, Copenhagen University Hospital – Rigshospitalet, 2100 Copenhagen, Denmark
2 Institute for Molecular Bacteriology, TWINCORE, Centre for Experimental and Clinical Infection Research, 30265 Hannover, Germany.
3 Department of Molecular Bacteriology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany.
4 Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, 30265 Hannover, Germany

The overall success of a pathogen depends on its ability to efficiently adapt to challenging conditions during infection. Although *Pseudomonas aeruginosa* is known for its remarkable adaptability, comprehensive knowledge of how it uses distinct adaptive mechanisms to increase its fitness is limited.

In this cross-sectional study, we analyzed transcriptional profiles and genomic sequence variations of >400 clinical *P. aeruginosa* isolates from various infection sites to dissect the driving forces of bacterial adaptability.

Convergent changes in gene expression patterns were found in different groups of clinical isolates when analyzing transcriptional profiles upon the switch from planktonic to biofilm growth state. We identified a group of biofilm pre-adapted clinical isolates. They exhibited increased levels of the intracellular second messenger c-di-GMP already in planktonic growth and changed the expression of 10-times less genes upon switching to biofilm conditions as compared to non-adapted strains. We also show that repeatedly observed adaptive gene expression patterns were linked to a non-functional quorum-sensing regulator LasR. Our results demonstrate that clinical *P. aeruginosa* isolates follow distinct evolutionary pathways to fix and thus constitutively express biofilm-adapted transcriptional profiles, which become independent of the respective environmental trigger.

The knowledge gained promises to provide a better understanding of the evolution of *P. aeruginosa* when adapting to the conditions encountered during biofilm-associated infection, and thus promises to identify potential targets to combat difficult-to-treat chronic infections.
S14-Breakthroughs into understanding how bacteria form biofilms

Julieanne L Vo¹, Gabriela C Martinez Ortiz¹, Makrina Totsika², Lilian Hor¹, Mickaël Desvaux³, Mark Schembri⁴, Jason Paxman¹, and Begoña Heras¹

1Department of Biochemistry and Chemistry, La Trobe Institute for Molecular Science, La Trobe University, Australia
2Centre for Immunology and Infection Control, School of Biomedical Sciences, Queensland University of Technology, Australia
3Université Clermont Auvergne, INRAE, Clermont-Ferrand, France
4Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, Australia

The formation of aggregates and biofilms enhances bacterial colonisation and infection progression by affording protection from antibiotics and host immune factors. Despite these advantages there is a trade-off, whereby bacterial dissemination is reduced. As such, biofilm development needs to be controlled to suit adaptation to different environments. We are interested in understanding the molecular mechanisms underlaying the function of one of largest groups of bacterial adhesins, the autotransporters, that play a critical role in the assembly of bacterial aggregates and biofilms [1-4]. We recently described the structural and functional characterisation of autotransporter Ag43 variants from different Escherichia coli pathotypes [1]. We showed that specific interactions between amino acids on the contacting interfaces of adjacent Ag43 proteins drives a common mode of trans-association that leads to cell clumping. Furthermore, subtle variation of these interactions alters aggregation kinetics and the degree of compacting within cell clusters. Together, our structure–function investigation reveals a universally conserved mechanism for autotransporter mediated aggregation and biofilm formation and the molecular basis for variations in the density of these bacterial communities. We are currently using these findings to inform the further development of our recently patented autotransporter targeted biofilm inhibitor [5].

References
Intra-colony channels have been recently identified in mature Escherichia coli biofilms, where they facilitate nutrient transport. The effect of substrate composition and stiffness on the morphology of these channels is however still unknown. We hypothesised that both of these variables affect channel structure and distribution inside mature biofilms. We used fluorescence mesoscopy, which provides sub-micron resolution within millimetre-size live biofilms, and a custom-made quantitative image analysis pipeline, making use of FIJI and a Python script, to study the effect of environmental growth conditions on biofilm and channel morphology. The mesoscopic effects were quantified by measuring biofilm base area, for which carbon concentration in the substrate was found to be the limiting factor. At the microscale, we found that channel width increased non-linearly with radial distance from the centre of the biofilm irrespective of the nutrient availability, and that channels were proportionally narrower at the centre of the biofilm with respect to the edges. Nutrient concentration affected absolute channel width, with channels forming on carbon-limited substrates being on average 50% wider than those forming on nitrogen-limited substrates. Substrate stiffness variation led to a change in channel density inside biofilms grown on rich medium, with soft substrates leading to narrower and more densely packed channels than hard substrates.

This work demonstrates the first quantitative analysis of intra-colony channels in E. coli biofilms. We found that both the type of substrate (rich or minimal medium) and the substrate composition (stiffness and nutrient availability) influence biofilm morphology. Thanks to its simplicity, our custom image analysis pipeline can be easily adapted for the study of internal patterns in a diverse range of biofilms.
Phage therapy to combat difficult-to-treat biofilm infections is gaining an increasing popularity due to growing number of successful clinical cases. The chronic nature of biofilm-related infections is ideal for a personalized phage therapy modality, providing a treatment time buffer that allows the proper development and preparation of personalized therapeutic phage cocktails. However, biofilms are challenging for therapeutic phages, mainly due to the protection conferred by the biofilm matrix and resistance mechanisms. Therefore, in vitro data and clinical reports suggest that the association of phages with other chemical/mechanical treatments may be beneficial to increase the efficacy of phage therapy against biofilm infections. Here I will present the major challenges imposed by biofilms to phage attack as well as the strategies that can be used to counteract biofilm defences. Moreover, I will also show other ways of exploiting phages as antibiofilm agents, namely the use of phage derived endolysins.
**S16-Design synthesis and therapeutic validation of a Quorum Quencher, UTIQQ to combat gestational UTI**

Adline Princy Solomon¹, Sahana Vasudevan¹ and Karthik Shanmugam¹

1Quorum Sensing Laboratory, Centre for Research in Infectious Diseases (CRID), School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, 613401, India

**Introduction:** Urinary Tract Infection (UTI) is a globally widespread human infection caused by an infestation of uropathogens. Even though Escherichia coli is often quoted as being the chief among them, Staphylococcus aureus involvement in UTIs, especially in gestational UTIs, is often understated.

**Hypothesis:** Developing a potential drug molecule targeting the SarA of *S. aureus* would combat biofilm-associated infections.

**Methodology:** The antibiofilm activity of the root extracts of Melia dubia against clinical strains of *S. aureus* was evaluated. The active ingredient, o-coumaric acid, was hybridized with the previously established SarA inhibitor, SarABI. The hybrid molecule, UTIQQ, was tested for its antibacterial activity by CLSI guidelines. Further, the antibiofilm activity was evaluated, followed by synergy studies with gentamicin. In vivo evaluation was done by catheterization to induce urinary tract infections in the pregnant rat. Bacterial burden and Histopathology analysis were carried out to evaluate the in vivo efficacy of UTIQQ.

**Results:** Our previous studies have reported the antibiofilm activity of SarA-based biofilm inhibitor (SarABI) and root extracts of *M. dubia* against *S. aureus* and uropathogenic *E. coli*, respectively. A hybrid molecule combining the molecule screened from *M. dubia* root extracts and a modified SarA-based inhibitor (SarABIM) was designed through in silico approach. The synthesized hybrid molecule, 4-(Benzylamino)cyclohexyl 2-hydroxycinnamate (UTI Quorum-Quencher, UTIQQ) was validated for anti-biofilm activity. The MBIC50 and MBIC90 of UTIQQ were 15 and 65 µg/mL, respectively. Confocal laser scanning microscopy (CLSM) images showed biofilm reduction. UTIQQ was proven to have synergistic activity with gentamicin. In vivo studies in a rat model corroborated the in vitro studies.

**Conclusion:** The drug candidate, UTIQQ would be a promising candidate when used alone or in combination with an antibiotic for staphylococcal-associated gestational UTI.
S17-The human Atrial Natriuretic Peptide as a powerful weapon against *Pseudomonas aeruginosaa* biofilm

Lesouhaitier Olivier¹, Louis Mélissande¹, Clamens Thomas,¹ Desriac Florie, Rodrigues Sophie¹, Harmer Nicholas J.², Lendon Courtney², Grandjean Teddy³, Kipnis Eric³, Gosset Philippe³, Barreau Magalie¹, Cornelis Pierre¹, Chevalier Sylvie¹, Feuilloley Marc G.J.¹, Tahrioui Ali¹

1Unité de Recherche Communication bactérienne et Stratégies Anti-infectieuses CBSA EA 4312, University of Rouen Normandy, 27000 Evreux, France
2School of Biosciences, University of Exeter, Exeter, EX4 4QD, UK
3U1019-UMR9017-CIIL-Centre d’Infection et d’Immunité de Lille, University Lille Lille F-59000, France

**Introduction:** *Pseudomonas aeruginosaa* is responsible for chronic infections in wounds or lungs where it forms biofilms that are impervious to antibiotics treatments. There is therefore a need for alternative treatments that prevent biofilm formation or disperse pre-established biofilms. The C-type natriuretic peptide (CNP), a member of the natriuretic peptide hormone family, was previously shown to strongly decrease *P. aeruginosaa* biofilm formation while slightly enhancing bacterial virulence. These effects are triggered by binding of CNP to the *P. aeruginosaa* AmiC sensor protein. A second member of this peptide family, the Brain Natriuretic Peptide (BNP), does not bind to AmiC.

**Hypothesis** and aims: In the present study, we evaluate the impact of the third member of this hormone family, the Atrial Natriuretic Peptide (ANP), on *P. aeruginosaa* biofilm formation and dispersion.

**Results:** ANP not only prevented the formation of a *P. aeruginosaa* biofilm, but also strongly disrupted pre-formed biofilms. This effect was dose-dependent, requiring low peptide concentrations (10 pM to 0.1 µM). This effect is ANP specific: neither BNP nor CNP impacted pre-formed biofilms. The ANP dispersal activity required the presence of both the *P. aeruginosaa* AmiC sensor protein and the AmiR anti-terminator regulator. Moreover, ANP acted as an adjuvant agent, enhancing the anti-biofilm action of different antibiotics, allowing almost full biofilm eradication. In addition, we showed that ANP had no impact on either *P. aeruginosaa* virulence traits or survival, and that ANP is active against numerous clinical strains. Finally, we are currently validating in vivo the effects of ANP on biofilm dispersion using two mouse models of chronic infections.

**Conclusion:** Altogether, these data suggest that ANP could be a new promising and powerful anti-biofilm weapon for dispersing the *P. aeruginosaa* biofilm. Our work establishes the activation of members of the ami pathway as a potential mechanism for *P. aeruginosaa* biofilm dispersion.
**S18-Photodynamic inactivation – an efficient approach for eradication of microbial biofilms.**

**Bujdáková Helena, Štefánek Matuš, Dadi Nitin Chandra Teja, Bilská Katarína, Vargová Jarmila, Kendra Samuel**

Comenius University in Bratislava, Faculty of Natural Sciences, Department of Microbiology and Virology, Ilkovičova 6, 842 15 Bratislava, Slovakia

**INTRODUCTION**

Photodynamic inactivation (PDI) is a promising approach for fighting microorganisms in both planktonic form and biofilm.

**HYPOTHESIS**

The effectiveness of PDI was studied on single-species and inter-kingdom biofilm represented by methicillin-resistant *Staphylococcus aureus* (MRSA) and azole-resistant *Candida albicans*, the looking for an answer, whether PDI can effectively eradicate biofilms. Of particular interest was the question, of how PDI interacts with efflux, the main mechanism of biofilm resistance.

**METHODOLOGY**

Three different models have been employed: 96-well microtitre plates and nanomaterial with functionalized photosensitizer (PS, in vitro), mouse tongues (ex vivo), and *Galleria mellonella* (in vivo). The effectiveness of PDI was tested in the presence of 2 selected PSs; methylene blue (MB) and phloxine B (PhB). Biofilms were evaluated by CFU/ml, CLSM, and SEM. Resistance was determined by MIC50 or SMIC50 (concentrations reducing the growth of planktonic or sessile biofilm cells by 50%). Gene expression was determined by qPCR.

**RESULTS**

PDI inhibited biofilm growth; 0.5-1 mM MB or 0.05 mM PhB showed 10 to over 1000-fold reduction in growth of MRSA strains compared to the control sample despite up-regulation of the NorA gene (up to 27-fold). Similarly, PDI effectively inhibited the *C. albicans* biofilm manifesting overregulation of the CDR and MDR1 genes. CLSM showed that the yeast-to-hyphae transition could play an important role in susceptibility to PDI and SEM imaging revealed cell walls disruptions. Moreover, PDI proved effectiveness to inter-kingdom biofilm, but this was lower compared to single-species one. Stress response based on measurement of reactive oxygen species showed a different response of prokaryotic MRSA compared to eukaryotic *Candida*. The highest therapeutic effect was achieved in the group of *Galleria mellonella* infected with *S. aureus* after PDI.

**CONCLUSION**

The obtained results contributed to the understanding of PDI principles and suggested a potential of this unique approach in eradication of resistant biofilms.
S19-Probiotics for treatment of skin wound infection: fighting biofilms and promoting tissue regeneration

Sixuan Zhang, Katharina Maniura, Zhihao Li, Qun Ren

The Laboratory of Biointerfaces, Empa, the Swiss Federal Laboratories for Materials Science and Technology
Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

Chronic wounds are prone to localized infection and form biofilm. Biofilms can promote chronic inflammation, thereby halting the normal wound healing, and protecting bacteria from immune attacks. The current clinically applied treatments rely on the application of antiseptics/antibiotics, which often cause irritation to healthy tissue even the generation of super bacteria. Probiotics have recently received increasing attention for their great potential in treating skin diseases. Here, we aim to investigate whether probiotics can influence the viability of pathogenic bacteria (Pseudomonas aeruginosa) and human dermal fibroblast cells (HDFs), and trigger the inflammatory response of skin related cells. HDFs were co-cultured with probiotic or P. aeruginosa, respectively for 24 h. Cell Counting Kit-8 (CCK-8) used to measure the cytotoxicity of bacteria to HDFs. It was found that probiotic in the tested concentration range (up to 106 CFU/mL) has no negative impact to HDFs, whereas HDFs were dead after incubation with P. aeruginosa of the same concentration (106 CFU/mL). Furthermore, significant inflammatory response was observed for cells exposed with either of the bacteria analyzed by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). These results suggest that probiotics can be a novel tool to combat bacterial pathogen and inhibit biofilm formation in skin wounds.
Orthopedic implant colonization is a hard-to-treat complication of arthroplasties and Staphylococcus aureus is the more prevalent pathogen encountered in orthopedic prosthesis-related infections. Current strategies consist of antibiotic treatment and surgical procedures, gentamicin being often used for local delivery but can adversely promote biofilm formation over planktonic state. To prevent S. aureus colonization of orthopedic implant, Wharton’s Jelly derived from the human umbilical cord could represent a valuable opportunity as this tissue recently showed a potential use in the field of regenerative medicine and for treatment of wound infections. Especially, a new decellularization process of Wharton’s Jelly can prevent rejection and alleviate legislative restriction.

Herein, we investigated the biofilm formation of S. aureus in the presence of classically devitalized Wharton’s Jelly (WJ) or decellularized ones (DWJ) in vitro. For this, S. aureus was cultured with a titanium disk, mimicking orthopedic implant, and WJ or DWJ. A reduction of more than 90% of the bacterial burden adherent to DWJ compared to WJ was observed and biofilm biomass quantity was reduced up to 25 times. Then, adding gentamicin into the medium together with DWJ induced a higher reduction (up to 5 log10) of the bacterial burden adhered to titanium, compared to titanium alone with gentamicin. Furthermore, we sought to identify the post-partum metabolites within the WJ which was separated in ten fractions by physicochemical techniques. Preliminary results showed that several fractions displayed antibiofilm properties.

These results highlight a particularly promising property of DWJ to avoid adverse side effect of antibiotic treatment: bacterial switch to biofilm form. Indeed, the study reveals a potential synergistic effect as DWJ significantly reduces the number of adherent bacteria induced by gentamicin treatment. Thus, umbilical cord might represent a valuable source of antibacterial and antibiofilm molecules to produce DWJ, which arises as an excellent candidate to prevent prosthesis-related infections.
S21-Signal to kill: Nitroxide-induced biofilm dispersal for enhanced antibiotic eradication

Makrina Totsika

Centre for Immunology and Infection Control, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Australia

Introduction: Bacterial biofilms are extremely tolerant to antimicrobials. This presents a significant clinical challenge, with biofilms responsible for up to 80% of bacterial infections. Bacterial cell-cell communication, known as quorum sensing, is central to biofilm formation and thus, molecules that interfere with these pathways hold great potential. Nitric oxide (NO) is known to act as a signal for biofilm dispersal in several species. While its use against biofilms is promising, NO’s chemical properties hinder its clinical, agricultural and industrial application.

Hypothesis: Nitroxides, which are stable NO mimics, can be used to disperse bacterial biofilms and synergistically potentiate antibiotic activity against biofilms.

Methodology: A variety of nitroxide functionalised ciprofloxacin derivatives were synthesised and evaluated for biofilm eradication and/or dispersal in several clinically important bacterial species. Standard methods were used to determine MIC and MBEC values for hybrids and stand-alone agents. The mechanism of action of nitroxide-ciprofloxacin hybrids was investigated using synthesized profluorescent probes and fluorescence and confocal laser scanning microscopy.

Results: Nitroxide functionalized ciprofloxacin exhibited significantly improved biofilm-eradication activity against Pseudomonas aeruginosa (up to 94% eradication) showcasing the advantage of covalently linking a nitroxide to an antibiotic. Sequential addition of nitroxides to the ciprofloxacin core via a lysine linker, produced a di-nitroxide-ciprofloxacin hybrid with improved biofilm-eradication activity against uropathogenic Escherichia coli, highlighting the importance of optimising the nitroxide to antibiotic ratio. Anti-biofilm activity of nitroxides extended to Staphylococcus aureus (99.9% eradication, 64-fold more potent than ciprofloxacin), suggesting a potentially universal mechanism of action. In all cases, the nitroxide free radical was fundamental to hybrid activity and appeared to involve an intracellular process. No human cell toxicity was observed in vitro.

Conclusion: Nitroxide functionalised antibiotics overcome many of the limitations of current biofilm-eradication agents and represent a promising anti-biofilm strategy against both Gram-negative and Gram-positive pathogens for further development as new antimicrobials.
Treating and controlling bacterial infections are becoming a real and significant concern in healthcare nowadays. On the one hand, the antibiotic resistance upsurge due to the misuse of antibiotics is an increasing public health threat, and on the other hand, chronic infections characterized by biofilm formation (i.e., lung, skin infections) greatly complicate the antimicrobial therapies for these patients. The lack of new clinically available therapeutic options hinders the advances toward better control of infectious diseases. Therefore, we urgently need multi-targeted therapeutic approaches to treat antibiotic multi-resistant (AMR) bacteria and bacterial biofilm infections and effectively deliver antimicrobials to the infection site. In addition, there is a need for easy-to-use methods of testing the antibiotic susceptibility of bacteria that form biofilms and for screening new possible antibiofilm strategies.

In this talk, I will review our latest laboratory results to combat bacterial biofilm infections by applying specific drug delivery systems and their interactions with the biofilm matrix. A revisiting today’s nanomedicine strategies to treat bacterial infections will be discussed.

In addition, I will describe our BiofilmChip, a microfluidic system to diagnose bacteria growing in biofilms. The device proved to be suitable for studying polymicrobial communities, as well as for measuring the effect of antimicrobials on biofilms and choosing the better personalized treatment. Our results demonstrate that BiofilmChip is a straightforward tool for antimicrobial biofilm susceptibility testing that could be easily implemented in routine clinical laboratories.

Acknowledgments

The group is supported by grants from the Ministerio de Economía, Industria y Competitividad, MINECO, and Agencia Estatal de Investigación (AEI), co-funded by Fondo Europeo de Desarrollo Regional, FEDER, European Union (RTI2018-098573-B-100 and PID2021-125801OB-100), the CERCA programme and AGAUR-Generallitat de Catalunya (2017SGR-1079), the European Regional Development Fund (FEDER), Catalan Cystic Fibrosis association and Obra Social “La Caixa”.

Eduard Torrents1,2*

1Bacterial Infections and Antimicrobial Therapies Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain
2Microbiology Section, Department of Genetics, Microbiology, and Statistics, Faculty of Biology, University of Barcelona, 08028 Barcelona, Spain

etorrents@ibecbarcelona.eu / eduard.torrents@ub.edu
References


S22-The Use of Raman Technologies for Biofilm Mapping

Lukas Krien¹, Steffen Rupp¹, Kevin Wright², Renzo Ccahuana-Vasquez³

¹Fraunhofer Institute for Interfacial Engineering and Biotechnology, Stuttgart, Germany
²Procter & Gamble, Reading, United Kingdom
³Procter & Gamble, Kronberg, Germany

**Introduction:** Oral biofilms play a key role in the development of oral diseases like gingivitis, periodontitis or dental plaque. While there is great knowledge regarding the biofilm’s architecture and dynamics, there is limited measurement of the spatial chemical composition in combination with microbes. Confocal Raman microscopy can give inside knowledge into the composition and structure of biofilms and provide some understanding on the strain’s role in the development and maturation of biofilm structures. Furthermore, this information can be helpful in understanding the impact of structure on both disease formation and the impact of therapies on biofilms, potentially making it possible to predict the design of health products for the future.

**Hypothesis and aims:** The theme of the research is to establish whether it is possible to differentiate bacterial species spatially in artificial biofilms. Coupling Raman spectra acquisition with multivariate statistical models should allow a differentiation resulting in the mapping of species non-destructively.

**Methodology:** Confocal Raman Microscopy was used for spectral acquisitions and multivariate statistical methods for mapping. In a first step, it was evaluated if different subgingival species are able to be differentiated using Raman spectra and least square analysis. In a second step, two species were cultured in-vitro and mapped using Raman spectra combined with cluster analysis. The workflow was then applied to other biofilms for applicability demonstration.

**Results:** Raman spectra show a specific fingerprint region (600-1800 cm⁻¹) where different strains were successfully differentiated statistically. This information was used to successfully differentiate two species in a subgingival biofilm (S. oralis and A. denticolens) and was further demonstrated on another biofilm (P. aeruginosa and C. albicans).

**Conclusion:** It was possible to show that confocal Raman microscopy can be used for the assessment and differentiation of biofilms consisting of two species. Optimizations were identified to improve the workflow of this proof-of-concept approach in the future.
Biofilm development on bone and prosthesis leads to hard-to-treat infection as therapeutic approaches are not adapted. Indeed, infectious environment is known to dramatically influence biofilm structure. Our project is to identify bone context factors that influence biofilm formation and structure. The final aim is to develop an in vitro model of biofilm development on prostheses in order to screen antibiofilm molecules or to assess biomaterials.

First, we observed differences in biofilm matrix composition of two methicillin-sensitive (MSSA: CIP 53.154 and SH1000) and one methicillin-resistant (MRSA: USA300) strains developed in a bone-like environment medium (excess of magnesium, lack of nutrients and hypoxia). Then, we compared the three strains biofilm formation on different supports prepared to mimic the periprosthetic environment: bone explant (mineral and organic parts, no living host cells) and on titanium covered with fibronectin, mimicking an implanted prosthesis. We measured different parameters: adhesion by counting methods, matrix production thanks to confocal microscopy and biofilm-related gene regulation by q-PCR. MRSA bacterial burden was important on bone explant contrary to both MSSA strains. We also noticed an influence on MSSA planktonic growth. The mechanisms involved in bacterial adhesion in bone context were strain-dependent: production of a matrix for MSSA vs metabolism responses for MRSA. The simultaneous presence of bone explant and fibronectin coating titanium was also found to change the bacterial behavior. We also evaluated the impact of osteoblast cells (SaOS-2 and primary cells) using the same methods and we concluded that MSSA biofilm formation is induced or inhibited by osteoblast secretome in a donor-dependent manner.

These results highlighted the need for new biofilm models, more representative of the infectious environment with adapted culture medium and presence of in situ supports in order to better evaluate therapeutic strategies on biofilm.

References/Acknowledgements: Project and Fabien Lamret were supported by the French Region Grand Est and the Fondation URCA.
Development of a three-dimensional human in vitro model of a biofilm-infected wound

Jana Wächter; Pia Kaiser; Maike Windbergs

1 Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt am Main, Germany, j.waechter@em.uni-frankfurt.de

Current approaches for investigating biofilm-related wound infections rarely involve the combination of biofilms with skin cells, as maintaining cell viability during the required time for biofilm formation is difficult to achieve. Although affords are described to combine mature biofilms with living cells, they entail only indirect contact or compromise biofilm integrity since the translocation of pre-grown biofilms to the tissue fails due to insufficient mechanical strength. Therefore, we developed a bacterial biofilm model on the basis of electrospun fibers that provides high mechanical stability to allow for a transfer of intact biofilms to human tissue models. Additionally, the nanofibrous scaffold, consisting of biocompatible polymers, closely resembles the structure and polymeric composition of the native biofilm matrix.

We inoculated the electrospun mats with Pseudomonas aeruginosa as a common wound pathogen and monitored the growth of adherent bacteria over the maturation period of 48h. Uniform bacterial distribution as well as matrix production throughout the fiber network was demonstrated by histological investigations and SEM imaging, highlighting the scaffold as an adequate growth substrate. Next, antibiotic susceptibility of the biofilm model was determined by the treatment with gentamicin, where, in accordance with in vivo biofilms, the developed model showed a highly enhanced tolerance compared to planktonic bacteria. Finally, pre-cultivated biofilms were transferred to wounded ex vivo human skin tissue, where a steady bacterial growth was observed for further 24h. The interaction of the biofilm and the host cells was assessed by histological investigations and SEM imaging, showing close contact of biofilm and wound bed.

We thereby were able to develop a novel biofilm model which exhibits main characteristics of native biofilms and provides high mechanical stability to enable the transfer of intact biofilms to human skin tissue to model wound infections in a physiological relevant setting.
S25-Label-free spatially resolved analysis of bacterial biofilms – effect of composition and maturation on drug penetration

Pia Kaiser¹; Jana Wächter¹; Maike Windbergs¹

1 Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University Frankfurt, Max-von Laue-Str. 9, 60438 Frankfurt am Main, Germany

Effective management of tissue infections still represents a severe therapeutic challenge in clinical practice mostly due to the presence of bacterial biofilms, for which increasing resistance to established antibiotics is observed. A comprehensive understanding of the key parameters influencing the bacterial response to antibiotic treatments is of major importance. In this context, development of predictive in vitro biofilm models mimicking infection-relevant key aspects combined with innovative analytical strategies for non-invasive characterization are urgently needed.

In this study, we developed non-destructive and label-free analytical approaches using confocal Raman microscopy for identifying bacteria and monitoring the formation of a mature biofilm. Based on an in-house established in vitro biofilm model comprising electrospun fibers as scaffold for inoculation with bacterial strains, we were able to identify key aspects influencing drug penetration into bacterial biofilms focusing on maturation and composition. In a proof-of-concept study, we selected representative bacterial strains accounting for severe wound tissue infections and investigated the penetration behaviour of gentamicin sulphate as common antibiotic active based on different development stages of biofilm formation and composition (Pseudomonas aeruginosa mono-species model vs. dual-species model by addition of Staphylococcus aureus). During biofilm maturation, a remarkable increase of bacterial extracellular substance could be visualized by Raman analysis. Drug penetration decreased as a function of maturation. Interestingly, differences in drug penetration could be correlated with the composition of the bacterial biofilm.

In conclusion, we successfully applied confocal Raman microscopy as chemically selective and label-free strategy for non-invasive bacterial biofilm microanalysis. We further identified key parameters influencing the penetration behaviour of antibiotics into a biofilm. The technique bears a high potential for basic research on bacterial biofilms as well as for development of novel anti-infectives.
S26- Identification and Antimicrobial Resistance Profiling of Respiratory Pathogens Using Multi-excitation Raman Spectroscopy

Dr Callum Highmore, Dr Adam Lister, Dr Niall Hanrahan, Professor Saul Faust, Professor Sumeet Mahajan, Professor Jeremy Webb

School of Biological Sciences, University of Southampton, National Biofilms Innovation Centre

Currently, antibiotic sensitivity testing relies on culture-based techniques, delaying diagnosis of antimicrobial resistant (AMR) infections. In addition, detection of biofilms, a mode of bacterial growth that facilitates AMR, relies on complex methods which are time, labour and cost intensive. Raman spectroscopy is a rapid, selective, and label-free method of fingerprinting a sample through interrogation of vibrational modes of molecules and offers the potential for real-time, close-to-patient diagnostics for biofilms and AMR. We have developed a multi-excitation Raman spectroscopy methodology that enhances Raman capacity for pathogen identification and characterisation. By analysing bacterial samples at wavelengths 532nm and 785nm, we can achieve rapid strain-level differentiation of respiratory pathogens Pseudomonas aeruginosa and Staphylococcus aureus. By combining multi-excitation Raman spectra with support vector machine (SVM) analysis, identification of both species following inoculation into artificial sputum medium was achieved with 99.75% accuracy, including 100% accuracy for drug-sensitive and drug-resistant S. aureus.

Analysis of Raman peaks associated with P. aeruginosa and S. aureus were applied to Raman images of mono- and dual-species biofilms, to work towards label-free identification of biofilm constituents.

AMR sensitivity profiles (for tobramycin, ceftazidime, ciprofloxacin, and imipenem) were generated for 21 P. aeruginosa strains and compared with their Raman spectral signatures using the multi-excitation methodology. Spectra could be correctly categorised by their antibiotic sensitivity profile using SVM, with >98% accuracy. Respiratory pathogens with unknown antibiotic sensitivity profiles will undergo similar analysis to determine the capability of the multi-excitation Raman methodology to predict AMR and recommend antibiotic treatments for bacterial respiratory infection. Together these data provide the basis for a potential new clinical diagnostic platform. With a rapid methodology that requires little sample preparation, it could have the transformative capability to save lives at scale and reduce the spread of AMR.
Parallel session 6: Management of endovascular and catheter-related infections.

Invited speaker:

Biofilm and catheter-related infections: can we improve prevention or treatment?

David Lebeaux

MD.; PhD, Unité Mobile d’Infectiologie, Service de Microbiologie, Hôpital Européen Georges Pompidou. Paris (FR)

The use of central venous catheters (CVC) is constantly increasing among patients in Intensive Care Unit (ICU) or for the long-term management of cancers, digestive diseases or chronic kidney diseases. Although CVCs improve patients’ care, their use is associated with the risk of device contamination by microorganisms subsequently forming biofilms, ultimately leading to catheter-related infections. Once established, bacterial biofilm (i.e. dense microbial communities associated with a surface) are able to better survive the host immune system and antimicrobial agents.

To avoid CVC contamination and biofilm formation, a bundle of routine preventive measures include hand hygiene, maximal sterile barrier precautions, skin preparation with alcoholic chlorhexidine, use of sterile, transparent, semi-permeable dressing and education/training of nurses and doctors. If the incidence of catheter-related infections remains too high in ICU, chlorhexidine dressings or antibiotic-containing catheters can be used. Among patients with long-term catheters, if the incidence of infection remains high despite the improvement of catheter handling procedures, preventive lock can be proposed such as the combination of minocycline and EDTA or taurolidine-containing solutions. Lock therapy relies on the instillation of a small volume of highly concentrated antibiotic (or anti-biofilm compound) in the catheter lumen to prevent (or eradicate) biofilm. Beside these clinically-validated treatments, data from basic research may help to identify more potent preventive strategies.

Once a catheter-related infection is diagnosed, catheter removal and systemic antimicrobials remain the cornerstones of the treatment. Beside, antibiotic lock therapy plus systemic antimicrobials is an effective and safe strategy to treat uncomplicated catheter-related infection without catheter removal. This conservative strategy should only be proposed to long-term CVC in stable patients and cannot be used to treat infections caused by Staphylococcus aureus or Candida spp. Recent experimental data suggest that anti-biofilm molecules could help improving the eradication of biofilms formed on the surface of CVC.
S27-Catheter related bloodstream infection caused by *E. cloacae* and *Candida parapsilosis*: Are biofilms guilty?

Matúš Dohál (1), Vitor Borges (2), Sigurd Wenner (3), Isabel Nogueira (4), Miguel Pinto (2), Isabel Faria (5), Maria Ana Pessanha (5), Cristina Verissimo (2), Raquel Sabino (2), Joao Rodrigues (2), Rui Matias (2), Patricia Carvalho (6), João Paulo Gomes (2), Helena Bujdáková (1) and Luisa Jordao (7).

1. Department of Microbiology and Virology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia. 2. Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge (INSA), Lisboa, Portugal. 3. SINTEF, SINTEF Industri, Materials and Nanotechnology, Trondheim, Norway. 4. Universidade de Lisboa, MicroLab, Instituto Superior Técnico (IST), Lisboa, Portugal. 5. Hospital Egas Moniz, Centro Hospitalar de Lisboa Ocidental (CHLO), Laboratório de Microbiologia Clínica e Biologia Molecular do Serviço de Patologia Clínica, Lisboa, Portugal. 6. SINTEF, SINTEF Industri, Materials Physics, Oslo, Norway. 7. Department of Environmental Health, INSA, Lisboa, Portugal.

Biofilm-associated infections is a public health concern in the context of healthcare associated infections (HAI) such as catheter related bloodstream infections (CRBSI). Here the dynamics of two top ten etiological agents of CRBSI, *Enterobacter cloacae* and *Candida parapsilosis* isolated from a CRBSI’s patient, were studied to get insights on the role played by biofilms on this HAI.

Antimicrobial susceptibility of CVC and HC’s isolates was evaluated according to EUCAST guidelines. Single and/or mixed biofilms assembled on different materials in Mueller-Hinton broth with 2% glucose were assessed by crystal violet assay and scanning electron microscopy (SEM). Fluorescence in situ hybridization (FISH) was used for identification purposes and to assess microorganisms distribution within the biofilm (3D reconstruction) complemented with Focus Ion Beam (FIB)-SEM to assess biofilms assembled on the inner/outer CVC’s surfaces (tomograms). Whole-genome sequencing (WGS) was performed for all isolates.

All isolates were antimicrobial resistant. Of note *E. cloacae* resistance to collistin and an additional resistance of the CVC compared to HC-isolate (ceftolozame-tazobactam) probably linked to a mutation in rpoB gene. *Candida* resistance to fluconazol might be explained by ERG11 gene mutation. Enterobacter and Candida assembled biofilms on glass, polystyrene and polyurethane being mixed biofilms denser when both microorganism were present from the beginning. FISH and SEM analysis showed that biofilm bottom layer was in all cases richer in *E. cloacae*. Using environmental isolates of the same species we showed that this biofilm phenotype is not a general feature. Using polyurethane catheters (shape/material factor), denser mixed biofilms richer in EPS were observed. A distinct phenotype was present on the patient’s CVC by SEM and FIB/SEM. WGS confirmed the genetic identity of the pair CVC/HC isolates, while corroborating the virulence potential and observed antimicrobial resistant character of the studied CRBSI-driving pathogens.

The results suggest that biofilms allow interaction and adaptation of microorganisms belonging to different kingdoms (Bacteria and Fungi). Adaptation might affect virulence in a
transitory or permanent fashion, with potential impact on microorganisms’ potential to cause CRBSI.

**S28-Novel Synthetic Antimicrobial and Anti-biofilm Peptides (SAAPs)-containing coatings to prevent biomaterial-associated infection**

_Martijn Riool^1_, Moniek Schmitz^2_, Leonie de Boer^1_, Robert Cordfunke^3_, Jan Wouter Drijfhout^3_, Patricia Dankers^2_, Sebastian A.J. Zaat^1_

1 Department of Medical Microbiology and Infection Prevention, Amsterdam institute for Infection and Immunity, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands
2 Department of Biomedical Engineering, Laboratory of Chemical Biology, Institute for Complex Molecular Systems, Eindhoven University of Technology, PO Box 513, 5600 MB, Eindhoven, The Netherlands
3 Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, 2300 RC, Leiden, The Netherlands

The use of medical devices has grown significantly over the last decades, and has become a major part of modern medicine and our daily life. Infection of implanted medical devices (biomaterials), like catheters, prosthetic heart valves or orthopaedic implants, can have disastrous consequences, including removal of the device. For still not well understood reasons, the presence of a foreign body strongly increases susceptibility to infection. These so-called biomaterial-associated infections (BAI) are mainly caused by _Staphylococcus aureus_ and _Staphylococcus epidermidis_. Formation of biofilms on the biomaterial surface is generally considered the main reason for these persistent infections, although bacteria may also enter the surrounding tissue and become internalized within host cells (Riool et al., Acta Biomater., 2014). Our work focuses on the development and characterization of novel antimicrobial agents and delivery systems, and their effectiveness in the prevention of BAI and other difficult-to-treat biofilm infections. The scarcity of current antibiotic-based strategies to prevent infections and their risk of resistance development prompted us to develop novel Synthetic Antimicrobial and Anti-biofilm Peptides (SAAPs) based on the primary sequences of the human antimicrobial proteins Thrombocin-1 and LL-37, and to test their potential in the fight against implant-associated and wound infections by multidrug-resistant bacteria. The lead peptide, SAAP-148, kills multidrug-resistant pathogens without inducing resistance, prevents biofilm formation and eliminates established biofilms and persister cells, and is effective against both acute and established skin infections (de Breij & Riool et al., Sci. Transl. Med., 2018). Currently, we are developing improved SAAPs. As a next step, we aim to develop antimicrobial coatings, such as a new polymeric supramolecular scaffold material, exerting two important functions: preventing microbial adhesion - by incorporating SAAPs - and thereby preventing biofilm formation, and inducing endogenous (eukaryotic) cells to adhere and propagate, as a first step towards functional tissue repair.
S29-Interactions of microorganisms within a urinary catheter four species biofilm model

Jontana Allkja (1,2), Darla M. Goeres (3), Andreia S. Azevedo (1,2,4,5), Nuno F. Azevedo (1,2)

Biofilms are often polymicrobial in nature, which can impact their behaviour and overall structure, often resulting in an increase in biomass and enhanced antimicrobial resistance. Using plate counts and locked nucleic acid/2′-O-methyl-RNA fluorescence in situ hybridization (LNA/2′OMe-FISH), we studied the interactions of four species commonly associated with CAUTI: Enterococcus faecalis, Escherichia coli, Candida albicans and Proteus mirabilis. Eleven combinations of biofilms were grown on silicone coupons placed in 24-well plates for 24 hrs, 37 °C, in artificial urine medium (AUM). The results showed that P. mirabilis was the dominant species when present and was able to inhibit both E. coli and C. albicans growth. In the absence of P. mirabilis, an antagonistic relationship between E. coli and C. albicans was observed, with the former being dominant. E. faecalis growth was not affected in any combination, showing a more mutualistic relationship with the other species. Imaging results correlated with the plate count data and provided visual verification of species undetected using the viable plate count. Moreover, the three bacterial species showed overall good repeatability (Sr values 0.1 – 0.54) in all combinations tested, whereas C. albicans had higher repeatability SD (Sr) values (0.36-1.18). The study showed the complexity of early-stage interactions in polymicrobial biofilms and that more sensitive, visual techniques can provide more information on these interactions and resulting structures which could inform improved control strategies.
S30-Graphene quantum dots for in situ treatment of vascular catheter biofilms

J. Scott VanEpps, Christopher Altheim, Shannon VanAken, Mische Hubbard, and Nicholas Kotov

University of Michigan, Departments of Emergency Medicine, Biomedical Engineering, Chemical Engineering, Biointerfaces Institute, and Weil Institute for Critical Care Research and Innovation.

Introduction: Despite ongoing initiatives to adopt or adhere to specific recommendations (e.g., care bundles) and increased use of antibiotic impregnated catheters, the incidence of central line associated bloodstream infection remains flat or increasing. Biofilm formation on catheter surfaces offer constituent bacteria significant protection from host response and antimicrobial therapy. Thus, definitive therapy frequently requires removal and replacement or guidewire exchange of catheters with associated morbidity, mortality, and cost. We recently described graphene quantum dots (GQDs) that effectively disrupt mature staphylococcal biofilms via direct interaction with phenol soluble modulins. In this study, we advance that work from the in vitro to the in vivo setting.

Hypothesis: GQDs will reduce bacterial load without increase in systemic dissemination in a rat central venous catheter infection model.

Methodology: Six Sprague-Dawley rats were surgically instrumented with a jugular venous catheter which was then inoculated three days later with S. epidermidis. After 7 days, a dense biofilm is present on the intraluminal catheter surface. Three animals were treated with a single 24-hour dose (500µg/ml) of GQDs as a catheter lock solution. The other three animals had a standard heparin lock as a control.

Results: A single catheter lock dose of GQDs results in ~0.4-log reduction (interquartile range of 1.8E4-1.6E5 for GQD vs 1.8E5-1.4E6 for control) in CFU/sq cm recovered from the catheter compared to untreated controls. There was no increase in bacterial load in end organs indicating no increased systemic dissemination. Laboratory studies confirmed no hepatic, renal or hematopoietic toxicity with GQD treatment.

Conclusions: GQDs are a promising in situ anti-biofilm treatment with potential to mitigate the need for removal and replacement of central venous catheters. Future work will be focus on optimized design of GQDs as well as treatment doses and duration to achieve eradication.
Catheter associated urinary tract infections (CAUTI) are a common clinical concern as they can lead to severe, persistent infections or bacteremia in long-term catheterized patients. This type of CAUTI are difficult to eradicate, as they are caused by multi-species biofilms that may be tolerant to various antibiotics. Many new strategies to tackle CAUTI have been proposed in the past decade, including antibiotic combination treatments, surface modification and probiotic usage. However, those strategies were mainly assessed on mono- or dual-species biofilms that could hardly represent the long-term CAUTI cases where, normally, 2-4 or even more species can be involved.

We have developed a four-species in vitro biofilm model on catheter involving clinical strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Proteus mirabilis* isolated from indwelling catheters. Interspecies interactions as well as responses to antibiotics and probiotics were quantitatively assessed. The four species were chromosomally tagged with genes expressing different fluorophores, enabling visualization of the spatial organization and attachment to the catheter material at the individual species level, using confocal microscopy and image analysis. Collaborative interactions as well as competition were found among members in our model biofilm and those interactions affected the individual species abundances upon exposure to antibiotics and probiotics as mono-, dual- or multi-species biofilms.

Our study shows complex interactions between species during assessment of CAUTI control strategies for biofilms and highlights the necessity of evaluating treatment and control regimes in a multi-species setting.
Parallel session 7: Optimizing antimicrobial efficacy to treat biofilm-related infection.

Invited speaker:

**Strategies to potentiate antibiotics and avoid resistance**

Oana Ciofu

Professor. MD, PhD, Dr. med. University of Copenhagen. Department of Immunology and Microbiology (DK)

The interaction between bacterial biofilms and antibiotics is complex and distinct from that of planktonic cultures. Detangling the mechanisms of tolerance and of resistance development in biofilms is a prerequisite for establishing strategies to optimize antimicrobial efficacy to treat biofilm-related infections. The actual knowledge of these mechanisms and an overview of possible ways to potentiate antibiotics and avoid resistance in *P. aeruginosa* biofilms will be presented. The role of conventional resistance mechanisms in bacterial biofilms will be discussed. Results from the studies of antibiotic combinations acting synergistically on biofilms of colistin resistant *P. aeruginosa* from cystic fibrosis (CF) patients and of combinations between antibiotics and bacteriophages against ciprofloxacin resistant *P. aeruginosa* from CF patients will be presented. To avoid development of resistance, the concept of anti-evolutionary drugs will be presented and preliminary results from ciprofloxacin- treated *P. aeruginosa* biofilms will be shown.
S32-Therapeutic strategies based on antagonistic resistance mechanisms between novel β-lactam and carbapenem antibiotics to combat XDR Pseudomonas aeruginosa biofilms

**Maria Fernández-Billón**, Elena Jordana-Lluch, Pamela Jael Colman, Rosa Torrandell, María D. Macià and Antonio Oliver.

Microbiology department and Instituto de Investigación Sanitaria de les Illes Balears (IdISBa). Son Espases Hospital. Palma. Spain.

**Introduction:** Chronic biofilm-related infections by Pseudomonas aeruginosa are almost impossible to eradicate and very difficult to treat. In this context, novel β-lactam–β-lactamase inhibitor combinations, such as ceftolozane/tazobactam (TL/TZ) seemed promising option; however, this antibiotic is not exempt from mutational resistance development. On the positive side, resistance to TL/TZ, mediated by specific mutations in the Ω-loop of AmpC entail susceptibility to carbapenems. On the other hand, the combination of the carbapenem imipenem (IM) with relebactam (RL) appears as an interesting option due to the stability against association of OprD inactivation and AmpC overexpression.

**Hypothesis:** Based on antagonistic antibiotic resistance mechanisms treatment of XDR P. aeruginosa biofilms with the alternation of TL/TZ, that leads to the resistance development but increases susceptibility to IM, followed by IM could avoid the development of TL/TZ resistant mutants maybe being a promising therapeutic strategy on P. aeruginosa biofilms.

**Methods:** Biofilms of XDR clinical strain C2 (only susceptible to TL/TZ and IM/RL) were grown at 30º C using the flow cell system. 48 hours-old biofilms were challenged during 6 days with either TL/TZ, 4/4 (μg/mL) or IM/RL, 2/4 (μg/mL) or the alternation of TL/TZ (2 days) plus IM (2 days) plus TL/TZ (2 days). At the end of the treatment biofilms were detached and collected by washing the channels with a 1 ml glass bead (Sigma) suspension in 0.9% NaCl. Then, serial dilutions were plated in Mueller-Hinton agar (MHA) to determine the numbers of viable cells and in MHA with TL/TZ 4/4 and with IM/RL 2/4 to determine the resistant mutants. Structural dynamics were monitored by confocal laser scanning microscopy (CLSM). Propidium iodide (PI) was used to visualize the bactericidal effect of the different antibiotic regimens. Pictures were processed by IMARIS software package (Bitplane AG, Zurich, Switzerland) and biofilm structural parameters were analysed by the COMSTAT program. At least, three independent triplicate experiments (three-channel per flow cell) per condition were performed.

**Results:** TL/TZ in monotherapy was not able to eradicate biofilms of XDR C2 strain and lead to the selection and amplification of resistant mutants (3 log approx. at day 6). On the contrary, alternation with IMI was able to achieve the eradication of TL/TZ resistant mutants. Overall, treatment with IM/RL during 6 days was the more effective treatment leading to the highest reduction in biofilm viable cells without antibiotic resistant mutant selection.

**Conclusion:** IM/RL was revealed as effective treatment of XDR P. aeruginosa biofilms.
S33-Comparative efficacy of meropenem in extended infusion and intermittent bolus alone and with colistin against *Pseudomonas aeruginosa* biofilm: a pharmacodynamic study

Eva Benavent¹, Heidi H Yu², Cristina El Haj³, Hasini Wickremasinghe⁴, Lynn Wang⁵, Mohammad A K Azad², Raul Rigo-Bonnin³, Jian Li², Oscar Murillo¹

1. Laboratory of Experimental Infection, Infectious Diseases Department, IDIBELL-Hospital Universitari Bellvitge, Barcelona, Spain.
2. Antimicrobial Systems Pharmacology Laboratory at the Biomedicine Discovery Institute, Monash University, Melbourne, Australia
3. Laboratori Clínic, IDIBELL, Hospital Universitari de Bellvitge, L’Hospitalet de Llobregat, Barcelona, Spain.

**Introduction:** Optimized administration of beta-lactams, alone or in combination, may improve efficacy when treating biofilm-related infections (BRI).

**Hypothesis:** As carbapenems seem to possess high anti-biofilm activity among beta-lactams, we aimed to evaluate the efficacy of meropenem administered in extended infusion (EI) and intermittent bolus (IB), alone and with colistin, against biofilms by *P. aeruginosa* (PAER) strains.

**Methodology:** We used a pharmacodynamic model (CDC-Biofilm Reactor) with two PAER strains: PAO1 (meropenem- and colistin-susceptible; MIC=1 and 1mg/L, respectively) and XDR-PAER clinical strain (AmpC ß-lactamase hyperproduction, OprD porin deletion; meropenem MIC=16mg/L, colistin MIC=0.5mg/L). Therapeutic experiments: i) monotherapies of meropenem every 8-hours in IB (free Cmax=90mg/L, t1/2=1h) and EI over 4h (freeCss=25mg/L), and colistin-continuous infusion (Css=3.50mg/L); ii) combinations of meropenem-IB/EI with colistin; and controls. Biofilm-embedded bacteria from coupons were obtained at 0, 6, 24, 30, 48 and 54 hours; decreases in bacterial counts (ΔlogCFU/mL-Xh) were used as criteria of efficacy. Resistance emergence was screened, and main pharmacodynamic indexes were evaluated.

**Results:** For PAO1 strain, meropenem-EI (T>MIC=100%, T>4xMIC=82.5%; ΔlogCFU/mL-54h=-4.66) was more effective than meropenem-IB (T>MIC=80%, T>4xMIC=47.5%; ΔlogCFU/mL-54h=-3.4, pMIC=25%) was ineffective (ΔlogCFU/mL-54h=0.5) whereas meropenem-EI (T>MIC=57.5%) was bactericidal (ΔlogCFU/mL-54h=-3.65; p0.001); the colistin addition, increased the activity for both monotherapies (meropenem-IB-colistin ΔlogCFU/mL-54h=2.35; meropenem-EI-colistin ΔlogCFU/mL-54h=-4.98; p0.001). Colistin-resistant strains were only detected in experiments using PAER-XDR strain treated with meropenem-IB in combination with colistin (MIC= 8-16mg/L).

**Conclusion:** Meropenem administered in EI achieved better PK/PD indexes than IB in both susceptible or resistant PAER strains, this leading to significant higher efficacy either in monotherapy or in combination with colistin. Meropenem-EI recovered bactericidal activity in monotherapy against XDR-PAER, and its combination with colistin improved the efficacy and offered greater protection from colistin-resistance development. Optimizing dosage of meropenem with EI should be encouraged for treating biofilm-related infections.
**S34-Understanding the killing dynamics of levofloxacin treated *Pseudomonas aeruginosa* biofilms.**

**Valentina Lember**(1), **Matthias Preuße**(2), **Mathias Müsken**(1)
(1) Central Facility for Microscopy, Helmholtz Centre for Infection Research, Germany
(2) Department of Molecular Bacteriology, Helmholtz Centre for Infection Research, Germany

Bacteria that are encased in biofilms show significantly enhanced resistance to antimicrobials and therefore represent a major challenge in infectious diseases. Bacterial biofilms play a substantial role in chronic lung infections, especially in cystic fibrosis patients, with the opportunistic pathogen *Pseudomonas aeruginosa* being one of the most predominant colonizers of the lung airways with the likelihood of infection reaching 80% for adult patients in Europe. Despite the increasing threat of biofilm-associated infections, adapted and biofilm-specific therapy is, however, still not applicable in routine diagnostics. In this study, the effect of levofloxacin (LEV) and other fluoroquinolones on the biofilms of *P. aeruginosa* strain PA14 was investigated in an optimized flow cell system by automated time-lapse confocal laser scanning microscopy to generate biofilm-specific killing kinetics. We also utilized scanning electron microscopy (SEM) to investigate the effect of LEV on morphological perturbations in PA14 biofilm structures.

Our results revealed that LEV treatment exhibits concentration-dependent killing of PA14 biofilms. At high concentrations, antibiotic was resulting in approximately 70% of dead bacteria, however, provoked an extensive biofilm regrowth after 16 hours of continuous treatment. Furthermore, other tested quinolones in their concentrations exceeding MICs were also inducing strong regrowth patterns in less than 24 h. The cell-size regulation was significantly aberrated in PA14 biofilm structures that underwent the treatment with LEV. SEM showed a high proportion of filamentous cells with an increased vesicle formation. To understand the molecular mechanism upon LEV treatment, RNA-seq at chosen time points was applied, revealing 5431 differentially expressed genes in treated PA14 biofilms. PA14 R-, F-, and S-type pyocin gene clusters occurred to be highly upregulated, whereas chemotaxis and energy metabolism were significantly impaired. The image data of killing kinetics is planned to be used to complement and optimize a previously established, agent-based biofilm model mimicking antibiotic exposure in silico.
S35-Ceftolozane/tazobactam plus tobramycin against planktonic and biofilm states of hypermutable Pseudomonas aeruginosa isolates from paediatric patients with cystic fibrosis


1 Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia; 2 Biomedicine Discovery Institute, Monash University, Melbourne, Australia; 3 Instituto de Investigación Sanitaria Illes Balears (IdISBa), Palma De Mallorca, Spain; 4 College of Pharmacy, University of Florida, Orlando, United States.

Biofilm-associated Pseudomonas aeruginosa infections in cystic fibrosis (CF) have limited treatment options. We hypothesised that ceftolozane/tazobactam (C/T) plus tobramycin (TOB) combination regimens provide synergistic activity and suppression of resistance emergence for the pharmacokinetics of paediatric patients simulated in a dynamic biofilm model.

Hypermutable paediatric CF isolates FQSE12 (MIC C/T 2mg/L, MIC TOB 4mg/L) and FQSE24 (MIC C/T 2mg/L, MIC TOB 2mg/L) were investigated in 168h dynamic biofilm studies (inoculum ~10^7 CFU/mL and ~10^7 CFU/cm2). The dynamic biofilm model simulated representative C/T and tobramycin pharmacokinetics in lung fluid of paediatric patients with CF based on population pharmacokinetic models and lung fluid penetration. Regimens were: C/T 4.5g/day as continuous IV infusion (penetrationLungFluid=48%); TOB (t1/2,LungFluid=3h, penetrationLungFluid=50%) 10mg/kg 24-hourly as 0.5h intravenous infusions; TOB (t1/2,LungFluid=3h) 300mg 12-hourly inhaled; C/T + intravenous TOB; C/T + inhaled TOB. Total and resistant counts of planktonic and biofilm bacteria were determined. C/T and TOB exposures were confirmed by LC-MS/MS. Bacterial viable counts were mathematically modelled.

All monotherapies of C/T and TOB resulted in amplification of resistance emergence compared to the growth control, although inhaled TOB was more effective than intravenous TOB. Against both isolates, the combination regimens demonstrated synergy and completely suppressed the emergence of C/T and TOB resistant planktonic and biofilm bacterial subpopulations. Mechanism-based modelling incorporating subpopulation and mechanistic synergy well described the antibacterial effects of the combination regimens against both the planktonic and biofilm bacteria. Parameter estimates from the mathematical model suggested longer mean generation times in the biofilm compared to the planktonic state of growth, and a lower maximum killing rate constant of tobramycin against biofilm compared to planktonic bacteria, for both isolates.

Clinically relevant regimens using C/T in combination with intravenous or inhaled TOB were synergistic against hypermutable P. aeruginosa isolates from paediatric patients with CF and warrant further investigation.
S36- Synergistic effects of synthetic nano-engineered antimicrobial-peptide polymers and antibiotics against S. aureus and P. aeruginosa biofilms in in vitro and ex vivo models

Ramón García Maset (a), Alexia Hapeshi (b), John Lapage (c), Niamh Harrington (c), Jenny Littter (c), Sébastien Perrier*(a,b,d), Freya Harrison* (c)

a Warwick Medical School, University of Warwick, Coventry, CV4 7AL, UK
b Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK
c School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK
d Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

Introduction: Biofilm infections are associated with a high mortality risk for patients. Antibiotics perform poorly against biofilm communities, and high doses and prolonged treatments are often used in clinical settings. Antimicrobial peptides (AMPs) have shown promising antimicrobial activity against biofilms, but their cytotoxicity against mammal cells and impermeant in biological conditions (pH, cations, protein-absorption, degradation by proteases) limits their clinical application. Synthetic AMPs have been suggested as a possible alternative to natural AMPs to improve their therapeutic index.

Hypothesis: We hypothesised that synthetic nano-engineered antimicrobial peptide polymers (SNAPPs) could synergise with antibiotics to combat biofilm infections, using clinically relevant models.

Methodology: 2 SNAPPs were synthesised for this study. Their antimicrobial activity alone and in pair-wise combinations with different antimicrobial agents were investigated using a checkerboard assay in different media (cation-adjusted Muller-Hinton broth, synthetic wound fluid and synthetic cystic fibrosis medium). Synergistic pairs were further analysed using statistical models to obtain 3D landscape synergy plots. Promising combinations of SNAPPs and antimicrobial agents were screened against biofilms in a soft tissue collagen wound model, an ex vivo porcine skin wound model and an ex vivo lung cystic fibrosis model. Visually, biofilm disruption was evaluated using scanning electron microscopy, and the penetration of fluorescently labelled SNAPPs was investigated in cross-sections of the porcine skin using confocal microscopy.

Conclusions: One of the SNAPPs selected was synergistic with penicillin and silver sulfadiazine against planktonic S. aureus (MRSA) in synthetic wound fluid. Furthermore, the combination of this SNAPP and silver sulfadiazine showed a potent synergistic antibiofilm activity against MRSA in in vitro and ex vivo wound biofilm models. The second SNAPP selected was synergistic with colistin against planktonic P. aeruginosa in cation-adjusted Muller-Hinton broth and synthetic cystic fibrosis medium. This pair showed a potent synergistic antibiofilm activity against P. aeruginosa in an ex vivo cystic fibrosis lung model.
Parallel session 8: Fungal biofilms in 2022 – how far we come?

Invited speaker:

From the ABCs to the XTTs of fungal biofilm research: two decades of progress

Gordon Ramage

School of Medicine, Dentistry and Nursing, University of Glasgow & Glasgow Biofilm Research Network.

In the early 2000s a handful of researchers acknowledged the importance of fungal biofilms. To date there are over 5000 peer reviewed publications that have contributed to our understanding of this field. This presentation will examine how the evolving work in Candida and Aspergillus biofilms was stimulated through the use of simple XTT metabolic assays to evaluate antifungal drugs efficacy, and discuss the clinical importance of these infections. It will also summarise the key discoveries on the way to understanding the molecular basis of fungal biofilm development, and discuss how our interest in interkingdom biofilm communities have been propelled by the growth of microbiome studies. The overall aim of the presentation is to provide an overview of the field.
Invited speaker:

Antifungal agents for the treatment of biofilms: past, present and future

Jose L Lopez-Ribot

Dept. of Molecular Microbiology and Immunology and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, Texas, US.

Fungal infections represent an increasing threat to a growing number of immune- and medically-compromised patients. These fungal infections are often associated with biofilm formation, which contributes to their ability to resist antifungal treatment, increasing the urgency to find new effective anti-biofilm therapies. Fungi are eukaryotic organisms and, as such, there is a limited number of selective targets which can be exploited for antifungal drug development. This is the major reason for the paucity of current antymycotics, and also constitutes a major hurdle for the development of novel antifungal agents. Moreover, the utility of available antifungals is limited by toxicity, drug interactions and the emergence of resistance, which contribute to high morbidity and mortality rates. This presentation will provide a brief summary on the landscape of current antifungals and those at different stages of clinical development, with emphasis on their biofilm activity. We will also discuss potential new targets and opportunities for the development of novel molecules and strategies to combat the threat of biofilm-associated fungal infections. Development of these novel approaches should have a profound impact on the management of patients suffering from these devastating infections.
S37-Pilocarpine interferes with sphingolipids metabolism in *Candida albicans*

Emerenziana Ottaviano, Michele Dei Cas, Silvia Ancona, Silvia Bianchi, Elisa Borghi

Department of Health Sciences, Università degli Studi di Milano, Milan (Italy)

*Candida albicans* is the most common human fungal pathogen with an estimated crude mortality rate of 40%. The ability of the organism to filament and to produce biofilms are important virulence factors, responsible for tissue invasion and antifungal tolerance, respectively. Pilocarpine hydrochloride (PHCl), a muscarinic receptor agonist, has been shown to inhibit *C. albicans* biofilm formation and in vivo pathogenicity in the *Galleria mellonella* model, but the mechanism underlying this effect is still unknown.

Due to the sphingolipids' (SLs) role in the yeast cell wall integrity signaling, filamentation and virulence, we investigated the possible effect of PHCl on *C. albicans* sphingolipid content.

Lipids from pellets of planktonic and biofilm-organized cells of *C. albicans* strain SC5314, treated or not with PHCl 25 uM, were isolated by extraction with methanol/chloroform mixture coupled to alkaline methanolysis for phospholipids removal. The sphingolipids profile was obtained by liquid chromatography tandem mass spectrometry (LC-MS/MS) using electrospray ionization.

Ceramide content was found reduced in both untreated and PHCl-treated biofilm-organized cells, despite no differences in the dihydroceramide precursor. On the contrary, PHCl reduced Cer concentration in planktonic cells. Phytoceramide (PHC) production was increased by PHCl in both planktonic and sessile cells, whereas the alpha-hydroxylated PHC was enriched only in treated sessile cells. Inositol phosphoryl ceramide (IPC), the precursor of mannosilated IPCs (MIPC) reported to be involved in *Candida* morphogenesis, was significantly higher in biofilms and not affected by PHCl treatment.

Biofilm itself causes severe sphingolipid content remodeling. PHCl seems to exert a modulatory effect on SL pathway in both sessile and planktonic cells. Further studies encompassing other sphingolipids, such as MIPC and M(IP)2C, are necessary to elucidate whether the PHCl-driven reduction in filamentation and biofilm formation could be SL-mediated.
Understanding biofilm heterogeneity and adaptation in *Candida*

**Christopher Delaney** (1), Bryn Short(1), Ranjith Rajendran(1), Dave Bradshaw (2), Craig Williams(3), and Gordon Ramage(1)

1 - School of Medicine, College of Medical, Veterinary and Life Sciences (MVLS), University of Glasgow, UK, 4 Institute of Healthcare Associated Infection, School of Health, Nursing and Midwifery, University of the West of Scotland, UK, 6 Aberdeen Fungal Group, MRC Centre for Medical Mycology, University of Aberdeen, UK.

2 - Microbiology Department, Lancaster Royal Infirmary, University of Lancaster, Lancaster, United Kingdom.

3 - Oral Health Research and Development, GlaxoSmithKline, St Georges Avenue, Weybridge, Surrey, UK.

*Candida* infections in hospitalised patients remains to be a significant cause of morbidity and mortality. Biofilm formation is an important virulence factor and has been shown to be heterogenous in clinical isolates. This biofilm heterogeneity has been demonstrated to be associated with poor clinical outcomes in candidemia patients. Moreover, the biofilm forming phenotype has been shown to be induced or modulated by numerous factors including nutrient, stress and interkingdom interactions. Therefore, we have aimed to investigate the clinical implications and the mechanisms that underpin biofilm heterogeneity.

From a total of 280 clinical isolates, we assessed the biofilm formation by standard biomass assays. Subsequently isolates were categorised as low, intermediate, or high biofilm formers (LBF, IBF and HBF respectively). Antifungal sensitivity assays showed that HBF and LBF isolates were differentially affected. LBF isolates were also shown by standard biomass and microscopic analysis to be highly inducible to biofilm forming from external factors such as serum. Similarly, biofilm formation of HBF and LBF has been shown to be variable with the coculture with pathogenic bacteria. Through a multi-Omic integrative approach we have discerned several important pathways that are modulated between HBF and LBF and functional differences during the induction of the biofilm phenotype. These included amino acid, fatty acid and glycerolipid metabolic pathways, in addition to individual metabolic perturbations in the arachidonic acid cascade.

Collectively our findings provide evidence that the *Candida* biofilm phenotype is highly variable and a clinically important factor. *Candida* biofilm heterogeneity is associated with antifungal resistance and patient outcome. We have also demonstrated several important factors such as nutrient and polymicrobial interactions that are able to modulate the biofilm phenotype through metabolic and transcriptional pathways, further demonstrating the high variability of biofilm formation in *Candida*. 
S39-Mixed biofilm of *Aspergillus fumigatus* and *Stenotrophomonas maltophilia*: microscopic visualization of galactosaminogalactan and galactomannan polysaccharides in the extracellular matrix

Isabel Valsecchi¹, Anne Debourgogne², Thierry Fontaine³, Françoise Botterel¹

1 Dynamyc - EA 7380, Université Paris-Est Créteil-Val de Marne (UPEC), Ecole nationale vétérinaire d’Alfort (EnvA), USC Anses, 94000 Créteil, France
2 Laboratoire Stress Immunité Pathogènes, EA7300, Faculté de Médecine, 9 avenue de la Forêt de Haye, 54505 Vandoeuvre-les-Nancy, France
3 Institut Pasteur, Université de Paris, INRAE, USC2019, Unité Biologie et Pathogénicité Fongiques, F-75015 Paris, France

**Introduction:** *A. fumigatus* (Af) and *S. maltophilia* (Sm) are commonly co-isolated from the airways of cystic fibrosis patients, especially in mixed biofilms. A mixed Af-Sm biofilm model, developed by our lab, demonstrated that Sm exhibits an antibiosis effect on Af by (I) inhibiting fungal growth, (II) rendering the fungal mycelia highly branched and (III) increasing the fungal cell wall thickness (1). The presence of three Af cell wall polysaccharides, galactomannan (GM), galactosaminogalactan (GAG) and α-1,3-glucan, have been described in the extracellular matrix (ECM) of Af biofilms (2). GM and GAG are known to be released out from the cell wall of Af (3). GAG contains N-acetylgalactosamines (GalNAc) which undergo partial deacetylation extracellularly to be converted to GalN, and thus transform this polysaccharide into a polycation with strong surface binding properties (4).

**Aim of the study:** Analyze the structure of fungal cell wall polysaccharides in the formation of Af biofilm alone or mixed with Sm.

**Material and Methods:** The Af-Sm co-culture was prepared on 8-chambers Lab-Tek slide as described previously (1). Anti-GAG and anti-GM monoclonal antibodies produced at the Aspergillus Unit at Pasteur Institute of Paris, were used to focus on 3D structure of GAG and GM in the ECM of the biofilm. These polysaccharides were analyzed by fluorescence confocal microscopy at 24h.

**Results and Conclusion:** GM was found, as expected, in the Af cell wall of the hyphae but was very little in the ECM. GAG was also found in the cell wall but mainly forming a beautiful fibrillary network between the hyphae, showing the importance of this polysaccharide in cell-cell interaction and in the structuration of Af biofilm. GAG could be the surface receptor for Sm, which would promote strong adhesion between Sm and Af in the biofilm.

Parallel session 9: Cystic fibrosis and chronic respiratory infections

Invited speaker:

Mycobacterium biofilms

Jaime Esteban
Dr. Med.Clinical Microbiologist at the Microbiology Department of the Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid (ES)

The genus *Mycobacterium* includes more than 180 species with different phenotypic characteristics. With the notable exception of the *M. tuberculosis* complex and *M. leprae*, the members of the genus are considered environmental microorganisms that can be found in nature forming part of polymicrobial biofilms, usually associated with water environments, and only occasionally cause human diseases. Biofilms can also be formed *in vitro* by some of the pathogenic species (like *M. avium* complex or the non-pigmented rapidly growing mycobacteria), and more recently, the ability to develop biofilms have been described in *M. tuberculosis*, increasing the interest in these field.

Biofilms have been described in clinical samples of some patients with chronic lung disease caused by some species of non-tuberculous mycobacteria (notably *M. abscessus*), and it has been hypothesized that biofilms could have a role in the pathogenesis of tuberculosis. Other infections, like biomaterial-related ones, are also considered as biofilm-related ones, as well as the presence of them in environmental sources that are the origin of outbreaks. More recently it has been demonstrated that mycobacteria can be part of polymicrobial biofilms that includes other human pathogens (like *P. aeruginosa*) that can have an important role in some human diseases.

Mycobacterial biofilms have an extracellular matrix formed by lipids in a higher amount than other microorganisms. Lipid metabolism have been demonstrated to have an important role in biofilm development, including the importance of glycopeptidolipids and other molecules. One clinically relevant property of these structures is the increasing resistance against antimicrobials of sessile organisms. Because some of these microorganisms are constitutively resistant against many antibiotics, this fact increases the importance of biofilms in human pathology. New strategies are needed to treat patients with biofilm-related diseases with good possibilities for cure.
Lungs of cystic fibrosis (CF) patients are prone to chronic Pseudomonas aeruginosa (Pa) infections accompanied with extensive biofilm formation. Women suffering from CF exhibit a significantly lower life expectancy compared to men due to an earlier impairment of lung function triggered by the occurrence of pulmonary exacerbations that correlates with a mucoid switch of Pa and fluctuations in estradiol (E2) blood serum concentrations.

However, further studies are needed to shed light on this sexual dimorphism in CF and the impact of the sex hormone E2 on Pa biofilm development in particular. Therefore, Pa isolates from CF patients were grown in the presence of E2, dissolved in EtOH or DMSO, or water soluble E2 complexed with cyclodextrin, in a microtitre plate based biofilm model. Biofilms have been examined in an experimental procedure allowing simultaneously both the quantification of attached biofilm mass via crystal violet assay and ultrastructural analysis of biofilms via field emission scanning electron microscopy. Additionally, supernatants were collected to monitor quorum sensing (QS) activity using N-acylhomoserine lactones luminescent reporter bacteria.

We observed that E2 modulates the development of biofilms derived from Pa-CF isolates with respect to (i) attached biofilm mass, (ii) ultrastructure of attached biofilm and (iii) QS activity. More than half of the screened Pa-CF isolates showed a significant increase in attached biofilm mass accompanied by ultrastructural changes of biofilms. These E2 induced changes were highly diverse among the isolates regarding overall biofilm structure and remodelling of extracellular polymeric substance. Ongoing studies aim to reveal the underlying mechanisms of E2 on Pa to identify central targets for E2 induced phenotype switches of Pa.

The obtained results may have strong impact on future personalized treatment of female CF patients and beyond, considering both the patients’ sex and hormonal status to improve clinical outcomes of Pa infections.
S41-Bacterial biofilms predominate in both acute and chronic human lung infections

Mette Kolpen (1), Kasper Nørskov Kragh (1,2), Juan Barraza Enciso (3), Daniel Faurholt-Jepsen (4), Birgitte Lindegaard (5), Gertrud Baunbæk Egelund (5), Andreas Vestergaard Jensen (5), Pernille Ravn (6), Inger Hee Mabuza Mathiesen(4), Alexandra Gabriella Gheorghe (7), Frederik Boëtius Hertz (8), Tavs Qvist (4), Marvin Whiteley (3,9,10), Peter Østrup Jensen (1,2) and Thomas Bjarnsholt (1,2)

(1) Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark;
(2) Costerton Biofilm Center, Institute of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.
(3) School of Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, USA.
(4) Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark.
(5) Department of Pulmonary and Infectious Diseases, Nordsjællands University Hospital, Hillerød, Denmark.
(6) Department of Medicine Section for Infectious Diseases, Herlev- Gentofte University Hospital, Hellerup.
(7) Department of Forensic Pathology, Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Background: A basic paradigm of human infection is that acute bacterial disease is caused by fast growing planktonic bacteria while chronic infections are caused by slow-growing, aggregated bacteria; a phenomenon known as a biofilm. For lung infections, this paradigm has been thought to be supported by observations of how bacteria proliferate in well-established growth media in the laboratory - the gold standard of microbiology.

Objective: To investigate the bacterial architecture in sputum from patients with acute and chronic lung infections.

Methods: Advanced imaging technology was used for quantification and direct comparison of infection types on fresh sputum samples thereby directly testing the acute versus chronic paradigm.

Results: In this study we compared the bacterial lifestyle (planktonic or biofilm), growth rate and inflammatory response of bacteria in freshly collected sputum (n=43) from patient groups presenting with acute or chronic lung infections. We found that both acute and chronic lung infections are dominated by biofilms (aggregates of bacteria within an extracellular matrix) although planktonic cells were observed in both sample types. Bacteria grew faster in sputum from acute infections, but these fast-growing bacteria were enriched in biofilms similar to the architecture thought to be reserved for chronic infections. Cellular inflammation in the lungs was also similar across patient groups, but systemic inflammatory markers were only elevated in acute infections.

Conclusions: Our findings indicate that the current paradigm of equating planktonic with acute and biofilm with chronic infection needs to be revisited as the difference lies primarily in metabolic rates, not bacterial architecture.
S42-Use of phages adapted to *Pseudomonas aeruginosa* biofilms to tackle respiratory tract infections in Cystic Fibrosis (CF)

Luciana Meneses 1,2, Diana Priscila Pires 1,2, Sílvio B. Santos 1,2, Tom Coenye 3, Joana Azeredo 1,2

1 CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal
2 LABBELS – Associate Laboratory, 4800-122 Braga/Guimarães, Portugal
3 Laboratory of Pharmaceutical Microbiology, Ghent University, Gent, Belgium

**Introduction**: The use of (bacterio)phages, viruses that selectively kill bacteria, could be a promising approach to tackle multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*. Besides their proven safety and efficacy against planktonic bacteria, phages can penetrate and degrade biofilms, which is essential for the treatment of biofilm-related infections, including respiratory tract infections in cystic fibrosis (CF) patients. However, the complete eradication of biofilms is almost impossible.

**Hypothesis**: Given the natural ability of phages to evolve and counterattack the bacterial defense mechanisms, the aim of the present study was to improve the anti-biofilm activity of phages through adaptative evolution.

**Methodology**: Phage adaptation towards biofilms was performed in 24-well plates during 8-days. For this, 24h-old biofilms formed by a *P. aeruginosa* clinical isolate recovered from a CF patient were treated with phage PE1, a *Pseudomonas* PB1-like phage. After 24h of infection, the phages were recovered from the wells and added to a fresh 24h-biofilm. This procedure was repeated for 8-days and the final biofilm-adapted phages were recovered for phage production and characterization.

**Results**: The phage evolution process resulted in an increased anti-biofilm activity of the adapted phages compared to the wild type (WT) phage. The two adapted phages with the highest anti-biofilm activity revealed an increased efficiency-of-plating against several clinical *P. aeruginosa* strains, as well as against *P. aeruginosa* colonies isolated from untreated biofilm and biofilms treated with WT phages. After genome analysis, two SNPs were identified in genes encoding proteins involved in host recognition and binding (tail-fiber and baseplate).

**Conclusion**: The occurrence of mutations in genes involved in bacterial recognition, together with the increased efficiency-of-plating, indicate that the biofilm evolution process increases phage host range and infectivity. Given the common heterogeneity of biofilms, the enhancement of bacterial recognition may be the reason for the increased anti-biofilm activity of the evolved phages.
S43-Deciphering genetic requirements for *Streptococcus pneumoniae* biofilm formation and maintenance.

Suyen Espinoza Miranda, Federico Rosconi, Tim van Opijnen.

Biology Department, Boston College, Chestnut Hill MA

**Introduction**

*Streptococcus pneumoniae* (*S. pneumoniae*), a colonizer of the upper respiratory tract in humans, can easily migrate and form biofilms in sterile tissues and organs causing acute and chronic infections. Since biofilms are important during different stages of (establishing) an infection it is critical to study bacteria in the context of a biofilm. Biofilm formation and maintenance have been studied in many species, but little is known for the opportunistic pathogen *S. pneumoniae*.

**Methodology/Hypothesis**

Here we develop a method to create a consistent long-term biofilm assay, which for the first time allows for monitoring population dynamics in *S. pneumoniae*'s biofilm. The major advantage of this novel method is that the biofilm can be maintained and reconstituted indefinitely, rather than hours/days (as in previously published assays). As a first step towards characterization, we use confocal microscopy to quantify four distinctive features of biofilm growth and maintenance: biomass, average and maximum thickness as well as roughness coefficient. We validated our assay using *S. pneumoniae* strains previously reported as having high and low biofilm forming index (BFI).

**Results**

Our validation results are consistent with previous reported data. Additionally, our data suggest high heterogeneity in the early stages of biofilm formation and a more controlled growth as the biofilm mature over time. Next, we use transposon mutant libraries in our biofilm model to uncover the genetic requirements for 1) initial bacterial attachment, 2) expansion and biofilm growth, 3) biofilm maintenance and 4) biofilm dispersal for the first time ever in a high throughput manner. We found a group of 15 genes essential for all stages of biofilm development as well as some genes specifically required for each of the stages mentioned above.

**Conclusion**

Our work aims to elucidate genetic requirements for biofilm survival to further establish a targeted treatment in *S. pneumoniae* biofilm-related infections.
S44-Modulation of Pseudomonas aeruginosa biofilm by corticosteroids in Chronic Obstructive Pulmonary Disease context

E. Jordana-Lluch\textsuperscript{1,2}, M. Escobar-Salom\textsuperscript{1,2}, C. Camps-Munar\textsuperscript{1}, C. López-Causapé\textsuperscript{1,2}, A. Iglesias\textsuperscript{3,4}, E. Rojo-Molinero\textsuperscript{1,2}, I.M. Barceló\textsuperscript{1,2}, B. G. Cosio\textsuperscript{3,4}, C. Juan\textsuperscript{1,2}, A. Oliver\textsuperscript{1,2}.

1 Servicio de Microbiología, Hospital Universitari Son Espases-Instituto de Investigación Sanitaria de las Islas Baleares (IdISBa) - Palma (Spain)
2 CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid (Spain)
3 Servicio de Neumología, Hospital Universitari Son Espases-Instituto de Investigación Sanitaria de las Islas Baleares (IdISBa), Palma (Spain)
4 CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III - Madrid (Spain)

Introduction/Hypothesis. Chronic Obstructive Pulmonary Disease (COPD) affects millions of people worldwide, being an economic burden for the health systems. These patients are prone to developing bronchiectasis and are easily colonized by pathogenic bacteria, causing chronic bacterial infections (CBIs) and leading to worse prognosis. Inhaled corticosteroids (ICs) are commonly used to reduce airway inflammation but are associated with an increased risk of Pseudomonas aeruginosa (PA) infection. We hypothesized that ICs could increase biofilm formation in PA, facilitating recurrent CBIs.

Methods. A co-culture model was used to generate a biofilm of seven strains (PAO1 and 6 isolates from COPD patients) onto a monolayer of A549 cells, incubated for 16h and imaged at confocal microscopy (CSLM). Viability of A549 was measured at endpoint with PrestoBlue (Thermofisher). Biofilm formation of PAO1 strain without A549 was also monitored by CSLM at 24h and 48h. All developed biofilms were treated with 1 µM of corticosteroids fluticasone, budesonide or 0.1% DMSO (corticosteroids’ solvent). CSLM images were analysed by COMSTAT to obtain biomass data. Paired one-way ANOVA tests (GraphPad7) were used for statistical analysis.

Results. Co-culture revealed 2 different phenotypes, non-virulent (>60% A549 viability after 16h) and virulent strains (30% A549 viability). All clinical strains showed a significant increase (p<0.05) of biofilm biomass after treatment with one or both corticosteroids in comparison with the DMSO control. Non-virulent strains showed higher biofilm biomass. PAO1 biofilm showed no significant differences in biomass after 16h co-cultured with A549. However, when treated with corticosteroids continuously for 48h (without A549) a significant increase in its biomass was observed.

Conclusion. Our results suggest that corticosteroids might be able to promote biofilm formation. By promoting this chronic trait, ICs could be involved in the persistence of PA infections in COPD patients. Further studies will be carried out to unravel the mechanistic behind the observed phenotypes.
Parallel session 10: Cooperation, competition or quite the opposite: The complex interactions in biofilm communities.

Invited speaker:

**Polymicrobial interactions in cystic fibrosis biofilm**

**Niam Harrington**

University of Liverpool (UK)

The microbial community of the cystic fibrosis (CF) lung is highly complex, including a diverse microbiota and a high incidence of infection with microbial pathogens. Chronic biofilm infections of the CF lung are characterised by extensive antimicrobial resistance and increased mortality rates. Polymicrobial interactions and their impact on disease outcomes in CF are not fully understood. Progress is limited by current laboratory models being unable to reliably recapitulate *in vivo* biofilms or to mimic the true infection environment. We have developed a clinically realistic, high-throughput model for chronic infection using *ex vivo* pig lung (EVPL) bronchiolar tissue. We have demonstrated that the dominate CF pathogen *Pseudomonas aeruginosa* forms a biofilm associated with the EVPL tissue that is morphologically representative of those seen on CF lung biopsies. We have also used RNA sequencing to show that *P. aeruginosa* gene expression is distinct in the EVPL model biofilm compared with *in vitro* growth, with functional importance for phenotypes linked to virulence and antibiotic tolerance comparable to human infection. We have now incorporated species of the *Burkholderia cepacia* complex in the model to investigate the incidence of co-infection with *P. aeruginosa* and potential interactions. We have shown the two species are able to co-exist in the lung model and quorum sensing may be involved in their interactions. Our work also highlights the importance of considering all aspects of human infection when investigating polymicrobial interactions in the lab.
S45-Epithelial Thrombin Modifies Gut Microbiota Biofilms And Fuels Dysbiosis

E. Meunier¹, G. Le Cosquer¹², C. Deraison¹, N. Vergnolle¹ and JP. Motta¹

¹ Institute of Digestive Health Research, IRSD, INSERM U1220, Toulouse, France. 2 Department of Gastroenterology and Pancreatology, Toulouse University Hospital, Toulouse 3 University, France. Corresponding Author: Jean Paul Motta, Institute of Digestive Health Research, IRSD, INSERM U1220, Toulouse, France. Mail: jean-paul.motta@inserm.fr

Introduction and objectives: Thrombin protease (F2) is released at high concentrations at gut mucosal surface and this is implicated in the pathophysiology of Inflammatory Bowel Disease (IBD) (Denadai-Souza 2018 Sc Rep, Motta 2020 J Crohn's colitis). Our study objectives were to determine whether thrombin can directly alter commensal biofilms (in vitro) and increase its pro-inflammatory behavior (microbiota transplantation into germfree mice).

Methods: Tissue-associated microbiota from human colonic biopsies (colon cancer screening, healthy, N=6) were cultured as polymicrobial anaerobic biofilms on a mucin-coated polystyrene Calgary Biofilm Device (methods described in Motta 2018 IBD). Mature biofilms were then exposed to increasing concentrations of active human thrombin (0 to 10 U.mL⁻¹) during 24 hours and total biomass was quantified (safranin-O assay). C57B6 mice were administered intracolonically with vehicle (PBS, N=7) or active thrombin (5U and 20U per animal, N=7 per group) during 10 days, after which tissue-associated microbiota was individually transplanted into adult C57B6 germfree mice (PBS, 5U and 20U, N=7 per group). Germfree mice were sacrificed after 4 days colonization. Macroscopic damage score (erythema, ulcer, edema), colonic wall thickness and bacterial translocation into spleen and mesenteric lymph nodes (blood agar plating) were recorded in thrombin-exposed mice, and transplanted germfree mice.

Results: At concentrations of 10U/ml of thrombin, the total biomass of human polymicrobial biofilms was reduced in all 6 individuals. One individual’s biofilm biomass started to reduce at the lowest concentration of thrombin (0.01 U/ml) while others started to reduce at 1 U/ml. Taxonomic analysis is undergoing in these samples. Animals administered with thrombin were characterized by increased macroscopic damage score, increased colonic wall thickness and increased bacterial translocation. Thrombin-induced effects were more pronounced in animals exposed to 20U compared to 5U. Colonos from transplanted germfree mice were characterized by mild signs of intestinal inflammation (macroscopic damage and colon wall thickness), with a more pronounced and dose-related effect in groups of mice transplanted with thrombin-exposed microbiota.

Conclusions: Our findings suggest that high concentration of thrombin directly alters gut biofilm organization and promotes an aggressive phenotype of microbiota when transplanted into germfree animals. Dysregulation of epithelial thrombin, such as this occurs in IBD, could thus directly contribute to the dysbiosis associated with chronic inflammation in these patients.
Polymicrobial biofilms are common throughout the healthcare setting and have complex interactions with spatial and physical components that are not fully understood. Pseudomonas aeruginosa, has three quorum sensing (QS) systems: two based on the use of N-acylhomoserine lactone signals and the Pseudomonas quinolone signalling (pqS) system based on the use of alkyquinolones (AQS). The pqS system has previously been shown to be impacted by Staphylococcus aureus and Candida albicans.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) has been used to identify QS molecules produced by biofilms but lacks spatial information. The 3D OrbiSIMS has recently been developed to provide label free imaging to visualise exogenous and endogenous metabolites. This instrument combines high spatial resolution of secondary ion mass spectrometry (SIMS) with the high mass-resolving power of an Orbitrap. This has a high spatial resolution of under 200 nm for inorganic species and 2 μm for intact biomolecules. Liquid extraction surface analysis mass spectrometry (LESA-MS) has a 2D spatial resolution of 1 mm and can be performed as either direct (biofilm) or indirect (diffused/secreted molecules). Our aim was to investigate the production of pqS signalling pathway molecules in monospecies and polymicrobial biofilms using 3DOrbiSIMS and LESA-MS.

Using polymicrobial models of C. albicans, S. aureus and P. aeruginosa we first validated the detection of the PQS signal and its precursor molecules by 3DOrbiSIMS and LESA-MS analysis of P. aeruginosa WT and pqS mutants. The results of this study demonstrated that 3DOrbiSIMS and LESA-MS can be used to investigate QS-mediated interactions and metabolites in polymicrobial biofilms. Our findings show that 1) S. aureus and C. albicans significantly modulate pqS QS signalling of P. aeruginosa 2) distribution of these QS molecules can be spatially imaged using these techniques 3) antagonist of the pqS QS regulator PqsR cause abrogation of PQS signal production.
S47-Development of a multi-species biofilm model suggests a potential role of bacterial osmotolerance in cystic fibrosis related infections

Jeanne Trognon¹, Maya Rima¹, Barbora Lajoie¹, Fatima El Garah¹*, Christine Roques¹,²*

¹ Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France
² CHU Toulouse, Hôpital Purpan, Service de bactériologie hygiène, Toulouse, France

Immunocompromised cystic fibrosis (CF) patients often suffer from chronic infections, mainly caused by opportunistic pathogens, such as the Gram-negative Pseudomonas aeruginosa and Burkholderia cepacia or the Gram-positive Staphylococcus aureus, which are able to colonise patients’ lungs and form resistant biofilms. The composition of the airway liquid plays a major role in infection and biofilm formation. In particular, an elevated concentration of NaCl is known to favour the development of osmotolerant bacteria, such as Staphylococcus aureus, and is also able to inhibit the antibacterial defence mechanism of the host, thus predisposing the patient’s lungs to infections. Here, we report the development of a multi-species in vitro model, based on P. aeruginosa, S. aureus and B. cepacia. First, a low-nutritive culture medium able to promote the biofilm growth was optimised by combining adherent CFU counts and qPCR (S. aureus ATCC 33591 and P. aeruginosa PAO1 CIP 104116) with confocal observations. The effect of modifications of the microenvironment, in particular hyperosmotic conditions encountered in CF patients’ lungs, on the biofilm settlement of both laboratory and clinical strains was also studied. This confirmed the difficulty that S. aureus ATCC 33591r encounters in growing in vitro in presence of PAO1 in 1:1 mixed biofilms when cultivated in minimal medium, and showed that an increase in S. aureus inoculum restored the corresponding adhered population. More interestingly, an elevated NaCl concentration (145 mM) not only favoured the co-existence of S. aureus ATCC 33591 and P. aeruginosa PAO1 and, ultimately, of these two bacteria with B. cepacia ATCC 25416r but dramatically changed the balance between the three strains, S. aureus becoming the dominant one. Furthermore, an NaCl positive effect was observed in a mucoid P. aeruginosa and, to a lesser extent, in two S. aureus clinical strains (MSSA and MRSA) from CF patients. This highlights the importance of the microenvironment during the colonisation/infection process. The drastic change in biofilm population among the three-species community in the presence of NaCl proves that hyperosmotic conditions are one of the key factors for better exploration and understanding of the genesis and behaviour of CF multi-species biofilms.
Biofilms are multicellular microbial communities, implicated in chronic wound infections, where they fuel the persistent, non-healing wound state. In clinical wounds, biofilms are seen as discrete, microbial aggregates distributed across, and in close association with, wound bed, including host cells, as well as matrix, biochemical and nutrient factors. Further, biofilms in wounds are most often polymicrobial; *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common bacterial pathogens.

While in vivo and ex vivo studies, and clinical specimen microscopy, have provided relevant insights into wound biofilms, such as bacterial aggregate dimensions and distribution, they are limited in their ability to provide a detailed characterization of biofilm structure and organization across the host-microbial interface. Recognizing this, there has been a push towards developing engineered in vitro approaches that recapitulate key features of the wound infection state, and enable the study of wound biofilms in the context of the complex microenvironment.

To study the structure and organization of mixed-species biofilms under conditions that mimic the infection state, we have built a four-dimensional (4-D) wound microenvironment consisting of a reconstructed host cell surface and an in vitro wound milieu (IVWM). Using confocal fluorescence microscopy and quantitative image analysis, we leveraged this reconstructed platform to characterize the structure and organization of mixed-species *P. aeruginosa* and *S. aureus* biofilms in wound-relevant conditions. In doing so, our platform not only provide insights into the features of mixed-species *P. aeruginosa* and *S. aureus* biofilms, but also enables the understanding of the role of interspecies interactions and host cell substrates in the development of mixed-species biofilm structure and organization. Our findings provide multi-level insights into mixed-species *P. aeruginosa* and *S. aureus* biofilm formation and features under wound-relevant conditions, which we incorporate to present a fine-tuned model of biofilm structure and organization in the wound bed.
S49-Methicillin-Resistant Staphylococcus aureus is Protective against Infection with Cystic Fibrosis-Adapted Pseudomonas aeruginosa in the CF-like Airway of Scnn1b Transgenic Mice

Kristen J. Brao (1), Casey Hofstaedter (1), Rachel Fanaroff (2), Brendan P. Wille (1), Peter Kim (1), Amanda M. Fischer (1), Robert K. Ernst (1), Janette M. Harro (1)

1 Department of Microbial Pathogenesis, School of Dentistry, University of Maryland-Baltimore, Baltimore, Maryland 21201
2 Department of Pathology, University of Maryland Medical Center, Baltimore Maryland 21201

Patients with cystic fibrosis (CF) develop thick mucus in their lungs that predispose them to recurrent and chronic infections. Young patients tend to be infected with Staphylococcus aureus (SA); however, as patients age, Pseudomonas aeruginosa (PA) predominates the airways causing morbidity and mortality. Patients are often co-infected with both pathogens, permitting the species to interact, either directly or via the immune system.

To determine the effects of SA on the ability of PA to infect the CF lung, we intranasally infected Scnn1b-Tg BALB/c mice with both organisms simultaneously. Scnn1b-Tg mice overexpress epithelial sodium channels in the lungs, resulting in dehydrated mucus, reduced mucociliary clearance, and neutrophilic inflammation recapitulating CF lung pathology. We previously established this strain as a model of CF-associated PA infection. When Scnn1b-Tg mice were co-infected with a USA300 methicillin-resistant SA (MRSA) and an early PA CF isolate (three-month-old patient), we found a positive correlation between the number of MRSA and PA in the lungs on day three post-infection. In contrast, when Scnn1b-Tg mice were co-infected with MRSA and a mucoid PA isolate (18-month-old patient), the burden of this PA isolate was reduced (2.8x10^8 vs 1.4x10^5 CFU/g lung tissue, p<0.001). These findings were replicated using a minimally passaged MRSA CF isolate. In contrast to the in vivo results, the early CF PA isolate inhibited the growth of MRSA in vitro, whereas the adapted PA isolate did not. Histological analysis found the adapted PA isolate alone increased neutrophilic infiltration into the bronchioles, with minimal involvement of the alveolar spaces, whereas coinfection with both MRSA and the adapted PA isolate was associated with less neutrophilic infiltration and more macrophage activity.

We have demonstrated that MRSA can play complex roles in the CF lung, potentially both aiding PA in initially colonizing the lung and inhibiting the pathogenicity of adapted PA isolates.
Poster session September 1st

P04- A marine *Rhodococcus* with anti-biofilm activity against *Pseudomonas aeruginosa*

**Alison Acosta**, Valentina González, Leonardo Zamora, Andrés Cumsille, Francisco Salvà-Sierra, Beatriz Cámara.

Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Valparaíso 2340000, Chile

Biofilms are an organized microbial ecosystem composed by either one or several species of microorganisms associated to a living or inert surface. These bacterial biofilms confer various benefits to associated microorganisms. For example, due to increased cell density during biofilm formation, an optimal environment for horizontal gene transfer can lead to acquiring resistance to antibiotics. Consequently, new therapeutic approaches to treat bacterial infections, where biofilms are eminent, are required. Chile is a country that has a very diverse geography and an extensive coast, of which marine ecosystems represent an important yet less explored source of natural bioactive compounds on the planet. *Actinomycetota* are a phylum of Gram-positive bacteria recognized for their ability to produce secondary metabolites, particularly antibiotics. Therefore, the hypothesis of our research work is A marine strain of *Actinomycetota* isolated from the coast of Chile produces metabolite(s) that can inhibit the formation of biofilm in *Pseudomonas aeruginosa*.

In this work, we began with the search of strains with antibiofilm activity by screening a strain collection of marine *Actinomycetota* from our laboratory. Bacterial growth was assessed in co-cultures between selected *Rhodococcus* strains and the model strain *Pseudomonas aeruginosa*. Due to interesting inhibition activities, three *Rhodococcus* strains, isolated from different coastal regions of Chile, were further selected. Secondary metabolites were assessed by crude extracts obtained from *Rhodococcus* cultures and tested against *Pseudomonas aeruginosa* cultures forming biofilm. One strain achieved a 60% inhibition of biofilm formation against *Pseudomonas aeruginosa*. Crude extracts obtained after 1 day of *Rhodococcus* culture presented a minimum inhibitory biofilm concentration of 0.06 mg/mL. This study suggests that a *Rhodococcus* strain isolated from the coast of Valparaíso, Chile produced antibiofilm metabolite(s) against *Pseudomonas aeruginosa*. The *Actinomycetota* Phylum represents an important resource of biofilm inhibitors.
Synthetic communities provide a proxy to reveal the ecological interaction between microbes, including metabolic cross feeding and inter-species signals that influence growth and differentiation. Among these laboratory communities, biofilm retains a spatially organised niche. Using bioinformatic, genetic, transcriptomic, and metabolomic analyses, we could recently uncover syntrophic cooperation between *Bacillus velezensis* and *Pseudomonas stutzeri* in biofilms and in their natural niche (Sun et al 2021 ISME J). The synergistic interaction of these two species is highly environmental dependent, the emergence of syntrophic cooperation was only evident in a static nutrient-rich niche, such as pellicle biofilms. Capitalizing on our understanding of the ecology in this synthetic community, we now also demonstrate that the presence of *P. stutzeri* alters the evolutionary diversification and fitness of evolving *B. velezensis* biofilm populations. Specifically, compared with the single species biofilm evolutionary path of *B. velezensis*, enhancement of *Bacillus* productivity in time is suppressed when evolving with a partner. In addition, complex interaction between Bacilli and Pseudomonads during biofilm development is also highlighted by our screening effort that tested 720 *Pseudomonas* isolates using high content imaging of mixed species biofilms. This screen revealed a set of *Pseudomonas* species inhibiting biofilms of Bacilli, while other species in this genus were able to promote biofilm formation and specifically expression of biofilm-related genes in *Bacillus subtilis*. 

P07-From competition to cooperation in multispecies biofilms of Bacillus and Pseudomonas

Xinli Sun [1], Marg Lyng [1], Zhihui Xu [2], Akos T Kovacs [1]

[1] Technical University of Denmark, Denmark
[2] Nanjing Agricultural University, China
P09- Mushroom-shaped structures formed in Acinetobacter baumannii biofilms grown in a roller bioreactor are associated with quorum sensing dependent Csu-pilus assembly

Manuel Romero¹, Celia Mayer¹,², Stephan Heeb², Krittanont Wattanavaekin³, Miguel Cámara¹, Ana Otero⁴, Paul Williams¹

¹National Biofilms Innovation Centre, Biodiscovery Institute and School of Life Sciences, University of Nottingham, Nottingham, United Kingdom.
²Instituto de Investigacion Sanitaria de Santiago de Compostela (IDIS), Santiago de Compostela, Spain.
³Sakaeo Crown Prince Hospital, Department of Internal Medicine, Sa Kaeo, Thailand.
⁴Departamento de Microbiología e Parasitoloxía, Facultade de Bioloxía, Edificio CIBUS, Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

There is currently a need to develop simple biofilm models that facilitate investigation of the architecture/biology of mature bacterial biofilms in a consistent/standardised manner given their environmental and clinical importance and the need for new anti-biofilm interventions. This study introduces a novel biofilm culture system termed the rolling biofilm bioreactor (RBB). This easily operated system allows adherent microbial cells to be repeatedly exposed to air/solid/liquid interfaces optimising biofilm growth. The RBB was exploited to investigate biofilm formation in Acinetobacter baumannii. High-levels of A. baumannii biofilm biomass reproducibly accumulate in the RBB and, importantly, undergo a maturation step to form large mushroom-shaped structures that had not been observed in other models. Based on image analysis of biofilm development and genetic manipulation, we show how N-acylhomoserine lactone-dependent quorum-sensing (QS) impacts on biofilm differentiation, composition, and antibiotic tolerance. Our results indicate that extracellular DNA (eDNA) is a key matrix component in mature Acinetobacter biofilms as the mushroom-like structures consist of dense cellular masses encased in an eDNA mesh. Moreover, this study reveals the contribution of QS to A. baumannii biofilm differentiation through Csu pilus assembly regulation. Understanding the mechanisms of structural development of mature biofilms helps to identify new biofilm eradication and removal strategies.
Approximately 60-80% of nosocomial infections are caused by biofilms, and a majority are caused by biofilm formation on medical devices. Three major sites of invasive device associated biofilm infections are vascular access graft infections (VGIs), catheter associated urinary tract infections (CAUTIs) and ventilator associated pneumonia (VAP).

To improve understanding of biofilm formation on medical devices, and assess anti-biofilm strategies, there is a need for clinically relevant models that accurately mimic real-world infection. Here, we used a modified Drip Flow Biofilm Reactor® to create clinically relevant in vitro models of biofilm formation in a CAUTI, VGI and VAP.

Biofilms were grown in and on the surface of catheters (CAUTI), endotracheal tubes (VAP) and vascular access grafts (VGI) in a modified Drip Flow Biofilm Reactor® to mimic the flow dynamics in and around the medical device when implanted. To simulate the real-world conditions for biofilm formation, we used physiologically relevant media within these models including; artificial urine medium (CAUTI), artificial saliva medium (VAP) and Plasma-like medium (VGI). The length of the experiment was tailored to match the course of the infections and analysis of the biofilms was performed via scanning electron microscopy. Staphylococcus aureus produced more developed biofilms in plasma-like medium than in Mueller-Hinton broth (MHB) in the VGI model, as assessed by SEM, whereas Acinetobacter baumannii produced smaller biofilms in artificial urine than in MHB in the CAUTI. For A. baumannii, the biofilms in the artificial urine were well structured, despite being smaller than those formed in MHB.

The use of infection site specific models, physiologically relevant media, and accurate flow dynamics is important to fully understand biofilm formation on medical devices and produced in vitro structures which resemble those in real-life. These models can be employed for the testing of anti-biofilm strategies such as development of antimicrobial medical device materials.
P11- Compositional differences in the tongue microbiota in hypertensive pregnancy disorders and nitrate biology in oral biofilm microcosms

Thomas Willmott, Andrew J McBain, Gavin Humphreys, Jenny Myers and Elizabeth Cottrell

All authors are affiliated with The University of Manchester

Background and Objectives. Chronic hypertension during pregnancy is associated with an increased risk of adverse pregnancy outcomes. Nitrate, found in green leafy vegetables, has emerged as a possible intervention for cardiovascular complications. Reduction of dietary nitrate to nitrite, in particular by commensal oral bacteria in sessile biofilms on the dorsal surface of the tongue, can increase nitric oxide (NO) within the vasculature, reducing systemic blood pressure (BP). To understand whether oral nitrate metabolism plays a role in the regulation of BP in pregnancy and whether targeting this pathway may have therapeutic potential, our studies aimed to 1) investigate nitrate reductase (NaR) activity and microbiota composition in normotensive versus hypertensive women and 2) determine using an in vitro biofilm model whether nitrate supplementation can alter the oral microbiota-derived microcosms to favour nitrate-reducing species.

Methods and Results. Plasma and salivary samples were collected in fasted pregnant and non-pregnant participants, with or without hypertension (n=55). Oral NaR activity and salivary nitrite were determined using the Griess reaction. Representative oral biofilm microcosms (n=3) were established in a continuous culture flow-through system. Hypertensive individuals demonstrated significantly lower levels of salivary nitrite (P=0.0060) and a trend towards a reduction in salivary NaR activity (P=0.0991). Abundances of the nitrate-reducing taxa Veillonella and Neisseria, were significantly lower in hypertensive participants (P<0.05). In vitro, an acute challenge with 15mM inorganic nitrate increased NaR activity 2-fold, combined with elevated nitrite.

Conclusions. These data indicate an association between BP status and oral NaR activity, confirming previous findings in non-pregnant adults. In vitro model data demonstrate the feasibility of modelling oral microcosms and suggest that NaR activity can be modulated with an acute inorganic nitrate challenge. Modulation of the nitrate-nitrite-NO pathway may be a feasible intervention in the future for the management of hypertensive pregnancies.
P12- Discovery and investigation of the C13 legumain-like protease in Acinetobacter baumannii

Bronagh Elmore, Dr James Burrows, Professor Brendan Gilmore.

Queen’s University Belfast.

Acinetobacter baumannii is a gram-negative bacterium whose nosocomial transmission rates and combined ability for antimicrobial resistance has made it a pathogen of high interest in recent years. A recent inhibitor screen showed that several cysteine protease inhibitors, particularly a legumain-specific inhibitor, were effective in reducing A. baumannii biofilm formation. This was unexpected as the C13 protease family, of which legumain is the main member, have been poorly characterised outside of plants, humans, and helminths where they are generally found in acidic vacuoles. Indeed, many bacteria do not even possess these proteases. However, a MEROPS database screen confirmed the presence of a single C13 family member in A. baumannii leading us to investigate if this was the target of the inhibitor. To investigate this legumain-like protein (LLP), we cloned its C13 protease domain into an expression construct for Escherichia coli and examined its activity against a legumain-specific substrate. Upon confirmation of legumain-like enzyme activity, cysteine inhibitors used in the previous screen for A. baumannii biofilm reduction were tested, and reduced the enzyme activity observed.

Further studies are currently underway to investigate the potential contribution of this C13 protease to A. baumannii’s antibiotic resistance in both planktonic and sessile cell forms.
P13- Structural investigation of the *Candida albicans/Staphylococcus aureus* dual biofilm by Mesolens 3D imaging

**Katherine Baxter; Gail McConnell; Paul A. Hoskisson**

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow

Co-infections of *Staphylococcus aureus* and *Candida albicans* are a cause of serious healthcare associated infections resulting in poor patient outcomes. Previous studies of *S. aureus/C. albicans* dual species biofilm have shown greater recalcitrance to antimicrobial treatments than either species alone, however the contribution of global biofilm structure and topology to this increased antimicrobial resistance is not fully understood. As a functional structure, a biofilm must contain architecture to provide suitable physiological conditions to allow the cells within to grow and survive. These may be macrostructures which cannot be visualised unless a large region of biofilm is studied.

Using the Mesolens, a next generation 3D imaging system which combines high numerical aperture with low magnification providing a wide field of view with subcellular resolution, we are investigating alterations in these intrinsic structures in *S. aureus/C. albicans* dual species biofilms. Visualising biofilm development from early to mature biofilm on a variety of growth substrates, we are developing our understanding of how global biofilm architecture is modulated in response to growth conditions and substrates. These data are informing on how the intrinsic heterogeneity of biofilms can promote virulence and AMR.

Preliminary results using an abiotic/biotic surface interface model with single species biofilms show greater biofilm mass on the interface model compared to the abiotic surface alone, suggesting adherence to abiotic surfaces may be more successful in the presence of a biotic component. Time course data reveals the overall structure of biofilms on agar, with a central *C. albicans* core peppered with *S. aureus* microcolonies, surrounded by a halo of *S. aureus*. Established early in biofilm growth, this structure will be used as a baseline as we explore the impact of growth conditions such as pH, and strain ratios in further study.
P14- Antibiofilm activity of biogenic silver nanoparticles against bacteria and yeast

Anna Miškovská, Alena Čejková, Olga Maťátková

University of Chemistry and Technology Prague

In recent years, medicine has faced problems related to biofilm-associated infections. Silver nanoparticles are known for their antimicrobial and antibiofilm activity against different pathogens and have already been proposed as antimicrobial agents in medical applications such as implants coatings or part of wound dressings. Metal nanoparticles can be prepared using physical, chemical, or biological methods. Biological approaches, unlike other mentioned ones, are usually eco-friendly, easy to implement, and inexpensive. The use of plant extracts for nanoparticle synthesis, which leads to the formation of highly stable and biocompatible nanoparticles, is particularly attractive. Biogenic nanoparticles could potentially replace chemically synthesized nanoparticles in many applications. This study deals with the biological synthesis of silver nanoparticles using grape vine extract and the subsequent determination of their antibiofilm activity against opportunistic pathogens. Nanoparticles of two different size distributions were prepared, and characterized using UV-vis, TEM, SEM, and DLS. Furthermore, the ability of nanoparticles to prevent the biofilm formation of pathogenic microorganisms was studied using a resazurin assay. Results presented in this study show that prepared nanoparticles were able to inhibit the growth of Candida sp. and Bacillus sp. biofilms, suggesting that biogenic silver nanoparticles have potential for the use in medical devices to avoid microbial colonization and biofilm formation.
Streptococcus mutans is one of the bacteria that initiates the colonization of the pellicle at the tooth surface. It forms a plaque, together with other bacteria, which gradually dissolves the pellicle and leaves the tooth surface unprotected against the acidic oral environment. Calcium phosphate ceramics are excellent synthetic materials for the study of biofilm formation in dentistry because they have a chemical composition and structure similar to that of teeth. Calcium phosphates can be processed to achieve a variety of crystalline compounds with biologically relevant ionic substitutions and structures that allow study of the effect of the surface chemistry and the topography independently. In our project, we prepared and characterized three types of calcium phosphate-based materials as a suitable surface for the formation of the $S. \text{mutans}$ biofilm: beta-tricalcium phosphate ($\beta$-TCP); sintered hydroxyapatite (SHA); and calcium-deficient hydroxyapatite (CDHA). The crystalline phase composition of the materials was determined by X-ray diffraction and compared with the Inorganic Crystal Structure Database (ICSD) for phase identification. The microstructure of the samples was observed using scanning electron microscopy (SEM). Moreover, the specific surface area (SSA) and porosity were determined. The biofilm formed on each material was quantified and morphologically and metabolically characterized by confocal laser scanning microscopy, SEM, CFU/ml counting and AlamarBlue assay. The densest biofilms were formed on the surfaces of SHA and CDHA, with no significant differences due to the stoichiometry or microstructure. In contrast, $\beta$-TCP showed a lower susceptibility to $S. \text{mutans}$ biofilm formation, suggesting that the crystalline structure is the controlling parameter. Subsequently, SHA was selected to develop a dental biofilm model that allowed study of $S. \text{mutans}$ biofilm susceptibility to chlorhexidine and ethanol.
**P17-Effects of stabilized HOCl on oral biofilms**

**Olivia Aherne** (1, 2), Roberto Ortiz (2), Magnus M. Fazli (3, 4) & Julia R. Davies (1)*

1. Section for Oral Biology and Pathology, Faculty of Odontology and Biofilms Research Center for Biointerfaces, Malmö University, Malmö S-20506, Sweden
2. CR Competence, Naturvetarvägen 14, 223 62 Lund, Sweden
3. Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
4. SoftOx Solutions AS, Oslo, Norway

Concerns regarding poor efficacy and biofilm penetration of current therapies in management of oral diseases have exacerbated the need for new anti-plaque chemical agents. Stabilized hypochlorous acid (HOCl) has shown efficacy as an anti-biofilm agent for treatment of acute and chronic wounds (Herruzo & Herruzo 2020, Burian et al., 2021) but its suitability for use in the oral cavity has not been well studied. Our aim was to investigate the effect of HOCl stabilized with acetic acid (HAc) buffer on oral biofilms and compare with that of chlorhexidine gluconate (CHX).

Multi-species biofilms representing supra-gingival (*Streptococcus mutans, Actinomyces odontolyticus, Veillonella parvula*) and sub-gingival (*Streptococcus gordonii, Parvimonas micra, Porphyromonas gingivalis*) communities were subjected to short-term exposure (5 minutes) with stabilized HOCl, pH 4.6 or CHX. To examine the bactericidal spectrum of HOCl and the effect of HAc-stabilization on HOCl efficacy, single-species biofilms of bacteria from the communities were also subjected to HOCl treatment with/without HAc buffer. Bacterial viability was determined using Baclight LIVE/DEAD staining and confocal microscopy.

A significant reduction in viability was seen in both supra- and sub-gingival biofilms on exposure to low concentrations (5 ppm) of stabilized HOCl whereas no equivalent effect was seen for CHX. In single-species biofilms, a similar HOCl susceptibility pattern was seen for almost all species, regardless of HAc concentration, suggesting that HAc buffer had no additive effect on HOCl bactericidal efficacy under the conditions investigated. No decrease in viability was detected in biofilms treated with HAc alone, possibly due to the exposure time and/or an elevated acid tolerance in the oral strains investigated here.

These findings show that even at low concentrations, HOCl stabilized with HAc, pH 4.6 has a bactericidal effect on oral biofilms and may thus offer potential as a candidate for treatment of biofilm-induced diseases in the oral cavity.
P18- Sorbed host proteins mediate neutrophil adhesion, motility, and discovery and clearance of Staphylococcus aureus on an abiotic surface

Brian A. Pettygrove,1,2 Timothy R. Borgogna,1,2 Jovanka M. Voyich,2 Philip S. Stewart1,3

1 Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
2 Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
3 Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT, USA

Video microscopy was applied to directly image the in vitro contest between bacteria (GFP-tagged S. aureus) attached to an abiotic surface (surrogate biomaterial) and human neutrophils. This approach allowed neutrophil functions including adherence, motility (random, chemotactic, and swarming), and bacterial discovery and killing to be visualized and quantified. In normal human serum, bacteria were readily cleared if neutrophils discovered the bacterial aggregate before it grew too large. When heat inactivated serum was used in place of normal human serum, neutrophil surface adherence, motility, and discovery were abolished, resulting in bacterial persistence. Whereas control wells coated with normal human serum enabled a 2.14 ± 0.82 log reduction in viable bacteria, only a 0.08 ± 0.12 log reduction was measured in wells coated with heat inactivated serum. This strongly suggests that heat labile proteins such as fibrinogen and complement are required for effective neutrophil function on biomaterials. Inhibition of the neutrophil adhesin MAC-1, a receptor that recognizes ligands include fibrinogen and certain complement proteins, reduced motility and efficacy (neutrophil track length 651 ± 113 µm in untreated wells vs 298 ± 33 µm in NIF treated wells), leading to delayed discovery and persistence of bacteria. Attachment and subsequent neutrophil motility were impaired in complement protein C3-depleted serum but not cobra venom factor depleted serum, suggesting that attachment to a serum coated surface is mediated by the complement C3 degradation product iC3b. Consistent with previous reports, adsorbed fibrinogen restored adhesion and motility, but a defect in S. aureus discovery persisted. In some instances, rapid, aggressive neutrophil chemotaxis was observed that resulted in fragmentation and phagocytosis of even relatively large bacterial aggregates. Together, these results highlight a critical role of sorbed host proteins in mediating effective neutrophil function on an implant surface and point to possible immunotherapeutic approaches for preventing medical device infection.
P20- Potential Pathogens in Shoulder Surgery

Bay, L.¹; Fritz, B.¹; Fatima, N.¹; Tranchant, E.E.¹; Christensen, M.H¹; Jensen, M.L.²; Juul, I.M.T.²; Olsen, B.S.¹; Hansen, A.J.³; Sørensen, A.K.B.¹; Bjarnsholt, T.¹,4.

¹Costerton Biofilm Center, Department of Immunology and Microbiology, Faculty of Health Science, University of Copenhagen, Denmark. ²Department of Joint and Bone Surgery, Herlev-Gentofte Hospital, Denmark. ³Center of Geogenetic, Globe Institute, Faculty of Health Science, University of Copenhagen, Denmark. ⁴Department of Clinical Microbiology, Rigshospitalet, Denmark.

Post-operative infections occur frequently in shoulder revision operations, with Cutibacterium acnes being the most prevalent pathogen involved. Microbial composition and distribution in incision skin and deeper tissues (bone, muscle, fascia, subcutis and synovium) was determined using biopsies from 40 primary and 18 revision alloplastic shoulder patients that were analyzed by cultivation, MALDI-TOF, whole genome sequencing, 16S rDNA amplicon sequencing, and confocal laser scanning microscopy.

Large bacterial aggregates were detected within hair follicles of the incision skin, while only a few, small aggregates were visualized in the deeper tissues. Viable bacteria up to 10⁷ CFU/biopsy was found in the surgical incision of 74% of patients. In 89% of revision patients, bacteria were cultured from the deep tissues. Among the most prevalent species were S. epidermidis, S. hominis, and C. acnes, with particularly the latter in high abundance in certain patients. Metagenomics identified a reduced microbiome diversity in revision patients that also demonstrated significantly less S. epidermidis, Corynebacterium spp. and Lactobacillus spp. relative to primary patients. More than 50% of the bacterial species in the deeper tissues were also found in the incision skin. This included specific C. acnes strains, suggesting an origin from the patients’ skin microbiota.

Overall, shoulder incision skin contained surprisingly high amount of bacteria, which may penetrate into deeper tissues during surgery and potentially cause infections.
P21- Design, synthesis and antimicrobial evaluation of novel quaternary ammonium salts against *Staphylococcus aureus* in biofilm and planktonic form

Aneta Markova\textsuperscript{a,b,*}, Michaela Hympanova\textsuperscript{a,b}, Lukas Prchal a, Lenka Pulkrabkova\textsuperscript{a,b}, Jan Marek\textsuperscript{a,b}

a Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
b Department of Toxicology and Military Pharmacy, CDepartment of Epidemiology, Faculty of Military Health Sciences, University of Defence in Brno, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic
*email: aneta.markova@fnhk.cz

**Introduction:** Quaternary ammonium salts (QACs) are widely spread cationic surfactants applicable in various industrial branches. QACs manifesting antibacterial effect contain hydrophilic core and a long alkyl chain in the structure. These compounds have a strong antimicrobial activity against certain bacteria strains especially non-spore forming, yeasts or filamentous fungi. *S. aureus* is a frequent cause of nosocomial infections and play an important role in the persistence of chronic infections, especially when it’s attached medical implants and host tissue, and developed in mature biofilm. Either way this pathogen represents a significant burden on the healthcare system and new antimicrobial compounds are always required in clinical practice.

**Hypothesis:** Our main research activity involves design, synthesis and antimicrobial evaluation of novel compounds based on QACs. The main goal is the evaluation of their eradication ability against pathogenic *S. aureus* in biofilm and free-floating form. Furthermore, the dependence of the antimicrobial effect on the structure of substances is monitored, as well as the difference in the resistance of both bacterial forms.

**Methodology:** All newly prepared compounds were prepared by nucleophilic reaction, verified by Nuclear Magnetic Resonance (NMR) and High-Resolution Mass Spectrometry (HR-MS). The antimicrobial properties were evaluated by microdilution broth assay and by Calgary device-based assay with MIC, MBC and MBEC as outcomes.

**Results and Conclusion:** The series of novel QACs was identified as promising active ingredients for biological decontamination of planktonic and more resistant biofilm form.
In recent years the systemic effects that oral health can have been recognised in the context of a myriad of conditions. This same connection has recently been made for chronic obstructive pulmonary disease (COPD), which may come as unsurprising considering the anatomical connection between the oral cavity and the lungs. With recent studies showing periodontal therapy reduces risk of acute exacerbations of COPD (AECOPD), the need to better understand the oropharyngeal microbiome is becoming more apparent. Therefore, using a biofilm model containing organisms found in the oral cavity and in the COPD lung was developed and characterised. Organisms were informed through cross-referencing organisms commonly found in both the oral cavity and the lungs of COPD patients. *Candida albicans*, *Staphylococcus aureus* and *Porphyromonas gingivalis* were grown as a multi-species consortium which was characterised by testing biofilm formation, antimicrobial susceptibility profiles and immunogenicity. Scanning electron microscopy and labelling biofilms with microbe-specific fluorescent probes allowed for easy identification of bacteria and fungi whilst revealing inter-kingdom biofilms possess a more complex structure compared to single species biofilms. This augmented biofilm complexity provided increased tolerance to antimicrobials such as erythromycin, amoxicillin and voriconazole. Immunofluorescence confirmed multi-species biofilms illicit a 2-fold increase in IL-8 production in epithelial cells. This inflammatory output was exacerbated following exposure to antimicrobials. This study addresses a large gap in the current literature as at present there are no multi-species biofilm models that can be applied to study COPD. Combining the ineffectiveness of antimicrobial therapies with the subsequent increase in cytokine production explains reasoning behind why antimicrobials show limited effectiveness in COPD therapy. The opportunity for repeat infection therefore remains. Therefore, maintaining good oral health can act as a prophylactic measure to prevent the migration of potentially pathogenic organisms to the lungs which could grow as biofilms, causing complications in COPD.
P23- Proteomic response in *Streptococcus gordonii* DL1 biofilm cells during attachment to salivary MUC5B

Carolina Robertsson (a), Gunnel Svensäter (a), Zoltan Blum (b), Magnus E Jakobsson (c) and Claes Wickström (a)

(a) Department of Oral Biology and Pathology, Faculty of Odontology, Malmö University, Malmö, Sweden
(b) Department of Biomedical Science, Faculty of Health and Society, Malmö University, Malmö, Sweden
(c) Department of Immunotechnology, Lund University, Lund, Sweden

**Introduction:** The salivary mucin MUC5B seems to induce synergistic ‘mucolytic’ activities in oral bacteria, and thereby support diversity in dental biofilms and promote oral health. Knowledge of how host salivary proteins may regulate the activity of early oral colonizers is integral to better understand the maturation of putatively harmful oral biofilms, and could provide key insights into biofilm physiology.

**Hypothesis:** The salivary mucin MUC5B is hypothesized to modulate biofilm activity in ways that promote biosis in oral microbial communities.

**Methodology:** The early oral colonizer *Streptococcus gordonii* DL1 was grown in planktonic cultures or in biofilm flow cell systems with uncoated, MUC5B or low-density salivary protein (LDP) coated surfaces. Cellular proteomes were studied using a quantitative mass spectrometry-based workflow, and variations in protein abundances between conditions were identified.

**Results:** Overall, the proteomic profiles of *S. gordonii* DL1 were similar between conditions. Six biofilm cell proteins absent in planktonic culture and three planktonic cell proteins absent in all biofilm cultures were identified. Salivary MUC5B also elicited specific responses in the biofilm cell proteomes. Compared to LDP, attachment to MUC5B elicited differences in abundance in thirteen proteins and absence of one.
The composition of the human gut microbiota is extremely diverse and differs not only from person to person but also spatial and temporal looking at one individual. Therefore, for reliable and reproducible microbiota research, there exists a growing demand for sophisticated in-vitro cultivation systems to overcome in-vivo limitations. The developed peristaltic mixed tubular bioreactor (PETR) aims to mimic in-vivo colonic conditions in a controllable and reproducible in-vitro environment, offering a versatile research tool for medical research applications. It consists of four stainless steel modules providing all required connections, e.g., for sampling and control. The modules are connected by flexible parts that are mixed periodically in a peristaltic manner simulating the intestinal peristaltic. The natural low shear stress mixing is beneficial for biofilm formation giving an environmental niche for sessile bacteria comparable to the mucus layer. After validating PETR from the biochemical engineer’s perspective, the biological applicability was shown. Therefore, PETR was inoculated with five bacterial strains as exemplary representatives of the human microbiota. After an initial batch phase and during a continuous cultivation of 8 d, PETR was able to create and maintain the desired pH gradient from 5.5 in the proximal to 7.0 in the distal part. The formation of small chain fatty acids as well as their resorption through an integrated dialysis membrane was measured using HPLC. The composition of the mixed culture was elucidated by qPCR using strain-specific primers showing different compositions regarding cultivation time, spatial location and between planktonic and sessile growth, with the latter being dominated by biofilm-forming Phocaicola dorei. The biofilm was also observed using CLSM-imaging. This proof of principle demonstrates PETR as a well-suited in-vitro system for microbiota research for planktonic and sessile growth. Further work will focus on integrating more representative microbiota and promoting even more sessile growth, e.g., through special carriers.
P25- Developing an in-vitro polymicrobial biofilm model for ventilator associated pneumonia

Dean Walsh, School of Life Sciences, University of Warwick, Coventry, UK
Trevor Lithgow, Monash Biomedicine Discovery Institute, University of Monash, Australia
Ana Traven, Monash Biomedicine Discovery Institute, University of Monash, Australia
Freya Harrison, School of Life Sciences, University of Warwick, Coventry, UK

Here, we showcase a reproducible model that simulates the chemical, biological, and material environment of biofilms implicated in ventilator associated pneumonia (VAP). VAP is defined as a pneumonia occurring after more than 48 hours of mechanical ventilation via an endotracheal tube (ETT) or tracheostomy tube. VAP results from biofilms that form on the ETT, thereby seeding the lower airways with pathogenic microbes. The prevalence of VAP varies between 9-65% with mortality rates as high as 76%. Furthermore, current treatment of VAP requires the use of broad-spectrum antibiotics, adding to already high selection pressures and further increasing antibiotic resistance. The current lack of accurate in vitro models of the VAP environment greatly limits our understanding of how the VAP environment alters pathogen physiology and the efficacy of existing and novel therapies. Numerous pathogens are capable of causing VAP, the most common being Pseudomonas aeruginosa. Other causative pathogens include Klebsiella pneumoniae, which is also commonly isolated, and Candida albicans, a fungus known to further complicate VAP treatment and recovery. Here, we add P. aeruginosa, K. pneumoniae, and C. albicans monocultures and polymicrobial cultures to novel formulations of synthetic airway surface liquid (ASL), in the absence or presence of sections of ETT. As ventilated patients can have a broad spectrum of comorbidities, we created multiple synthetic ASL formulations with differing compositions: healthy (no comorbidities), diabetic, and smoker. Sections of ex-vivo porcine trachea may also be added to the model. Our model can be used to inform on how the VAP environment alters biofilm formation, antibiotic susceptibility, and polymicrobial interactions. Confocal microscopy and scanning electron microscopy can be used to characterize the matrix composition, species distribution, and response to antimicrobial therapy in VAP polymicrobial biofilms.
The extensive genome that contains several virulence factors including the ability to form biofilms, combined with high-level resistance mechanisms, makes *Pseudomonas aeruginosa* a threat for infections, especially in hospital environments where often antibiotic-resistant strains are being found. The growing incidence of highly resistant strains, makes it difficult to select appropriate antibiotics and narrows the therapeutic options. For example, according to ECDC, 31.8% of *P. aeruginosa* found in hospitals was resistant to carbapenems, making it particularly life-threatening considering that many hospital patients have weak immune system. Therefore, much of the current research is focused to develop new substances that would help to treat and prevent infections, while contributing to reduce the use of antibiotics. Promising alternatives to conventional antimicrobials are, for example, silver nanoparticles. Nanoparticles have a large surface area that allows for increased antimicrobial efficacy and bioavailability compared to bulk metal. The effects and properties of silver nanoparticles vary depending on their size, shape, and caping agent. In this work, we prepared spherical silver nanoparticles covered by lignin, a biocompatible polymer with antimicrobial and antioxidant effects. The lignin used was isolated by the organosolv process from beechwood sawdust and plays a crucial role during the preparation of nanoparticles, where it acts as a reducing and stabilizing agent. The presence of a phenolic structure on the surface of nanoparticles can be expected to extend the antimicrobial properties of silver nanoparticles. This combined effect of silver and lignin will be demonstrated on the ability of nanoparticles to prevent biofilm formation of several selected clinical isolates of *P. aeruginosa*. 
P27- Microtopography-Driven Prevention of Bacterial Biofilm Formation.

Elizabeth Ison - School of Pharmacy, University of Nottingham & School of Life Sciences, University of Nottingham
Paul Williams - School of Life Sciences, University of Nottingham
Morgan Alexander - School of Pharmacy, University of Nottingham

Introduction: Inhibiting bacterial surface attachment is essential for preventing medical device-associated infections. Microtopographies have been shown to be capable of deterring biofilm formation and therefore could offer a unique non-biocidal strategy for reducing this burden (1). However, understanding of how specific microtopographies successfully modulate bacterial surface sensing to prevent attachment and subsequent biofilm formation remains lacking.

Methods: Unbiased screening of 2,176 micro-topographies was achieved using Topo-chips (1). Staphylococcus aureus and Pseudomonas aeruginosa were chosen as model pathogens to screen the anti- or pro-attachment nature of each pattern. Scaled up, hot embossed, topographies were also probed with P. aeruginosa strains with mutations in flagella (fliC) and type IV pilus (pilA) genes given the contribution of these organelles to surface sensing and early-stage biofilm formation. Integrating differential interference contrast (DIC) and internal reflectance microscopy further improved observations of real-time surface colonisation by single cells.

Results & Discussion: “Hit” anti-attachment microtopographies reduced bacterial colonisation up to 15-fold compared with flat and pro-attachment surfaces (1). These anti-attachment microtopographies were independent of surface chemistry. Confocal and DIC microscopy of P. aeruginosa showed that the bacteria had a pronounced preference for large gaps between surface pillars but were deterred from surface colonisation between pillars where the distance separating the pillars was less than that of P. aeruginosa’s long axis. If confined to grooves finer than a long axis, the cells tended to orientate themselves on their poles. Although differences were observed in the colonization of pro-attachment microtopographies by the P. aeruginosa fliC and pilA mutants in common with the wild type, neither mutant could colonize the anti-attachment microtopographies. Therefore, this surface patterning technique shows promise for biofilm resistant medical devices.

P29- Anti-quorum-sensing and anti-biofilm activities of marine microorganisms and tropical sponges extracts.

Flore Caudal¹,², Sophie Rodrigues¹, Sébastien Artigaud³, Gwenaëlle Le Blay⁴, Sylvain Petek⁵, and Alexis Bazire¹.

¹ Laboratoire Biotechnologie et Chimie Marines, Université Bretagne Sud, UR3884, LBCM, IUEM, CEDEX, 56321 Lorient, France
² Laboratoire des Sciences de l’Environnement Marin (LEMAR), UMR 6539 CNRS/UBO/IRD/IFREMER, BP 70, 29280 Plouzané, France
³ IRD, Univ Brest, CNRS, Ifremer, LEMAR, F-29280 Plouzané, France

Introduction: Pathogenic bacteria and their biofilms, are involved in many diseases, human or animal, and are a major public health problem with antibiotic resistance. Natural products are one of the most important sources for the development of new active molecule. The marine environment is full of organisms capable of producing original and bioactive metabolites, generally different from those found in the terrestrial world.

Hypothesis: The aim of this study was to evaluate potential antibiofilm and/or anti-quorum activity of Pseudoalteromonas supernatants and sponge extracts on the biofilm of 2 pathogens, Pseudomonas aeruginosa (PA) and Vibrio harveyi (VH).

Methodology: We first ensured that the 47 extracts and 14 supernatants did not exhibit any biocidal activity. Antibiofilm effects were screened using crystal violet method and then under hydrodynamic conditions, in a flow-cell system combined to confocal laser scanning microscope analysis (SCLM). Their anti-quorum effects have also been tested using bioreporters with quorum dependent luminescence.

Results: None of our sponge extracts or Pseudoalteromonas supernatants showed biocidal activity against PA and VH. None of the extracts were active on biofilm formation of PA, but 10 of them showed more than 75% inhibition in the formation of VH biofilm under static condition. Pseudoalteromonas supernatants showed no activity on VH biofilm, but a large majority resulted in more than 75% inhibition of PA biofilm formation, 3 of which were also active in a 2-hour treatment on pre-formed biofilms. Using SCLM, we observed impacted on biofilm structures of VH and PA by active extracts previously identified.

Conclusion: There seems to be a specificity of action of supernatants and extracts on their targets, this could be related to the differences in biofilm formation mechanisms between PA and VH. The next steps will be to purify/characterize these extracts and supernatants and then explore their mechanism of action.

P30- G-quadruplexes and Z-DNA in the extracellular matrix of *Staphylococcus epidermidis* biofilm

**Gabriel Antonio S. Minero, Andreas Møllebjerg, Rikke L. Meyer**

Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Gustav Wieds Vej 14, 8000 Aarhus, Denmark

*Staphylococcus epidermidis* is a commensal skin bacterium, which can be pathogenic and tolerant to antibiotics due to formation of a biofilm – multicellular community encased into extracellular polymeric matrix composed of proteins, nucleic acids, and polysaccharides. The extracellular DNA (eDNA) appears to become resistant to enzymatic digestion as the biofilm matures [1]. Recently, left-handed Z-DNA and G-quadruplex G4-DNA were identified in biofilms [1,2], and these secondary structures can explain why DNases fail to disperse biofilms. The aim of this study was to determine if secondary structures exist in the matrix of *S. epidermidis* biofilms, and what affects their formation. Immunolabeling and confocal laser scanning microscopy were used to visualize G4-DNA and Z-DNA and their co-localization with B-DNA in 60 h biofilms of the clinical isolate *S. epidermidis* AUH4567 [3].

Some eDNA was closely associated with the cell surface, and some was present as spiderweb-like strings connecting microcolonies. G4-DNA was present in both locations, while Z-DNA was exclusively in the web-like strings that suggests that stretching could affect Z-DNA formation in biofilms. In *S. epidermidis* biofilms, the B- to Z-DNA transition appears to involve interaction with the extracellular polysaccharide poly-N-acetyl-glucosamine (PNAG), which is typically 20% deacetylated and is thus a polycationic biofilm matrix component. Comparison of wild type *S. epidermidis* and mutant strains lacking PNAG revealed that more eDNA was flipped into Z-form in the presence of PNAG. This discrepancy did not apply to G4-DNA. DNA-intercalating molecules can facilitate Z- to B-DNA transition [1], and we demonstrate that an extracellular DNA-binding stain caused this transition. Our findings in the biofilm matrix were confirmed using synthetic DNA sequences and circular dichroism.

In conclusion, we show abundant secondary DNA structures in the biofilm matrix, and identify previously unknown contributors to their reversible formation.

The development of biofouling is a major problem for marine industries. The conception of antifouling and fouling release coatings, with controlled physico-chemical properties is a promising strategy. Among them, amphiphilic systems, such as those composed of a hydrophobic polydimethylsiloxane matrix and a hydrophilic polyethylene glycol additive are the most efficient up to date. Despite their effectiveness, these systems are questioned due to the petrochemical origin of PDMS. The aim of this project is to substitute PDMS matrix with a biopolymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and to improve its anti-adhesion properties through the elaboration of an amphiphilic system, via the addition of PEG or PHBHHx-b-PEG copolymer. Our results, including physico-chemical properties of PHBV based coatings, static and dynamic adhesion tests on two bacteria, Pseudomonas aeruginosa and Staphylococcus aureus are compared to those of PDMS and PEG-modified PDMS coatings. A real antiadhesion activity and fouling-release capacity were obtained for the PHBV/PHBHHx-b-PEG system for a promising eco-friendly strategy.
P33- Candida albicans anti-biofilm compounds from endolichenic fungi: research guided by a molecular networking approach

Seinde Toure†, Guillaume Hamion§, Lucie Ory‡, Catherine Roulier‡, Zineb Khaldi†, Valentin Pichon†, Christine Imbert§, Marion Girardot§, Marion Millot†, Lengo Mambu†

† EA 7500, Laboratoire PEIRENE, Université de Limoges, Limoges, France
‡ EA 2160, Equipe Mer Molécules et Santé (MMS), Université de Nantes, Nantes, France
§ UMR CNRS 7267, Laboratoire Ecologie et Biologie des Interactions (EBI), Université de Poitiers, Poitiers, France

Endolichenic fungi (EF) are an unexplored source for the search of original molecules active against Candida albicans biofilms. Phytochemical research in this area is currently increasing and has already demonstrated that these fungi synthesize original compounds endowed with biological activities such as anticancer or antimicrobial activities. The present work aims to study their potential as a source of anti-biofilm compounds using the innovative method of molecular networking.

Fourteen strains of EF were grown on three culture media and extracted with ethyl acetate. Antimutation and antibiofilm assays were performed in microplates by treating 2h- or 24h old biofilms of C. albicans (ATCC 28367 or clinical strains) for 24h or 48h. The residual biofilm after-treatment was quantified using XTT assay. All extracts were analysed by HPLC-ESI(-)HRMS/MS to investigate their chemical composition. After peak picking, a feature-based molecular network was built using both Metgem and the GNPS platform in order to link the anti-biofilm activity to the inventoried chemical components.

Thus, thirty-eight extracts were tested against C. albicans biofilms. After a treatment of 48h at 100 µg/mL, ten extracts reduced significantly (p≤0.0002) the maturation phase by at least 50%. Among them, four extracts were also capable of inhibiting (p0.0001) a pre-formed biofilm after a 48h treatment, using both the reference strain and clinical isolates of C. albicans. Preussia persica extract was the most active, inducing a non-strain-dependent activity (inhibition of at least 57%) against preformed biofilms. Molecular networks highlighted interesting and biologically active molecular groups like oxygenated fatty acids and cyclopeptides. The MS-targeted isolation performed on P. persica extract allowed the identification of a new highly oxygenated fatty acid. This study showed a poor number of annotated compounds and revealed the treasure of EF as a source of new chemical entities. The biological potential of EF compounds as anti-biofilm agents was also highlighted.
P34- Valuation of invasive plants against the bi-species biofilm *Staphylococcus aureus-Candida albicans*

**Guillaume Hamion**¹, Willy Aucher¹, Charles Tardif², Julie Miranda², Stéphanie Crapart¹, Clément Bernard¹, Caroline Rouger², Marion Girardot¹, Christine Imbert¹

¹ Université de Poitiers, UMR CNRS 7267, Laboratoire EBI, Poitiers, France;  
² Institut des Sciences de la Vigne et du Vin (ISVV), USC 1366 INRAE, Unité de recherche Œnologie, Bordeaux, France

Biofilms are poorly susceptible to most conventional drugs. Our study aimed to look for original molecules able to inhibit bi-species *S. aureus* plus *C. albicans* (Sa-Ca) biofilms. Invasive plants are efficient for colonizing non-native territories, suggesting a great production of secondary metabolites. Our postulate was that these metabolites could be effective anti-biofilm weapons.

Five invasive aquatic plants were harvested in the Nouvelle-Aquitaine France region: *Ludwigia peploides*, *Ludwigia grandiflora*, *Myriophyllum aquaticum*, *Lagarosiphon major*, *Egeria densa*. Forty extracts were prepared from leaves, stems and submerged parts, and tested (50-200µg/mL, treatment: 24h) against 24h old biofilms of *S. aureus* ATCC29213 and *C. albicans* ATCC28367 in microplates. Their anti-biofilm activity was assessed using crystal violet (CV). The activity of extracts reducing ≥50% of the biofilm biomass was comparatively analyzed on each microbial species forming the biofilm, by Flow Cytometry and Scanning Electronic Microscopy. Other clinical and ATCC strains were used to produce biofilms to insure that activity was strain-independent. Active extracts were fractionated, and the fractions’ activity was evaluated by CV. UHPLC-MS/MS analyses were performed to investigate their chemical composition. A Bioactivity-Based Molecular Networking (BBMN) was implemented, combining data of UHPLC-MS/MS analyses and anti-biofilm tests, to identify the potentially active compounds and then study their anti-biofilm activity by CV, FCM and SEM. Among the forty extracts obtained, the stem one of *L. grandiflora* showed the highest anti-biofilm activity (>50% inhibition at 50µg/mL). Its fractionation and the biological, chemical and BBMN investigations highlighted nine compounds correlated with this activity. The most correlated compound, LUg1, significantly reduced preformed Ca-Sa biofilms, whatever the strains used, reference or clinical (>40% inhibition at 55µM).

In conclusion, *L. grandiflora* synthesizes active compounds, in particular LUg1, which is a promising anti-biofilm candidate. Moreover, results highlighted BBMN as a rapid and efficient method to discriminate and identify active compounds in complex matrices.
The necessity for strong in vitro models, in which novel treatments can be tested, is an ever-relevant issue. Here we present a new in vitro model resembling a biofilm-infected chronic wound, which features two different bacterial species, two compositely different layers, and a continuous addition of nutrients. The model uses collagen instead of agar as the secondary matrix, is inoculated with Staphylococcus aureus and Pseudomonas aeruginosa, and is cast in transwell inserts before being placed in wound simulating media. This allowed for the continuous exchange of nutrients and waste products across a filter, as the media was renewed every 24 hours. The model was followed for 6 days and was found to maintain a stable number of both species over the full duration. Confocal microscope images were captured which confirmed the presence of biofilm microcolonies in the model. Microsensor measurements were performed repeatedly which uncovered steep oxygen gradients that had developed within the model due to bacterial respiration, as well as an alkaline pH that had increased to >8. These properties are commonly observed in clinical chronic wounds, emphasizing the realism of the microenvironment developed in the new in vitro model. Three hydrogels made from biodegradable starch microspheres loaded with different active compounds were tested as potential new wound care treatments. Chlorhexidine digluconate 2% solution, which is a commonly used wound antiseptic, was tested in the model as well and served as a control. The treatments were topically applied and left for 2 days, followed either by CFU enumeration or in some cases, a second round of treatments. The three hydrogels reduced the number of CFUs within the model significantly, whilst the chlorhexidine digluconate only caused a small CFU reduction. The hydrogels also decreased the pH levels remarkably and alleviated the bacterial oxygen consumption, restoring the oxygen levels within the model.
P36- Development of a three-dimensional human in vitro model of a biofilm-infected wound

Jana Wächter¹; Pia Kaiser¹; Maike Windbergs¹

¹ Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt am Main, Germany, j.waechter@em.uni-frankfurt.de

Current approaches for investigating biofilm-related wound infections rarely involve the combination of biofilms with skin cells, as maintaining cell viability during the required time for biofilm formation is difficult to achieve. Although affords are described to combine mature biofilms with living cells, they entail only indirect contact or compromise biofilm integrity since the translocation of pre-grown biofilms to the tissue fails due to insufficient mechanical strength. Therefore, we developed a bacterial biofilm model on the basis of electrospun fibers that provides high mechanical stability to allow for a transfer of intact biofilms to human tissue models. Additionally, the nanofibrous scaffold, consisting of biocompatible polymers, closely resembles the structure and polymeric composition of the native biofilm matrix.

We inoculated the electrospun mats with Pseudomonas aeruginosa as a common wound pathogen and monitored the growth of adherent bacteria over the maturation period of 48h. Uniform bacterial distribution as well as matrix production throughout the fiber network was demonstrated by histological investigations and SEM imaging, highlighting the scaffold as an adequate growth substrate. Next, antibiotic susceptibility of the biofilm model was determined by the treatment with gentamicin, where, in accordance with in vivo biofilms, the developed model showed a highly enhanced tolerance compared to planktonic bacteria. Finally, pre-cultivated biofilms were transferred to wounded ex vivo human skin tissue, where a steady bacterial growth was observed for further 24h. The interaction of the biofilm and the host cells was assessed by histological investigations and SEM imaging, showing close contact of biofilm and wound bed.

We thereby were able to develop a novel biofilm model which exhibits main characteristics of native biofilms and provides high mechanical stability to enable the transfer of intact biofilms to human skin tissue to model wound infections in a physiological relevant setting.
P37- Developing a Dental Implant Surface Characterization Model

Jon J. Vernon 1, El-Mostafa Raif 1, Jensen Aw 2, Ed Attenborough 2, Animesh Jha 3 and Thuy Do 1

1. Division of Oral Biology, School of Dentistry, University of Leeds, UK 2. Attenborough Dental Laboratories Ltd., Nottingham, UK. 3. School of Chemical and Process Engineering, University of Leeds, UK

Introduction. Peri-implantitis, a condition characterised by the inflammation of tissue surrounding dental implants and subsequent bone loss, is caused by oral biofilm accumulation and is the primary aetiology of late implant failure. Developing surfaces augmented for antimicrobial efficacy is crucial for the long-term persistence of implants. 

Hypothesis. Characterizing the physical and chemical properties of dental implant will inform best manufacturing processes for the development of anti-biofilm surfaces. Selected methods may be then implemented for process quality control.

Methodology. Roughness and surface chemical properties of laser-sintered CoCr and titanium disks (Al2O3 sandblasted, high and electrolytic polished) were determined through non-contact confocal chromatic profilometry and energy-dispersive X-ray spectroscopy (EDS). Cytotoxicity was assessed via contact effect and extract-based MTT cell viability assays. Dual- and five-species oral biofilms (Streptococcus salivarius, Actinomyces naeslundii, Fusobacterium nucleatum, Prevotella intermedia and Porphyromonas gingivalis) were cultured on implant materials for up to 12 days. Agar enumeration and scanning electron microscopy were used to assess biofilm differences.

Results. Total viable counts from five-species biofilms indicated a decreasing abundance trend from sandblasted (7.83±1.38 x 108) through to high (7.26±1.18 x 108) and electrolytic polished (5.28±0.59 x 108) surfaces, correlating with roughness, (XY Ra = 18.3, 17.2 and 1.7 Ra, respectively; p>0.05). Bacterial enumerations on implant surfaces correlated with hydroxyapatite controls suggesting that this model may be a useful tool for the assessment of the antimicrobial efficacy of future surface coatings. No evidence of cytotoxicity was detected, with no observed detriment to cells and 93-100% cell viability. EDS revealed the impact of post-production on surface chemistry, with Al2O3, molybdenum and silicon retained on implant surfaces after sandblasting or polishing. These may alter interactions between microorganisms, potentially impacting on early-stage biofilm formation.

Conclusion. Correlating microbiological models with physico-chemical parameters of implant materials has the potential to assist the optimization of anti-biofilm implant surfaces.
P38- Development of an oral biofilm model to incorporate mechanical chewing and its impact on antimicrobial efficacy against *Streptococcus mutans*.

Katherine Roe\(^1,^2\), Dr Beth Green\(^3\), Dr Ben Dias\(^3\), Dr Bob Faller\(^3\), Professor Jeremy Webb\(^1,^2\), and Professor Paul Stoodley\(^4\)

1 Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, 2National Biofilm Innovation Centre, University of Southampton, SO17 1BJ, UK, 3 Mondelez, 3 Parkway N, Deerfield, Illinois, 60015, USA, 4 The Ohio State University, 460 West 12th Avenue, Columbus OH 43210.

Approximately 50% of the global population suffer with oral diseases. With the average person harbouring roughly 250 bacterial species in their mouth, maintaining a healthy oral microbiome is key to mitigate diseases associated with oral pathogenesis. Few oral models incorporate an automated mechanical chewing action with this study aiming to develop a standardised model to feature chewing.

We hypothesised that when mechanical forces are applied to biofilms to mimic chewing within the oral cavity, there is enhanced antimicrobial efficacy, increased biofilm removal, and an impact on the architecture of the biofilm.

Initially, a range of media and reactors were investigated to see if supplements or static growth compared to in a CDC bioreactor altered the number of viable *Streptococcus mutans* cells.

*S. mutans* growth was compared between a range of media, reactors, and supplements. Additionally, static growth and continuous culture were compared. A E1000 Electropuls indenter mimicked chewing by replicating the rate and force of chewing under controlled conditions. The rate of bacterial removal using the E1000 was compared to a published manual chewing method using viable cell counts as a measure. Several antimicrobial compounds were tested on *S. mutans* to assess planktonic colony forming unit data and the impact on biofilms. Mechanical action was then introduced with the antimicrobials to observe potential synergism.

*S. mutans* growth in the CDC reactors resulted in thicker biofilm and a higher viable cell count compared to the statically grown bacteria. The *S. mutans* biofilms exhibited increased tolerance to antimicrobials compared to the planktonic *S. mutans* cultures. Incorporation of mechanical action is now allowing for studies of biofilm removal under simulated chewing action. In conclusion, we have developed a novel model that incorporates mechanical forces and that allows for the study of antimicrobial efficacy against oral biofilms.
The connection between gut microbiota and colorectal cancer (CRC) carcinogenesis has recently gained much attention, and several bacterial species have been linked to CRC, including the oral pathogens *Fusobacterium nucleatum* and *Bacteroides fragilis*. Despite correlations, we do not know much about the biogeography of the bacteria in the tissue or whether they aggregate in mixed-species biofilms, as seen in some chronic infections, e.g., periodontitis. In addition, the composition of bacteria is greatly affected by many factors; thus, we do not know whether the observation of *B. fragilis* and *F. nucleatum* applies to a Danish cohort.

Therefore, we investigated the in situ prevalence of these two bacteria utilizing species-targeted fluorescent in situ hybridization (FISH) on tissue biopsies from a cohort of 39 patients with CRC, both primary tumor (PT) and paired normal tissue (PN) and healthy (HT) tissue biopsies from 40 subjects with no known gastrointestinal disease. Here, we found that higher bacterial biomass was present in biopsies from PT as compared to PN ($p = 0.0001$) and HT biopsies ($p = 0.0001$). In addition, it was observed that bacteria displayed a tissue-invasive phenotype, meaning that large mixed-species biofilms were growing in or on the tissue. Finally, we observed a higher prevalence of *F. nucleatum* in PT biopsies than in HT biopsies ($p = 0.0009$), which was not the case for *B. fragilis*. Collectively, these results indicate an overrepresentation of bacterial biomass and *F. nucleatum* on PT biopsies compared to HT biopsies. These findings suggest that *F. nucleatum* plays a role in CRC carcinogenesis and may provide a therapeutic target for improving patient outcomes in a Danish cohort. In addition, our results also highlight the need for more research regarding the role of bacteria in CRC carcinogenesis.
P43- Biofilm modulation activity and machine learning of essential oils on *Pseudomonas aeruginosa* isolates from cystic fibrosis patients.

Rosanna Papa[^1], Gianluca Vrenna[^1], Rino Ragno[^2], Marco Artini[^1], and Laura Selan[^1]

1. Department of Public Health and Infectious Diseases, Sapienza University, p.le Aldo Moro 5, 00185 Rome, Italy
2. Rome Center for Molecular Design, Department of Drug Chemistry and Technology, Sapienza University, p.le Aldo Moro 5, 00185 Rome, Italy

*Pseudomonas aeruginosa* can cause both acute and chronic infections, since its pathogenic profile originates from a large and variable arsenal of virulence factors and antibiotic resistance determinants. In the airway of cystic fibrosis (CF) patients, it persists inducing chronic infection; furthermore, it is widely known that CF pulmonary environment confers multiple advantages to *P. aeruginosa* over other pathogens, such as *Staphylococcus aureus* and *Klebsiella pneumoniae*. The ability to form biofilm plays a pivotal role in CF airways colonization by *P. aeruginosa*. Indeed, among its various virulence factors, the ability to produce highly structured biofilms confers important advantages, including phenotypic resistance to host defenses, antibiotics and disinfectants. These characteristics prevent bacterial clearance and allow the establishment of highly recalcitrant chronic infections. Bacterial virulence mitigation and bacterial cell adhesion hampering and/or biofilm reduced formation could represent a major target for the development of new therapeutic treatments for infection control. Essential oils (EOs) are being considered as a potential alternative in clinical setting for prevention, treatment and control of infections sustained by microbial biofilm. EOs are complex mixtures of different classes of organic compounds, usually used for the treatment of upper respiratory tract infections in traditional medicine. Recently, we have investigated a wide series of EOs for their ability to modulate biofilm production by different pathogens comprising *S. aureus*, *S. epidermidis* and *P. aeruginosa* strains. Machine Learning (ML) algorithms were applied to develop classification models, in order to suggest a possible antibiofilm action for each chemical component of the studied EOs. In the present study we assessed the biofilm growth modulation exerted by 61 commercial EOs on a selected number of *P. aeruginosa* strains isolated from CF patients. Furthermore, ML has been used to shed light on the EOs chemical components likely responsible for positive or negative modulation of bacterial biofilm formation.
The biofilm lifestyle of bacterial pathogens is a hallmark of chronic lung infections in cystic fibrosis (CF) patients. Bacterial adaptation to the complex conditions in CF-affected lungs and repeated antibiotic therapies lead to increasingly tolerant and hard-to-treat biofilms. In the context of growing antimicrobial resistance and restricted therapeutic options, antimicrobial photodynamic therapy (aPDT) shows great promise as an alternative to conventional antimicrobial modalities. Typically, aPDT consists in irradiating a non-toxic photosensitizer (PS) to generate reactive oxygen species (ROS), which kill pathogens in the surrounding environment. In a previous study, we reported that some Ruthenium(II) polypyridyl ([Ru(II)]) complexes can demonstrate potent aPDT against planktonic cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus* clinical isolates. In the present work, [Ru(II)]-based aPDT was further evaluated for its capacity to inhibit and/or eradicate biofilms formed by the same bacteria. For this purpose, some selected [Ru(II)] complexes were used to assess their ability to diffuse and produce ROS in artificial sputum medium and biofilms. Antibiofilm effects were evaluated using crystal violet staining and colony-forming-unit counting. Altogether, our results suggest that [Ru(II)] complexes are promising candidates to combat biofilms formed by bacterial clinical isolates. This study thus provides further proof of the relevance of [Ru(II)]-based aPDT in CF lung airways while highlighting the need for optimized PS nano-complexes.
P45- A semi-synthetic cocktail based on Bald’s eyesalve: antibacterial activity and interactions with other antimicrobials

Oluwatosin Qawiy Orababa¹, Jessica Furner-Pardoe¹,²*, Freya Harrison¹*

¹ School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry CV4 7AL, UK
² Warwick medical school, Gibbet Hill Campus, University of Warwick, Coventry CV4 7AL, UK
* These authors contributed equally

Antimicrobial resistance is a major global challenge due to the increase in disease mortality, increased hospital stay, and cost of treatment. Natural products from plants amongst others are some of the alternatives that have been suggested to treat drug-resistant infections. Bald’s eyesalve is a medieval remedy for treating eye infections that has shown broad-spectrum antibacterial activity. Biofilm eradication by Bald’s eyesalve was previously shown to require the presence of all four ingredients in the remedy. We have now found that this biofilm eradication activity can be recapitulated by a semi-synthetic cocktail comprising a compound purified from one ingredient (compound A) and a second ingredient from the remedy (compound B). This study shows that the semi-synthetic cocktail has good antibacterial activity against both planktonic and biofilm-associated populations of Staphylococcus aureus and Pseudomonas aeruginosa. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the cocktail in different media (cation-adjusted Mueller Hinton Broth, synthetic wound fluid, and synthetic cystic fibrosis sputum medium) were determined with the broth-microdilution method, while in vitro wound model and ex vivo pig lung models were used to determine its biofilm eradication activity. We confirmed that the cocktail has better activity than its individual components, especially in biofilm models. The cocktail showed >4-log killing against S. aureus Newman and P. aeruginosa LESB58 in the wound biofilm and lung biofilm respectively. Interactions with other antimicrobials (silver sulfadiazine, vancomycin, meropenem, and honey) were classified as antagonistic, additive, and synergistic with checkerboard assay. The cocktail showed good antibacterial activity against planktonic S. aureus and P. aeruginosa. It also showed synergistic activity with silver sulfadiazine against P. aeruginosa, and additive effects with other antimicrobials against other planktonic cells. This study showed that the cocktail might be useful as a sustainable treatment for chronic wound and cystic fibrosis infections in the future.
P46- Growth of filamentous microscopic fungi biofilms: Finding the right methodology

Kulišová M.*; Kolouchová I.*

*Department of Biotechnology, University of Chemistry and Technology Prague, Czech Republic

The incidence of contaminating microorganisms in the food processing industry and the risk of food contamination depends on the complex hygiene standards of food processing plants. If contamination occurs, the source of problems is often related to biofilm formation. Biofilm is a widespread life strategy among microorganisms due to number of benefits for them (e.g. increased resistance to physical-chemical stress and inactivation). In addition to bacteria and yeast biofilms, microscopic fungi are also able to form biofilms, but this biofilm formation is still a relatively poorly explored area of microbiology. Our study focuses on developing a suitable methodology for quantifying the growth of fungal biofilms. Crystal violet staining, MTT assay, XTT assay and resazurin assay were the methods selected for fungal biofilm determination based on their applicability in bacterial biofilms quantification. These assays were tested on single species biofilms consisting of common microscopic fungi contaminants in the food industry (Alternaria alternata, Aspergillus niger, Fusarium culmorum, Fusarium graminearum). The biofilm growth was also analyzed using spinning disc confocal microscopy. Crystal violet staining (determination of total biofilm biomass) was found to be suitable for the evaluation of fungal biofilms. For the determination of the metabolic activity of biofilm cells, the MTT and XTT assays were suitable, but after the addition of menadione. This compound that helps with transfer of electrons through the cell membrane is not necessary when working with bacteria/yeast biofilms. The resazurin assay was found to be inapplicable for determining fungal biofilm metabolic activity.
P47- Real-Time monitoring biofilm growth dynamics using confocal laser scanning microscopy as a tool for understanding the diagnosis of catheter-related bloodstream infection

Marta Díaz-Navarro, Rafael Samaniego, Rafael Diez, Juan Carlos Piqueras, Marta Tormo, María Guembe.

Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Spain. Gregorio Marañón Institute of Health Research, Spain. School of Biology, Universidad Complutense de Madrid, Spain.

Introduction: In Catheter-Related Bloodstream Infection (C-RBSI), differential time to positivity (DTTP) is the most used conservative diagnostic technique, which consists of obtaining blood from all catheter lumens and from a peripheral vein to determine microbial growth in an automatized system. As microbial load from blood obtained through the catheter is higher when catheter is source of the infection, it be detected in a shorter time than blood obtained from the bloodstream (120 min difference). However, this cut-off has demonstrated to be not as reliably applied on Candida spp. and S. aureus.

Hypothesis: The dispersion of sessile cells in the biofilm may be faster in Candida spp. and S. aureus, which means that similar amount of yeasts/bacteria is present both in "catheter" and "blood" compartments in early stages of infection. Therefore, our aim was to establish the "cell spreading time" of the biofilm from different species using confocal laser scanning microscopy (CLSM).

Methodology: Biofilm formation of Candida albicans ATCC14053, Staphylococcus epidermidis ATCC 35984, and Staphylococcus aureus ATCC 29213 were performed on silicon disks and were analyzed by real-time CLSM. The time lapse-images of biofilms were recovered for 24h at a rate of 1 frame/1.5 min and processed using software FIJI/Image J. Data was plotted and analyzed using software GraphPad Prism v7. The dispersion time of cells of biofilm was calculated.

Results:

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mean±SD thickness (µm) 1h</th>
<th>p</th>
<th>Mean±SD thickness (µm) 17h</th>
<th>p-value</th>
<th>Mean±SD Dispersal time (min)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>80.68±9.49</td>
<td>&lt;0.001</td>
<td>48.27±21.60</td>
<td>0.004</td>
<td>90.00±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. aureus</td>
<td>7.75±0.85</td>
<td>&lt;0.001</td>
<td>15.48±1.48</td>
<td>0.253</td>
<td>95.23±0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>0.17±0.29</td>
<td>NA</td>
<td>8.61±3.55</td>
<td>NA</td>
<td>265.30±0.73</td>
<td>NA</td>
</tr>
</tbody>
</table>

Conclusion: Our findings could explain why early dissemination of cells in C. albicans and S. aureus make us unable to confirm or rule out the catheter as the source of the bloodstream infection.
The National Biofilms Innovation Centre aims to harness the UK’s academic and industrial strengths in biofilms and to build a biofilm network focussed on research, innovation and training across the UK and internationally. A critical unmet need for innovation across industry sectors is the infrastructure and support needed to demonstrate alignment to relevant standards and the associated analytical competencies. Our national and international academic-industry roadmapping has consistently identified the establishment of global standards in biofilms as a priority need. NBIC is actively addressing this need by establishing networks and collaborations and engaging in a variety of initiatives and research projects. In February 2020 NBIC, along with the US Center for Biofilm Engineering, the Singapore Centre for Environmental Life Sciences Engineering and an EU Cooperation in Science and Technology (COST) action group AMICI, formed an International Biofilms Standard Task Group (IBSTG) to drive the international development and acceptance of standardised biofilm test methods in health care, the built environment and industrial systems. The mission of the IBSTG is “To drive the international development and acceptance of standardized biofilm test methods in health care, the built environment and industrial systems.”

Here we will present an overview of the industrial and academic perspectives on the standard biofilm models, test methods and regulations. We will also provide the results of our progress to date through roadmapping exercises, working with standards bodies and direct stakeholder consultations that will support NBIC and IBSTG drive to progress biofilm standardisation.
PS1- SadB is a global regulator in *P. aeruginosa* that affects biofilm formation, nitrate respiration and cell to cell communication

Maria Papangeli¹,², Jean-Frédéric Dubern¹,², Stephan Heeb¹,², Morgan R. Alexander³ and Paul Williams¹,²

1 Biodiscovery Institute, 2 School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK, 3 School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD

Bacteria can adhere to almost any surface and form resistant biofilm communities. These pose a major clinical burden as they can form on medical devices such as catheters and are hard to prevent. Catheter-associated urinary tract infections (CAUTIs) are the most common nosocomial infection as they represent ~40% of all nosocomial infections (Mittal et al., 2009).

We have developed a series of polymers that prevent biofilm formation (Hook et al., 2012). Following a screen of 20,000 polymers and copolymers and subsequent scale-up, the hit acrylate polymer, EGdPEA has been clinically approved for the coating of urinary tract catheters and presents superior antibiofilm properties compared with silver-embedded silicone catheters. The bacterial biofilm resistance mechanism and surface sensing pathways involved are under investigation. In *P. aeruginosa* this includes the flagellar stators, type IV pili and SadB(Laventie et al., 2019). The latter is involved in the switch from reversible to irreversible surface attachment. Expression of sadB under a constitutive promoter overcomes the resistance of EGdPEA to biofilm formation (Carabelli et al., 2022).

Here we investigate the regulation of sadB and its downstream targets. The sadB gene was found to be differentially transcribed on EGdPEA compared with NGPDA indicating the importance of sadB in surface recognition. The absence of sadB inhibits biofilm formation but the function and pathway of sadB are currently unknown(Caiazza & O'Toole, 2004). We employed whole transcriptome analysis (RNAseq) to investigate the genes that are regulated by sadB in an untargeted manner and RT-PCR and phenotypic assay to validate the data. SadB was shown to negatively affect the PQS system and subsequently the rhl system, rhamnolipid and pyocyanin production and the denitrification pathway. Furthermore, sadB positively affected biofilm related genes such as Psl and c-di-GMP production. Consequently, SadB is a global regulator controlling up to 50% of the *P. aeruginosa* transcriptome.
PS3- Rapid and sensitive test for *E. coli* O157:H7 detection

Shayesteh Bazsefidpar, Gemma Gutierrez, Esther Serrano, Alberto Sánchez Calvo, María Matos and María Carmen Blanco-López

1 Department of Physical and Analytical Chemistry & Institute of Biotechnology of Asturias, University of Oviedo, c/Julián Clavería 8, 33006 Oviedo, Spain
2 Department of Chemical and Environmental Engineering & Institute of Biotechnology of Asturias, University of Oviedo, Spain

*Escherichia coli* (*E. coli*) is the most common species of gram-negative bacillus in fecal flora. It has the ability to do colonization for the initial adhesion of bacteria cells to form a biofilm in human, animal hosts, and the environment[1]. Therefore, there is a need for early detection of *E.coli* bacteria before starting to make the biofilm. The great challenge is to achieve a low limit of detection and capture of planktonic cells before starting bacterial colonization. Lateral flow immunoassays (LFIA) are known for rapid, low-cost, and simple detection. Silver deposition on gold by mixing a silver salt with a reducing agent (hydroquinone buffered to acid pH by citric acid) is a strategy that causes the generation of silver nanoparticles on the surface of gold nanoparticles. It allows the enhancement of visual output signal which can be detected with the naked eye[2], [3]. In this work, a silver enhancement procedure has been developed with the aim of amplifying the signal of colloidal gold on the test strip to detect *E. coli* O157:H7 in the sandwich format. The best results were obtained when a mix of a solution containing silver nitrate and hydroquinone/citrate buffer in proportion 1:1 was introduced to the strips for 4 minutes. It was possible to decrease the limits of detection of the biosensor down to 2 x 10^3 (cfu/ml) using dilutions of an *E. coli* O157:H7 cultured in Tryptic Soy Broth (TSB) in PB buffer.

Funding: This work is part of a project that has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No. 813439 (Break Biofilms). The work was also supported by the project SV-PA-21-AYUD/2021/52132.


**PS4- Biofilm formation of *Staphylococcus aureus* from pets, livestock and wild animals**

**Vanessa Silva**¹,²,³,⁴, Elisete Correia⁵, José Eduardo Pereira⁶,⁷, Luís Maltez⁶,⁷, Gilberto Igrejas²,³,⁴, Patrícia Poeta¹,⁶,⁷

1 Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
2 Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
3 Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
4 LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
5 Center for Computational and Stochastic Mathematics (CEMAT), Department of Mathematics, UTAD, Vila Real, Portugal
6 CECAV—Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal
7 Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

*Staphylococcus aureus* is a ubiquitous organism that colonizes and infects humans and a wide range of other mammals and birds. In addition to the antimicrobial resistance commonly found among *S. aureus* isolates, their ability to form biofilms plays an important role in the maintenance of the infection. Biofilms are responsible for up to 80% of all human chronic and recurrent infections. However, in veterinary medicine, only a few studies report the role of biofilm in animal infections. In this study we aimed to compare the biofilm formation ability of *S. aureus* isolated from a wide range of animals, study the association between biofilm formation and antimicrobial resistance and genetic lineages, and investigate the effect of conventional antibiotics on biofilm mass reduction. A total of 214 *S. aureus* isolated from pets, livestock and wild animals were evaluated regarding their ability to form biofilms. Statistical analysis was used to find an association between biofilm formation and antimicrobial resistance, multidrug resistance, sequence types (STs), spa- and agr-types of the isolates. The antimicrobial susceptibility of 24h-old biofilms was assessed against minimum inhibitory concentrations (MIC) and 10xMIC of amikacin and tetracycline and the biomass reduction was measured. The metabolic activity of biofilm cells was evaluated by the XTT assay. All isolates formed biofilms. Yet, significant differences in biofilm biomass production were detected among animal species. Multidrug resistance had a positive association with biofilm formation as well as methicillin-resistance. Significant differences were also detected among the clonal lineages of the isolates. Both tetracycline and amikacin were able to significantly reduce the biofilm mass. However, none of the antimicrobials were able to eradicate the biofilm at the maximum concentration used. Our results provide important information on the biofilm-forming capacity of animal-adapted *S. aureus* isolates which may have potential implications for the development of new biofilm-targeted therapeutics.
Coagulase-negative staphylococci (CoNS) are commensal organisms that colonize the skin and mucous membranes of humans and animals but, in recent years, it has been found that CoNS are also opportunistic pathogens with ability to form biofilms on indwelling medical devices, making these infections extremely difficult to treat. The ability of biofilm formation seems to play an important role in the virulence of staphylococci. However, studies reporting biofilm formation of coagulase-negative staphylococci isolated from animals are still very scarce. Thus, we aimed to evaluate the biofilm formation capacity of CoNS isolated from several animal species and to investigate the effect of conventional antimicrobials of biofilm reduction. A total of 192 CoNS, comprising 12 different species, were included in this study. Biofilm formation was assessed by the microtiter plate assay and the biofilms were stained by crystal violet. Association between biofilm formation and staphylococci species and antimicrobial resistance was also performed. Biofilm susceptibility testing was performed with tetracycline and amikacin at minimum inhibitory concentration (MIC) and 10xMIC. The metabolic activity of biofilm cells after antimicrobial treatment was accessed by the XTT assay. All isolates formed biofilm with S. urealyticus producing the most biofilm biomass and S. hominis producing the least biomass. There was a positive association between biofilm formation and multidrug resistance as well as resistance to individual antimicrobials. Neither tetracycline nor amikacin were able to eradicate the biofilm not even at the highest concentration used. This study provides new insights into biofilm formation and effects of antimicrobials on CoNS species.
Bacterial infections are increasingly hard to treat with antibiotics due to antimicrobial resistance (AMR) and/or biofilm formation. Novel agents to combat such infections are urgently needed. Based on our successful pilot results which comprised various experiments, we first determined the in vitro bactericidal activity of the naturally derived compound HHV-001. In addition, a time- and dose-dependent bacterial killing by the compound was observed. Importantly, HHV-001 did not induce resistance in vitro in A. baumannii, E. coli and poorly in S. aureus using 25 serial passages, while being effective in killing AMR ESKAPE bacteria and colistin-resistant E. coli in human plasma and in other relevant bodily fluids. Furthermore, HHV-001 dose-dependently degraded 24 hrs and 7 days mature AMR bacterial biofilms. The effects of HHV-001 on bacterial biofilms were confirmed using quantitative imaging techniques like confocal laser scanning and atomic force microscopy. Interestingly, the compound was equally effective against bacteria in bimicrobial biofilms as in monomicrobial biofilms. In addition, HHV-001 was highly effective against a panel of 18 clinical E. coli isolates. Moreover, transcriptomics experiments provided some insight in the antibacterial activities of the compound. Finally, the in vivo safety and efficacy of HHV-001 in animal models will be presented. Collectively, this data indicates that HHV-001 has potential as a novel agent for treatment of bacterial biofilm-associated infections that are hard to treat with current antibiotics.
PS7- Development of an alternative high-throughput method to assess antimicrobial susceptibility of *Pseudomonas aeruginosa* biofilms

**Amber De Bleeckere**¹, Sara Van den Bossche¹, Aurélie Crabbé¹, Tom Coenye¹

1 Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

The presence of biofilms in cystic fibrosis (CF) patients suffering from chronic lung infections, contributes to failure of antimicrobial therapy. Determination of the MIC is conventionally used to assess the antimicrobial susceptibility of a pathogen but whereas MICs can predict treatment success in infections involving planktonic cells, they do not always predict success in treating biofilm-associated infections. This discrepancy can be attributed to disregarding environmental conditions within a biofilm when evaluating antimicrobial susceptibility. To overcome this problem conventional microbial growth media could be replaced by media that better mimic the in vivo conditions, e.g. the synthetic cystic fibrosis sputum medium (SCFM) designed to mimic the microenvironment of the CF lung.

We determined the minimal biofilm inhibiting concentration (MBIC) of different antibiotics (tobramycin, ciprofloxacin and colistin) for a selection of *Pseudomonas aeruginosa* CF isolates, using a microtiter plate-based assay with SCFM as a growth medium and a high-throughput resazurin-based viability staining as read-out. For control purposes, the content of all wells was plated in parallel. MBICs were compared to MICs determined using microbroth dilution according to EUCAST guidelines.

For validation purposes, the correlation between the resazurin-derived fluorescence and CFU counts was assessed. A significant correlation was observed for all strains, suggesting this fluorometric assay is a reliable alternative for plating. For all isolates investigated (n=9), a clear difference between MICs and MBICs of all antibiotics tested was observed, with the latter being consistently higher, although the extent of the difference between MBIC and MIC was antibiotic- and strain-dependent.

Our alternative approach allows to obtain reliable measures about *P. aeruginosa* biofilm susceptibility within 24h and can easily be implemented in a routine clinical microbiology lab in high-throughput format. However, whether results obtained with our alternative assay better predict the in vivo antimicrobial susceptibility in *P. aeruginosa* biofilms remains to be confirmed.
**PS8- Staphylococcus aureus forms biofilm aggregates with reduced antimicrobial susceptibility in a synthetic synovial fluid model**

Amber De Bleeckere¹, Frits Van Charante¹, Fabien Lamret³, Aurélie Crabbé¹, Fany Reffuveille³, Tom Coenye¹

1 Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium
2 Laboratory EA 4691 Biomaterials and Inflammation in Bone Site (BIOS), Reims, France

Along with the rising number of periprosthetic joint implants, the risk of periprosthetic joint infections (PJIs) is increasing. There is growing evidence that bacteria form surface attached biofilms on prostheses, as well as biofilm aggregates embedded in the synovial fluid present in human joints. However, tools to investigate these biofilms in a context relevant for PJIs (including antimicrobial susceptibility testing) are currently lacking. To address this, we developed a synthetic synovial fluid (SSF) model, containing proteins, organic compounds, amino acids and hyaluronic acid.

In the present study we have focused on *Staphylococcus aureus*, as this species is the main causative organism in PJIs (four strains were included). Firstly, growth rates in SSF were determined and compared to those in TSB. Secondly, the presence of aggregates was examined using light microscopy. Finally, antimicrobial susceptibility to antibiotics relevant for the treatment of PJIs was investigated.

Slow growth of *S. aureus* was observed in SSF compared to growth in TSB, which resembles the situation in vivo. Microscopy showed suspended biofilm aggregates, similar to those observed in vivo. These aggregates showed a reduced susceptibility to the antibiotics tested. Our results suggest that this in vitro SSF model is a valuable alternative for growing *S. aureus* biofilms in conditions that resemble those encountered in PJIs and that the model allows antimicrobial susceptibility testing.

However, future experiments are needed to optimize this in vitro SFF model. Firstly, the influence of the components fibrinogen and fibronectin (recently reported to affect *S. aureus* biofilm formation), on aggregate formation in SSF will be studied. Furthermore, the effect of incubation time and oxygen concentrations on aggregate formation will be assessed. After optimization of the SSF we will investigate its ability to support aggregate formation in a larger collection of *S. aureus* isolates, as well as isolates from other PJI-related species.
PS9- Acid-tolerance in dental biofilms from caries-active and caries-free pre-school children in Sweden

Gabriella Boisen¹, Jessica Neilands¹, Susanne Brogårdh-Roth², Julia R. Davies¹

1. Section of Oral Biology and Pathology, Faculty of Odontology, Malmö University, Malmö, SWEDEN
2. Section of Pediatric Dentistry, Faculty of Odontology, Malmö University, Malmö, SWEDEN

Introduction
During caries development the selection of acid-tolerant bacteria eventually results in highly aciduric dental plaque biofilms, which promote enamel demineralization. Consequently, individuals with highly acid tolerant plaque would be expected to have an increased risk of developing caries compared to those with non-acid tolerant biofilms. As part of a wider investigation to explore whether plaque acid tolerance could represent an early biomarker to predict dental caries, in this study, the acid tolerance of dental plaque from two extreme groups – children with severe caries and healthy subjects was compared.

Hypothesis
Acid tolerance (AT) in dental plaque is higher in caries-active individuals compared to those who are caries-free.

Methodology
Dental plaque samples were collected from pre-school children (2-5 years); 15 with severe caries (CA) and 15 who were caries-free (CF). AT was analyzed by exposing the samples to pH 3.5 for 2 hours, followed by staining with LIVE/DEAD® BacLight Viability stain. Samples were examined with CLSM and bacteria viable after the challenge were considered as acid tolerant (AT). Levels of AT were evaluated using a scoring system ranging from 1 (no/low AT), to 5 (high/all AT).

Results
The AT scores of the CA group was significantly higher than those of the CF group (p<0.01). All individuals with the highest AT scores (score 4-5) belonged to the CA group. The AT scores of the CF group ranged from 1-3, where 73% had an AT score of 1-2.

Conclusion
Our results suggest that plaque AT is increased in individuals with active caries compared to caries-free individuals. Further studies will be aimed at elucidating the potential of plaque AT as a predictive biomarker for caries by investigating whether high AT in plaque precedes disease development.
P61- In vitro evaluation of the antibacterial activity of four daily-use mouthrinses with different active ingredient concentrations

Sergio Isabal, Dentaid Research Center
Vanessa Blanc, Dentaid Research Center
Rubén León, Dentaid Research Center

DENTAID, in Cerdanyola del Valles (Barcelona), Spain

Introduction: Chlorhexidine (CHX), Cetylpyridinium Chloride (CPC) and molecular iodine (I2) are antiseptic molecules formulated in mouthwashes for daily use. In the present study, we compared the antimicrobial activity on multispecies oral biofilms of four mouthrinses containing CHX, CPC and I2 (A: 0.12% CHX and 0.05% CPC; B: 0.5% CHX and 0.05% CPC; C: 50 ppm I2; D: 100 ppm I2)

Material and Methods: Oral biofilms formed by S. oralis, A. naeslundii, V. parvula, F. nucleatum, A. actinomycetemcomitans and P. gingivalis grew on hydroxyapatite discs for 96 hours at 37°C in anaerobiosis. Treatment with the mouthrinses lasted for 2 minutes. The biofilms were then mechanically disaggregated for 5 minutes in 1 ml of phosphate-buffered saline. Survival was calculated by counting viable colonies grown on blood agar and Dentaid-1 culture plates. Kruskall-Wallis and Dunntest were used for all comparisons.

Results: Significant differences in terms of antibiofilm efficacy were observed between the four products tested (p-value 0.001). Mouthwashes A and B caused a significantly higher absolute mortality than the other formulations (p-value A vs C 0.001; p-value A vs D 0.001; p-value B vs C 0.001; p-value B vs D 0.001). The percentage of mortality was also significantly higher for mouthrinses A and B (A: 99.48%, B: 98.57%, C: 26.76%; D: 40.66%). No significant differences were observed when comparing the antimicrobial activity of A vs B and of C vs D (p-value A vs B: 0.198; p-value C vs D: 0.0558).

Conclusion: Mouthrinse A showed the greatest antibiofilm power. The mouthrinses containing CHX and CPC in their formulation showed a higher antimicrobial capacity. The bactericidal power of molecular iodine on biofilm is low, and therefore, may not be effective in mouthrinses.
P63- New insight into the discovery of novel antibiofilm agents against *Pseudomonas aeruginosa* based on seaweed extracts

Maya Rima 1,2, Asma Chbani 2,3, Christine Roques 1, and Fatima El Garah 1

1 Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France; maya.rima@univ-tlse3.fr (M.R.); jeanne.trognon@univ-tlse3.fr (J.T.); latapie@chimie.ups-tlse.fr (L.L.)
2 Laboratory of Applied Biotechnology, AZM Center for Research in Biotechnology and Its Applications, Doctoral School of Science and Technology, Lebanese University, El Mittein Street, Tripoli, Lebanon; asmashbani61@gmail.com
3 Faculty of Public Health III, Lebanese University, Tripoli, Lebanon

The “superbug” *Pseudomonas aeruginosa*, member of “ESKAPE pathogens” group and defined by the WHO as a critical priority, is known as an opportunistic human pathogen often associated with chronic and nosocomial infections [1, 2]. Its increased ability to form a resilient biofilm provides it with a powerful armor against host defenses, as well as against anti-*Pseudomonas* antibiotics [3]. Thus, the search for new antibiofilm agents to effectively prevent and/or treat *Pseudomonas* biofilm associated infections, especially of natural origin, is currently the object of much attention. In the present study, we have focused on the exploration of extracts derived from three seaweed: the green *Ulva lactuca*, the brown *Stypocaulon scoparium*, and the red *Pterocladiella capillacea*, in terms of their potential antibiofilm activity against *P. aeruginosa* [4]. Various extracts were prepared by successive maceration in solvents with increasing polarity (cyclohexane, dichloromethane, ethyl acetate, and methanol). Then, their potential biofilm inhibition and eradication abilities were assessed using two complementary methods: crystal violet staining and quantification of adherent bacteria. An epifluorescence microscopic analysis of biofilm formed in presence of the active extracts was also performed. Interestingly, two extracts (cyclohexane and ethyl acetate) derived from *U. lactuca* exhibited a promising antibiofilm activity by involving two distinct mechanisms of action, which was supported by the microscopic analysis. The possible synergistic activity between the ethyl acetate extract, potential disruptor of the protective biofilm matrix, and tobramycin and colistin was then evaluated. Interestingly, this extract showed a promising synergistic effect with tobramycin. Concerning the chemical composition of extracts, first analyses by GC-MS permitted the identification of several molecules particularly phenolic compounds. These findings suggest the richness of the green alga *U. lactuca* in antibiofilm candidates with preventive and curative effects whether used alone or in combination with conventional antibiotics.

The role of non-typeable *Haemophilus influenzae* in cystic fibrosis lung infection

**Phoebe Do Carmo Silva** (University of Warwick), Dr. Darryl Hill (University of Bristol) and Dr. Freya Harrison (University of Warwick)

Non-typeable *Haemophilus influenzae* is a relatively understudied pathogen that colonises the lungs of people with cystic fibrosis (CF), a disease leading to the build-up of viscous mucus within the lungs. In CF, *H. influenzae* is one of the first pathogens to invade the lungs prior to later stage, more severe pathogens such as *Pseudomonas aeruginosa* which are life-threatening. As such, *H. influenzae* may be paving the way for these later stage pathogens to colonise the lungs and alter disease progression.

Within my PhD, I am using the ex-vivo pig lung model as a realistic model for respiratory infections, combined with synthetic CF sputum media to mimic the nutritional composition of CF sputum. This model will aim to answer key questions relating to how *H. influenzae* infection affects disease progression of CF, including:

- Investigating into the biofilm formation of *H. influenzae* and its structure on host lung tissue.
- Determining whether the growth of *H. influenzae* as a biofilm in the lungs affects susceptibility to standard antibiotic treatment.
- Determining what host factors in the CF lung cue clinically-relevant environmental changes that potentially alter colonisation of later-stage, more severe pathogens.

In this poster, I will present my preliminary observations from the first stages of my PhD to answer these questions on *H. influenzae* biology with the use of our realistic CF lung model.

An understanding of early bacterial colonisers in the CF lung becomes increasingly important as more people with CF receive treatment and modulator therapy that aid them to not progress to “classic” chronic infection with later stage pathogens. As such, the understanding of the physiology and ecology of early-stage pathogen infection will help to shed light on how *H. influenzae* alters the course of CF disease progression.
Non-healing chronic wounds are a considerable worldwide healthcare burden. Biofilms have been strongly implicated in delayed wound healing. Microorganisms within biofilms are less sensitive to antimicrobials than their planktonic counterparts. Slow microbial growth rates are a recognized driver of tolerance towards antimicrobial agents and poor treatment outcomes. We have developed an in vivo excisional murine wound model to investigate the mechanisms of wound biofilm formation and tolerance. Here, we compared biofilm formation within wounds infected with preformed biofilms of *Staphylococcus aureus* with wounds infected by the same bacterium in planktonic form. Bromodeoxyuridine (BrdU), a nucleotide analogue that incorporates into nascent DNA in actively dividing cells was used to visualise proliferating. Biofilms were exposed to BrdU for 4 h before harvesting at 1, 3 and 7 days. Wound healing was characterised by quantification of wound area and re-epithelialisation from histological sections, and neutrophils/macrophage marker immunohistochemistry. Wound biofilms were characterised by anti-BrdU immunofluorescence, tissue Gram stain, quantitative bacterial culture, and scanning electron microscopy. Uninoculated control wounds healed within 7 days, whereas wounds infected with preformed biofilm and planktonic *S. aureus* showed delayed healing to a similar extent. Over 7 days, re-epithelisation was comparable in wounds infected with planktonic and preformed biofilms, but lower than in uninfected wounds. The number of neutrophils and macrophages were significantly higher in wounds infected with preformed biofilm than in wounds infected with planktonic bacteria. Strong BrdU staining was observed corresponding to the active growth of *S. aureus*, one- and three-days post-wounding. In contrast, slow metabolic rates occurred seven days post-wounding. The presence of *S. aureus* on the surface of both planktonic and biofilm infected wounds was markedly increased after seven days. These models can be used to evaluate the pre-clinical efficacy of antimicrobial wound dressings against wound biofilm infections.
The biofilm-induced oral disease, periodontitis, affects around 60% of Swedish adults and in around 10% of these, tissue destruction is so extensive that they risk losing teeth. Currently there is no effective way of predicting which patients will develop severe disease and a significant number are falsely identified as being “at risk” and subject to costly and unnecessary preventive treatment.

We hypothesize that levels of proteolytic activity in fluid sampled from subgingival biofilms (GCF) could be used to distinguish patients at risk of further extensive periodontal tissue destruction from those with low risk.

Two patient groups: 48 identified as having risk for further tissue destruction and 48 identified as having low risk were included in the study. The risk group had bleeding on probing (BOP) and a probing depth of ≥4mm with evidence of ongoing bone loss over the past year while the low-risk group had no BOP, a probing depth of ≥4mm and no signs of bone loss. Samples of GCF collected from 5 sites per patient using a capillary tube were pooled and proteolytic activity assessed using a fluorescent substrate-based assay. Activity was compared with a trypsin standard curve.

Overall levels of proteolytic activity ranged between 0 and 49755 relative fluorescent units (RFU) corresponding to 0 - 0,28µg trypsin/ml. The median activity in samples from the patients deemed to be at risk or with low risk were 10697 RFU and 360.5 RFU, respectively. This difference was significant at the 0.1% level (Mann Whitney test).

These results show that levels of proteolytic activity in fluid from subgingival biofilms differ significantly between patient groups and can be correlated to their risk for further periodontal tissue destruction as predicted by clinical assessment. Proteolytic activity may thus be a good potential biomarker to aid risk assessment in periodontitis.
P67- Wound Infection On A Chip: A Human Relevant, Biomimetic Platform To Recapitulate The Dynamic Wound Infection Microenvironment

Nijamuddin Shaikh, Karishma Kaushik
Department of Biotechnology, Savitribai Phule Pune University, INDIA

Chronic wounds incur a significant expense on the healthcare sector and the problem of cutaneous wounds is further exacerbated by increasing trends in surgical intervention, diabetes, obesity, and an aging population. Bacterial biofilms formed in the wound microenvironment is a prominent reason for progression of wounds into non-healing, chronic state. The wound infection state is a dynamic microenvironment with intricate interplay across host elements, microbial biofilms, chemical cues, and biophysical factors. Forces such as shear stress and fluid flow influence a range of wound healing outcomes including cell proliferation, migration, and communication. However, the effects of biophysical forces on wound biofilms, particularly in context of the composite microenvironment, remains to be explored. Current wound infection studies present a range of scientific, technical, and ethical limitations, ergo the need to develop a platform closely mimicking the wound infection microenvironment. Using CAD software and 3D printing, we have developed a palm-sized ‘macrofluidic device’, which we term as ‘wound infection on a chip’. The device is made of polylactic acid, a biocompatible polymer, and consists of a transparent centrally placed chamber, with inlet and outlet channels connected to an in-house developed, battery-operated, semi-automated peristaltic pump, facilitating flow of growth medium and hence creating shear stress. Human epidermal keratinocytes (HaCaT) and dermal fibroblasts (HDFa) are seeded from the top portion of the chamber. The central chamber of the reconstituted ‘wound infection on a chip’ supports the proliferation of HaCaT and HDFa cells, alone and in co-culture. Using the scratch assay, to mimic wound injury, the co-cultured ‘wound bed’ demonstrates robust host cell migration and wound closure. The host-microbe interactions are currently being studied with host scaffolds and mixed species wound pathogens. The next steps include leveraging the ‘wound infection on a chip’ system to study the effects of biophysical forces on the formation and development biofilms grown on host cells and dissect host-microbe interactions, with the inclusion of a relevant wound chemical milieu. In doing so, the platform will support the development of a composite infection microenvironment, enabling hitherto unknown host-pathogen insights, such as the formation of biofilms in close proximity with host cells, and serve as a relevant approach to evaluating novel and composite wound infection treatments.
Oral biofilms can cause inflammation of the gum, gingivitis and periodontal disease if left untreated. Current methods of biofilm characterisation are destructive, time consuming, and do not allow the same biofilm to be analysed over time. Here we present a non-destructive method for in-situ characterisation of multispecies oral biofilms using Raman Spectroscopy (RS) and Microfluidics. RS is a form of vibrational spectroscopy where light interacts inelastically with matter to produce a spectrum showing the structural fingerprint of that sample. Growing biofilms using microfluidics allows the whole experiment to take place on one chip, and has the advantages of being able to control microenvironments, small samples sizes, non-destructive rapid analysis and low cost.

Single channel PDMS microfluidic devices were made with 2 inlets and 1 waste outlet. A salivary pellicle was formed before Streptococcus salivarius (S.s) and Actinomyces naeslundii (A.n) were injected into the chamber, at 1:3 ratio. Biofilms were grown under flow for 5 days. Raman Spectra and confocal images were taken every 24hrs. Images were taken after live/dead staining. PCA and LDA were used to process Raman spectra.

PCA is shown to differentiate between both planktonic species, with peaks at 746 and 1128 cm⁻¹ being associated with A.n. Cluster analysis allows the individual species within the biofilm to be mapped. LDA of Raman spectra from each day of the biofilm shows clear clustering of each day, with the clearest separation between Day 1 and Day 5. Live/dead staining shows an increase proportion of dead cells as the biofilm matures, with S.s appearing to die off first.

A non-destructive method for biofilm analysis was successfully created. Future works include using FISH to map the species within the biofilm, combining Microbubbles and ultrasound to treat and remove the biofilm, and developing computational models to follow the maturation of species over time.
Chemical environments determine the molecular interactions among bacterial populations and their physiological responses within biofilms. Electrochemical methodologies have emerged as robust ways to study chemical interactions, capable of providing information of the chemical environment spatially resolved at micron-scale resolution. However, scanning electrochemistry experimental platforms require specialized equipment that performs only one or a few techniques. In recent studies, the Whiteley lab has bridged the gap between microbiological methods of understanding bacterial physiology and electrochemical techniques to study biofilms. We developed an inexpensive instrumental setup for microbiological laboratories. Our design positions in-house fabricated electrodes on the micron-scale and scans biofilm surfaces to measure 3D concentration gradients of molecules, assess kinetic parameters including molecular detoxification rates, and map redox potentials of biofilms. We investigated the physiology of two model biofilm systems (1) the oxygen consumption of the bacterial pathogen *Pseudomonas aeruginosa* with and without antibiotics present and (2) the redox interactions in a multispecies oral biofilm. We developed an electrode capable of sensing oxygen and observed that nascent *P. aeruginosa* biofilms consume oxygen at maximum rates and continue to do so despite ciprofloxacin treatment where 99% of cells are killed. In oral biofilms, the oral pathogen *Aggregatibacter actinomycetemcomitans* and the oral commensal *Streptococcus gordonii* interact via redox molecules. Our results indicate that *S. gordonii* monoculture and coculture biofilms exhibit a higher activity of oxidized species 100-150 µm away from the biofilm as compared to *A. actinomycetemcomitans* monocultures. Collectively, this novel electrochemical framework is capable of providing fundamental insights into biofilm physiology in a non-invasive fashion in real-time.
P70- Assessing the role of biofilms in tonsillar diseases using optical mesoscopy

Megan Clapperton [1], Catriona Douglas [2], Gail McConnell [1]

[1] Department of Physics, University of Strathclyde, 16 Richmond St, Glasgow G1 1XQ
[2] Department of Otolaryngology, Head and Neck Surgery, Queen Elizabeth University Hospital, 1345 Govan Road, Glasgow, G51 4TF

The palatine tonsil is an immune organ located at the opening to the pharynx and is known to be associated with childhood illness. Despite this, the pathogenesis of such common childhood diseases are relatively understudied. Diseases such as tonsillitis and obstructive sleep apnoea (OSA) result in many tonsillectomies per year in the UK, causing a huge economic burden to the NHS [1].

Biofilms are known to be involved in chronic infections and to be resistant to antibiotic treatment. Biofilms have been demonstrated to inhabit the tonsils, however, studies have been limited to small fields of view, allowing only to visualise a small region of the tonsil at a time [2].

We report the first demonstration of mesoscopic imaging of biofilms in the palatine tonsil with use of the Mesolens, with a view to understanding the role of biofilms in tonsillar disease. The Mesolens offers the unique combination of a low magnification (4x) and high numerical aperture (0.47) lens, and gives sub-cellular resolution imaging within a tissue volume exceeding 100 mm3 [3]. We have used this to image fluorescently labelled biofilms in tonsils.

Tonsils were removed via routine tonsillectomy due to either tonsillitis or OSA. They were collected from theatre at the Royal Hospital for Children, Glasgow, and transported to the University of Strathclyde for fluorescent labelling and imaging with the Mesolens within 3 hours of excision from the patient. To our knowledge this is the most rapid turnaround of tonsil tissue from theatre to fluorescence microscope. Data were rendered for visualisation and all analysis was performed in Imaris.

We will present details of our methods for the preparation, staining and imaging of ultra-thick, living palatine tonsil tissue and our data analysis pipeline, together with data confirming biofilms are not limited to tonsil crypts but are more widespread than previously reported.
P71- Exploration of selected Traditional Asian phytomolecules as anti-caries agents against *Streptococcus mutans*

Sahana Vasudevan¹, Prasanna Neelakantan² and Adline Princy Solomon¹

1. Quorum Sensing Laboratory, Centre for Research in Infectious Diseases (CRID), School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur, 613401, India.

2. Faculty of Dentistry, The University of Hong Kong, Hong Kong, SAR

**Introduction:**
Dental caries is a silent epidemic affecting over 4 billion people worldwide and is a significant public healthcare concern. *Streptococcus mutans* is the primary etiological agent which contributes to oral dysbiosis, leading to the progression of the disease. Biologically active compounds from medicinal plants hold a promise of effective antimicrobials and are currently in international research hotspots as alternates to antibiotics.

**Hypothesis:**
trans-Cinnamaldehyde, Baicalin and Baicalein downregulate the *S. mutans* virulence associated with biofilm and acid production.

**Methodology:**
*Streptococcus mutans* was treated with trans- Cinnamaldehyde (TC), Baicalin (BIN) and Baicalein (BEN) to evaluate its antimicrobial activity per CLSI guidelines. At sub-inhibitory concentrations, the antibiofilm activity was assessed. Further, the effect on the acid accumulation in the presence of the phytomolecules was estimated using a glycolytic pH-drop assay. The anti-biofilm activity was qualitatively evaluated through microscopic imaging analysis – CLSM and SEM. The ability to potentiate the non-specific antimicrobials was tested using a checkerboard assay. The mechanistic studies were carried out by qRTPCR studies.

**Results:**
TC, BIN and BEN significantly inhibited biofilm formation without having any effect on bacterial growth. The acid production was considerably reduced till 8 h in the treatment conditions. SEM micrographs confirmed the reduction in biofilm biomass and reduction in the chain length of *S. mutans*. CLSM analysis of the treated biofilm further confirmed the biofilm inhibition and reduced biofilm thickness. TC and BEN potentiated the antimicrobial activity of fluoride. Gene expression studies revealed the downregulation of the genes responsible for biofilm, acid production and associated virulence characteristics.

**Conclusion:**
trans-Cinnamaldehyde, Baicalin and Baicalein are proven to have promising anti-caries activity against *S. mutans* and have the potential to be developed as anti-caries agents.
Dental unit water systems (DUWS) contain a complex inter-kingdom biofilm comprising of bacteria, fungi and protozoa. Biofilm control procedures for DUWS are mainly focused on the disinfection of the bacterial content, while removal of the whole biofilm is desirable. Amoeba present in the DUWS can feed off the remaining biofilm and play an important role in bacterial virulence and antimicrobial resistance. The aim of this study, therefore, was to investigate the biofilm removal potential and amoebicidal activity of commercially available DUWS cleaning agents.

Using the BioFlux platform, multiple DUWS cleaning products were assessed for their biofilm removal efficacy on 5-day old water biofilms. None of the products tested resulted in consistent biofilm removal, however Oxygenal (containing 0.02% hydrogen peroxide) had an anti-amoeba activity on the trophozoite, albeit not on the cyst form. Testing higher concentrations of H2O2 did result in lysis of the amoebal cyst form. Subsequently, 4-week old biofilms, grown in an in-vitro dynamic flow DUWS biofilm model, were subjected to 3 different types of cleaning regimens. These regimens, a combination of a daily low dose (DLD, 0.02% H2O2) with or without a weekly high dose (WHD, 0.25% H2O2), were tested longitudinally for 7 weeks. Treatment efficacy was monitored by analyzing ‘proxy’ biofilm samples for heterotrophic plate counts (HPC) and, using quantitative PCR, for the gene copy number (GCN) of *Vermamoeba vermiformis*.

At week 7, all DLD regimens, when compared to the negative control, resulted in a significant reduction of the *V. vermiformis* GCN to below the detection limit (p4 LOG10 CFU/ml, p0.005).

To conclude, only consistent DLD treatment, combined with a WHD is sufficient to reduce both the biofilm viability and the presence of amoeba in DUWS biofilm.
P77- Investigating anti-biofilm properties of doped bioactive glass fibres on an in vitro chronic wound biofilm model

Sandeep Shirgill, School of Dentistry, School of Mathematics, University of Birmingham
Dr Sarah Kuehne, School of Dentistry, University of Birmingham
Dr Gowsihan Pooologasundarampillai, School of Dentistry, University of Birmingham
Dr Sara Jabbari, School of Mathematics, University of Birmingham
Dr John Ward, Department of Mathematics Sciences, Loughborough University

Chronic wounds (principally pressure sores, venous leg ulcers and diabetic foot ulcers) are a drain on global health services and remain a major area of unmet clinical need. Chronic wounds are characterised by a stable and stubborn bacterial biofilm which hinders innate immune response and delays or prevents wound healing. Recently, research has delved into “biofilm-based wound care” as an effective strategy for healing wounds, where the first step of treatment is to disrupt the biofilm. Bioactive glass fibres, which have a “cotton-wool” like appearance, offer a promising biofilm-based wound care treatment for chronic wounds. When fibres are placed in fluid, they undergo multiple chemical reactions such as: leeching, dissolution, and precipitation. Si4+ and Ca2+ ions are released during dissolution and can promote wound healing. Fibres may also be doped with other inorganic ions, for example Ag+ and Cu2+, which are known to have antimicrobial activity.

Here, an in vitro chronic wound biofilm model is developed. This model is used to test the antimicrobial activity of normal fibres compared to fibres doped with Ag+ and Cu2+ on single species and co-culture biofilms containing common bacterial species found in chronic wound biofilms, namely Pseudomonas aeruginosa and Staphylococcus aureus.

Fibres doped with inorganic ions are more successful than undoped fibres at inhibiting biofilm formation and demonstrate higher antimicrobial activity against 24hr biofilms. However, physical properties of the fibres themselves, e.g., sharp glass debris, contribute to the antimicrobial action as it was found that placing fibres on top of inoculated membranes was more effective at inhibiting biofilm formation than using the fibre dissolution products. These results demonstrate that bioactive glass fibres could act as a potential treatment for infected chronic wounds.
P78- codY deletion impacts S. epidermidis 1457 biomass production and VBNC cells formation

Nathalie Lopes¹, Nuno Cerca¹, Ângela França¹

1 LIBRO, CEB, University of Minho, Braga, Portugal

Introduction and Hypothesis: CodY, a transcription regulator, was previously found to repress hundreds of genes in Staphylococcus aureus under nutrient-limited conditions. Under these conditions, bacteria reduce their metabolic activity by entering a viable but non-culturable (VBNC) state, which consequently reduces antibiotics efficiency and increases the recurrence of biofilm-related infections. The alteration to this metabolic state was also observed in S. epidermidis and preliminary evidence suggest that CodY might have a role in VBNC state emergence, both in biofilm and planktonic cells. Herein, we proposed to construct an S. epidermidis strain lacking the gene codY to determine its role in VBNC state induction and other virulence factors.

Methodology: codY deletion mutant was constructed by allelic replacement in strain S. epidermidis 1457. The impact of codY deletion in (i) bacterial growth (OD, CFU), (ii) biofilm formation (biomass, CFU), (iii) VBNC state induction (OD, CFU), (iv) susceptibility to antibiotics (time kill-curves) and (v) interaction with human blood (CFU) was assessed. All analyses were performed with both the wild type (WT) and complemented strains.

Results: A reduced growth rate and biofilm biomass production were found in the codY mutant in comparison to the WT strain. More importantly, a higher number of culturable cells was found in the biofilms formed by the codY-deficient strain under VBNC-inducing conditions. Additionally, cells lacking codY were more susceptible to rifampicin. No other phenotypic differences were found between the mutant and the WT strain.

Conclusion: This is the first study that characterizes a codY mutant in S. epidermidis and the results obtained suggest, as reported before for S. aureus, that CodY repressor is involved in biofilm accumulation. Moreover, it seems that CodY may have an important role in VBNC cells formation in S. epidermidis. Nevertheless, further studies are necessary to underpin CodY regulatory genetic network leading to this phenotype.
P79- Attachment to host ECM at high flow: Staphylococcus epidermidis uses velcro-like attachment to fibronectin via the giant protein Embp


1: Interdisciplinary Nanoscience Center (iNANO), Aarhus University, 8000 Aarhus, Denmark.
2: Institute for Medical Microbiology, Virology and Hygiene, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany.
3: Department of Chemistry, Aarhus University, 8000 Aarhus C, Denmark.
4: Van ‘t Hoff Institute of Molecular Sciences, University of Amsterdam, 1098XH, Amsterdam, the Netherlands.
* Corresponding author: rikke.meyer@inano.au.dk

Introduction: The commensal skin bacterium Staphylococcus epidermidis is a main culprit of implant-associated infections because it forms biofilms. Implants are hotspots for biofilm infections, and S. epidermidis attaches to the implant via adhesins that bind to host proteins adsorbed to the implant surface. One such adhesin is the giant extracellular matrix-binding protein Embp. Embp interacts with fibronectin (Fn), and we wondered how its interaction can be directed towards adsorbed Fn, when soluble Fn is abundant in the blood.

Hypothesis: Soluble Fn is globular while adsorbed Fn can form fibrils, and we therefore hypothesized that Embp interacts selectively with fibrillated Fn to promote attachment.

Aim: The aim of this study was to determine the mechanism of Embp-mediated bacterial attachment to adsorbed Fn.

Method: We produced a model system in which Fn adsorbed to polymer-coated surfaces in either globular or fibrillated conformation. This was achieved on (poly)methyl acrylate (PMA) and (poly)ethyl acrylate (PEA), as Fn only fibrillates upon adsorption to PEA. Embp is a giant 1 MDa protein, which is anchored in the membrane of S. epidermidis. It contains 10 F and 40 FG domains that are involved in the interaction with Fn.

Results: We show that Embp-mediated attachment occur exclusively to fibrillated Fn, and involves the Fn domain FNIII 12th-14th, which is buried in globular Fn but exposed in fibrillated Fn. Single-molecule force spectroscopy revealed a Velcro-like interaction, as a single of the 50 Fn-binding FG domains in Embp interacted weakly, while 15 domains attached strongly with the fibrillated ligand. Expression of Embp was decisive for the cells’ adherence to Fn under high flow.

Conclusion: Embp enables selective and strong attachment to immobilized Fn via a single surface protein, and this mechanism is critical for attachment under high shear stress. Embp may thus be important for attachment in the vascular system.
P80- Endovascular aortic repair (EVAR) prosthesis infections – an in vitro model of biofilm formation

Torgny Sunnerhagen; Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; Clinical microbiology and Disease Control, Office for medical Services, Region Skåne, Lund, Sweden; Division of Infection Medicin, Department of Clinical Sciences Lund, Lund University, Lund, Sweden
Franziska Schwartz; Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark
Lars Christophersen; Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark
Nikolaj Eldrup, Department of Vascular Surgery Rigshospitalet, Copenhagen, Denmark
Katja Vogt, Department of Vascular Surgery Rigshospitalet, Copenhagen, Denmark
Claus Moser, Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark;
Department of Immunology and Microbiology, Costerton Biofilm Center, University of Copenhagen, Copenhagen, Denmark

Introduction
Endovascular aortic repair (EVAR) is one of several catheter-implanted vascular prosthesis and is the preferred treatment for suitable patients. If such a foreign body becomes the focus of an infection, the treatment possibilities are limited as it is technical difficult to remove the EVAR to obtain source control. The propensity of bacteria to form a biofilm on foreign material probably also plays a part in such infections, making them difficult to eliminate completely due to antibiotic tolerance.

Hypothesis and methods
Hypothesis, S. aureus and P. aeruginosa form biofilm on EVAR prostheses resulting in antibiotic tolerance. This was investigated using 100 mg EVAR pieces exposed to bacteria in vitro in LB medium at 37 °C, with the bacterial count being assessed by culturing of EVAR pieces and broth at 0, 4, 18 and 24, and 48 hours. Antibiotics tested were penicillin G, tobramycin and ciprofloxacin, at 10 times MIC. Tests were performed in duplicates.

Results
Five minutes of exposure to bacteria at 100 CFU/ml, followed by washing, established stable colonization in 50% of grafts. Exposure for 15 minutes established biofilm in 100% of prostheses. Already after 4 hours, bacteria were stably attached to the EVAR prostheses and resisted being washed away when dipped three times in sterile saline. The number of bacteria attached to the EVAR grafts increased until 24 hours of incubation to a level of approximately 109 CFU/gram. The bacteria became more tolerant to antibiotics on the EVAR grafts over time, without any differences of MIC being detected when tested not attached to the EVAR.

Conclusion
The results indicate that bacteria can adhere to EVAR shortly after an exposure that is comparable to what is seen in transient bacteremia in vivo, and that the bacteria rapidly form a stable adhesion with biofilm properties, including antibiotic tolerance.
P82- Impact of NirA on *Pseudomonas aeruginosa* on biofilm development under reduced oxygenation

**Samuel Fenn** (1), JF Dubern (1), L Eberl (2), A Brangonzi (3) and Miguel Cámara (1)
1 National Biofilms Innovation Centre, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom.
2 Department of Microbiology, Institute of Plant Biology, University of Zürich, Zürich, Switzerland.
3 Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy.
4 Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany

*P. aeruginosa* (PA) nitrate metabolism is essential for survival at reduced oxygenations encountered in biofilms and the host environment. We recently uncovered a new nitrite reductase nirA (PA4130) to be essential for PA infection in multiple disease models. Expression of nirA is induced by cyanide alongside the PA4129-34 gene cluster under low oxygenation. Cyanide binds to heme proteins such as the electron donor cytochrome c and cytochrome c oxidases, inhibiting aerobic respiration and denitrification. NirA is also dependent on siroheme for electron transfer, indicating this reductase is up-regulated by its own inhibitor. The discovery that cyanide up-regulated PA4133-34 encodes a cyanide-tolerant aerobic respiratory chain sub-unit, led us to hypothesise that NirA encodes a cyanide-resistant nitrite reductase.

Using purified NirA and the homologous PA sulphite reductase CysI (62% amino acid homology), we assessed nitrite/sulphite reduction in the presence of increasing concentrations of cyanide. NirA demonstrated activity up to 600μM cyanide, whilst CysI sulphite reductase activity was abolished at 50μM. To determine the biological relevance of NirA cyanide-tolerance, we compared the response of wild-type strains with a series of nirA mutants constructed in clinical strains, when exposed to reduced oxygenation using planktonic culture and colony biofilms. In the presence of nitrate under reduced oxygenation, colony biofilm development is impeded with reduction in colony-forming units observed in all strains harbouring the nirA deletion. Similarly, culture of nirA is compromised in planktonic culture in the presence of nitrate and reduced oxygenation, whilst aerobic growth demonstrated wild-type kinetics. We suggest that this inhibition in colony biofilm formation and planktonic culture in the presence of nitrate is due to enhanced cyanide production under low oxygen tension. With alternative nitrite reductases NirB and NirS also heme-dependent cyanide would inhibition reduction, resulting in nitrite accumulation in the absence of NirA.
P83- Efficacy of a metal chelator as therapeutic agent against staphylococci biofilms

Laurine Kaul¹,²,³, Regine Süss², Andrew Zannettino³,⁴, Katharina Richter¹,³

1 Richter Lab, Department of Surgery, Basil Hetzel Institute for Translational Health Research, University of Adelaide, Adelaide, Australia
2 Institute of Pharmaceutical Sciences, University of Freiburg, Freiburg, Germany
3 Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia
4 Precision Medicine Theme, South Australian Health & Medical Research Institute, Adelaide, Australia

Introduction: Staphylococcus aureus and Staphylococcus epidermidis are associated with many clinical infections, such as surgical site infections. These major infectious diseases frequently lead to clinical complications and increased healthcare costs, as standard treatments fail due to the rise of antibiotic resistance and the formation of biofilms. Therefore, new antibacterial treatments are urgently needed.

Hypothesis: A newly patented combination therapy comprising the metal chelator diethyldithiocarbamate (DDC) and copper ions (Cu²⁺) are effective against staphylococci biofilms.

Methodology: DDC and Cu²⁺ were tested in vitro for their antibacterial activity against methicillin-resistant S. aureus and S. epidermidis using the AlamarBlue viability assay. Prevention of bacterial attachment was assessed with the xCELLigence system and prevention of biofilm growth was observed with the Bioflux model. In vivo toxicity and efficacy of the DDC-Cu²⁺ combination was investigated in infected Galleria mellonella larvae over 4 days. Statistical analysis: log-rank test with Holm-Bonferroni adjustment of Kaplan-Meier survival curves.

Results: While DDC and Cu²⁺ alone failed to show substantial antibacterial activity, the DDC-Cu²⁺ combination demonstrated concentration-dependent antibacterial properties, with 99% MRSA and 87% S. epidermidis biofilm killing. When left untreated, staphylococci attached to surfaces over 48 h (cell index of MRSA: 0.50; S. epidermidis: 0.46), though attachment was prevented when treated with DDC-Cu²⁺ (cell index of MRSA: 0.04; S. epidermidis: 0.03). Extensive MRSA biofilm formation was prevented in the Bioflux system when treated with DDC-Cu²⁺ over 24 h. DDC-Cu²⁺ showed no toxicity in Galleria mellonella larvae and significantly increased the survival of MRSA infected larvae over 4 days (87% survival of infected, treated larvae vs. 47% survival of infected, untreated larvae, p<0.001).

Conclusion: DDC-Cu²⁺ inhibited the formation of staphylococci biofilms in vitro and showed non-toxicity and antimicrobial activity in vivo. Delivered in a thermosensitive gel, DDC-Cu²⁺ can progress to mammalian animal studies for the treatment of surgical site infections.
Antimicrobial resistance of various pathogens has put a heavy burden on the healthcare system and the general well-being of human beings. Probiotics have been widely studied for their antimicrobial effects and potential applications in wound treatment as an alternative to antibiotics. However, an effective and efficient carrier system has not been developed so far. We hypothesize that by encapsulating probiotics with proper materials, they can continuously secrete antibacterial molecules and deliver them to the infection site, resulting in the alleviation of infection and biofilm formation. The incorporation of probiotics suspension into a commercial wound dressing has shown significantly improved antibacterial effects against Pseudomonas aeruginosa and Staphylococcus aureus, two pathogens widely encountered in chronic wounds. Smarter materials, e.g., pH-responsive or thermo-responsive polymers, and better formulation of the encapsulation are now being investigated. pH-responsive materials allow the controlled release of the encapsulated probiotics according to the pH change of the wound. Thermo-responsive polymers, such as Pluronics F127, allow easy administration at room temperature and form a gel at body temperature. The success of this study should provide a potential carrier system for topical bacterial therapy.
**P86- Role of the mechanosensitive ion channel CmpX in biofilm formation under dynamic conditions in *Pseudomonas aeruginosa* H103**


Laboratory of Bacterial Communication and Anti-infectious Strategies, CBSA UR4312, Université de Rouen Normandie, Université de Caen Normandie, Normandie University, Evreux, France.

*Pseudomonas aeruginosa* is an opportunistic pathogen causing chronic infections that are related to its ability to form biofilms. Cells within these biofilms are subjected to a wide range of mechanical forces, and the shear stresses applied from the fluid flow regulate the communities. Mechanosensitive ion channels (Msc) are inner membrane proteins involved in response to osmotic shocks, whose opening relies on membrane tensions. In *P. aeruginosa*, CmpX is predicted to belong to the small Msc family (MscS), and the gene cmpX belongs to the cfrX-cmpX operon, with cfrX encoding a putative anti-sigma factor of the extracytoplasmic function sigma factor SigX. The cell envelope stress response sigma factor SigX controls expression of the cfrX-cmpX operon, is activated upon membrane tensions, and is involved in biofilm formation. To investigate the effect of CmpX on biofilm formation, an isogenic deletion mutant (PAOPX) and an overexpression strain (H103cmpX) were grown for 24 h at 37°C with a constant and regular supply of LB medium (3 mL.h⁻¹). Cells and matrix were observed by confocal laser scanning microscopy. Remarkably, H103cmpX showed increased biofilm formation with a significant change in architecture and the presence of numerous elongated cells. Most of these elongated cells displayed an increased permeability to propidium iodide and calcofluor white labeling, suggesting cell envelope alterations and accumulation of exopolysaccharides, respectively. In addition, a significant amount of extracellular DNA and proteins were detected within H103cmpX microcolonies compared to the wildtype strain, whose biofilm remained thin and homogeneous. Overall, these results suggest the involvement of CmpX in the biofilm architecture in dynamic conditions. These phenotypes were not observed when the flow was reduced to 1.2 mL.h⁻¹, suggesting that the mechanical constraints on bacteria generated by the medium flow could be at the origin of these modifications. The molecular mechanisms leading to such phenotypes are under investigation.
P87- *Pseudomonas protegens* controls *Pseudomonas aeruginosa* by multiple secreted factors to inhibit growth and induce biofilm dispersal

Sujatha Subramoni 1, Clarice Lee Zi Qi 2, Kwok Zi Rou 2 and Scott A. Rice 1,3

1 Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore
2 School of Biological Sciences, Nanyang Technological University, Singapore
3 CSIRO, Agriculture and Food, Microbiomes for One Systems Health, Australia

In nature, bacteria commonly occur as complex, multispecies communities that form biofilms. We have developed a defined mixed species community consisting of *Pseudomonas aeruginosa* PAO1, *Pseudomonas protegens* Pf-5 and *Klebsiella pneumoniae* KP1 that mimics several features of naturally occurring biofilms. We have reported a competitive interaction between *P. aeruginosa* and *P. protegens* in this biofilm community, mediated in part by the *P. aeruginosa* N-acyl homoserine lactone quorum sensing (QS) system. Here, we investigated the role of GacA-dependent secondary metabolites of *P. protegens* in this competitive interaction. One of these metabolites, orfamide A, mimicked *P. protegens*-induced growth inhibition of a *P. aeruginosa* lasRrhlR mutant in an agar plate growth assay. Mutants in gacA and ofaA were not growth inhibitory. Similarly, growth of the *P. aeruginosa* lasRhlR mutant was inhibited when grown in the presence of *P. protegens* wild type spent supernatant extract, but this effect was absent or reduced in the presence of gacA and ofaA supernatant extracts. Furthermore, both *P. protegens* wild type spent supernatant, and orfamide A at micromolar concentrations, inhibited biofilm formation by the *P. aeruginosa* wild type and lasRhlR mutant. Interestingly, *P. protegens* wild type supernatant dispersed *P. aeruginosa* lasRhlR mutant biofilms better than *P. aeruginosa* wild type biofilms. This effect was gacA-dependent, but only partly orfamide A-dependent, suggesting that factors affecting growth and biofilm dispersion are different. Our results suggest that *P. protegens* affects growth and biofilm dispersion of *P. aeruginosa* through several factors which together determine the steady state composition and function of the community.
Biofilms are significant problem in healthcare and our inability to effectively treat established biofilm infections hampers the treatment of chronic wound infections. One major problem in patient management is identifying when bacteria have a biofilm phenotype. Most methods of biofilm analysis destroy biofilm architecture which makes interpretation of results difficult. We have used Fourier-Transform Infra-Red (FTIR) spectroscopy to identify markers of the biofilm phenotype in an intact biofilm. 

*Staphylococcus epidermidis* (RP62A strain) was grown onto calcium fluoride substrate in a range of nutrient broths and analysed in triplicate at intervals from 1 hour to 96 hours using FTIR spectroscopy. The resulting spectra were analysed using Principal Component Analysis (PCA).

At 24 hours, differences were detected in the spectra of planktonic and biofilm cultures. There were distinct wavenumber shifts in the spectra in the Amide I region and peak shape changes in the key phospholipid and DNA backbone regions. This indicates the presence of new proteins in the sample at 24 hours of biofilm development. The spectra also showed changes in the amounts of pre-existing proteins at the various stages of biofilm growth.

FTIR spectroscopy has been used in a novel application to evaluate the chemical composition of biofilms without disrupting biofilm architecture. This will allow the non-destructive monitoring of the growth of biofilms in real time, to understand and chemically define the timeframe for irreversible attachment in the early stage of development. We expect this work to move towards examining the interaction of biofilms with antimicrobials.
P89- A novel formulation combining peracetic acid and reuterin to control biofilms in dairy processing plants

Nissa Niboucha¹ ² ³, Coralie Goetz¹ ² ³, Laurie Sanschagrin¹ ² ³, Steve Labrie¹ ², Ismaïl Fliss¹ ² ³ and Julie Jean¹ ² ³

¹ Département des Sciences des Aliments, Université Laval, Québec,
² Institut sur la Nutrition et les Aliments Fonctionnels (INAF), Université Laval, Québec
³ Regroupement Op+lait, Québec

Biofilms may become a recurrent problem in dairy processing plants, especially when control strategies are not completely effective in eliminating them. The use of conventional sanitizers leads to their persistence and regeneration; hence there is a need to develop disinfectant formulations more specific and adapted to biofilms of the dairy sector.

This study aims to develop a novel antibiofilm formulation by combining peracetic acid (PAA) or a PAA-based commercial disinfectant with reuterin and evaluate their effectiveness on single- and multi-species biofilms composed of dairy origin biofilmogenic bacteria.

To this end, single- and multi-species biofilms, including Pseudomonas azotoformans, Serratia liquefaciens and Bacillus licheniformis isolates were formed using MBEC (minimum biofilm eradication concentration) Assay® plates and the CDC biofilm reactor in the TSB medium. Simultaneously, the minimum inhibitory concentrations (MICs) of PAA and PAA-based disinfectant alone and then in combination with reuterin were calculated to subsequently determine the MBECs for single- and multi-species biofilms, using the MBEC Assay® and the single tube standard methods.

Our results showed that bacterial densities of the three isolates in multi-species biofilms corresponded to 5.87, 6.01 and 4.67 log CFU/mm² when grown on MBEC Assay® plates and to 7.41, 8.33, 6.09 log CFU/cm² using the CDC biofilm reactor for P. azotoformans, S. liquefaciens and B. licheniformis, respectively. Moreover, the MICs of PAA, PAA-based disinfectant and reuterin did not exceed 152 ppm, 45 ppm and 4.77 mM for the three isolates. Preliminary data revealed an additive effect for PAA when used in combination with reuterin, reducing their MICs to 38 ppm and 2.39 mM, respectively. Furthermore, ongoing investigations would allow MBECs determination.

Used at lower concentration in combination with reuterin, PAA could be a promising strategy, as well as more environmentally friendly approach for eliminating biofilms on stainless food contact surfaces in the dairy industry.
P91- Involvement of the antitermination factor AmiR and the ami operon in *Pseudomonas aeruginosa* biofilm regulation

Mélissande Louis¹, Ali Tahrioui¹, Thomas Clamens¹, Amine M. Boukerb¹, Julien Verdon², Izelenn Dufour², Romain Villéger², Lisa Wallart¹, Sophie Rodrigues¹, Alain Dufour³, Laure Taupin³, Julie Hardouin⁴, Rafael Ruiz de la Haba⁵, Emeline Bouffartigues¹, Marc G.J. Feuilloley¹, Pierre Cornelis³, Sylvie Chevalier³, Olivier Lesouhaitier¹

1Unité de Recherche Communication bactérienne et Stratégies Anti-infectieuses CBSA UR4312, University of Rouen Normandy, 27000 Evreux, France
2Laboratoire Ecologie & Biologie des Interactions, UMR CNRS 7267, Université de Poitiers, Poitiers, France.
3Laboratoire « Polymères, Biopolymères, Surfaces » (UMR 6270 CNRS), Proteomic Platform PISSARO, Normandie Univ, UNIROUEN, Mont-Saint-Aignan, France
4Laboratory « Polymères, Biopolymères, Surfaces » (UMR 6270 CNRS), Proteomic Platform PISSARO, Normandie Univ, UNIROUEN, Mont-Saint-Aignan, France
5Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Sevilla, Sevilla, Spain.

**Introduction:**
We have previously shown that the human natriuretic peptide hormones are able to bind specifically the *Pseudomonas aeruginosa* sensor AmiC, leading to decreased biofilm formation and enhanced biofilm dispersion. In addition, we observed that the amidase AmiE, which is the final product of the ami operon, is involved in virulence regulation and mostly supports these effects.

**Hypothesis and aims:**
Since natriuretic peptides binding on AmiC induces AmiR release allowing the over-transcription of the whole ami operon, we decided in the present study, to decipher the key role played by AmiR in *P. aeruginosa PA14* biofilm regulation using an AmiR over-expressing strain (AmiR+), and an amiR-deletion mutant strain (ΔamiR).

**Results:**
The AmiR+ strain was strongly affected in its ability to form a biofilm, whereas the ΔamiR strain enhanced biofilm formation, and the biofilm matrix organization was affected in both the AmiR over-expressing strain and the amiR deletion mutant. Whole proteomic and transcriptomic studies were performed to identify potential new AmiR-targets. Most of them are involved in metabolism regulation explaining in some extent the diverse physiological phenotypes that we observed. Interestingly, exposure of *P. aeruginosa* pre-formed biofilms to numerous metabolites of the arginine metabolism pathway led to differentially disperses the PA14 biofilm.

**Conclusion:**
Altogether, our results suggest that the ami operon, through its AmiR regulator may contribute to biofilm regulation involving more complex regulatory processes than previously thought, including amino-acids metabolism and RNAs control processing.
P92- The secret world of udders from dairy cows – microbiota and biofilm in chronic bovine mastitis

Regitze Renee Pedersen¹, Emma Hornemann¹, Volker Krömker², Kirstin Dahl-Pedersen¹, Rikke Buhl¹, Elin Jørgensen³ and Thomas Bjarnsholt³,⁴

1 Department of Veterinary Clinical Sciences, University of Copenhagen, Copenhagen, Denmark,
2 Department of Veterinary and Animal Sciences, University of Copenhagen, Copenhagen, Denmark,
3 Department Immunology and Microbiology, Costerton Biofilm Center, University of Copenhagen, Copenhagen, Denmark,
4 Department of Clinical Microbiology, Copenhagen University Hospital, Copenhagen, Denmark

Introduction:
Bovine mastitis is one of the most paramount diseases in the dairy industry and has adverse effects on the economy, the use of antibiotics, and animal welfare. Some cases of mastitis cannot be eradicated using traditional antibiotic treatments and some cows suffer from recurrent or chronic infections. Biofilm formation in the udders can explain why these infections reoccur and are difficult to treat. This study uses novel methods to contribute to the limited research of biofilm in bovine mastitis and is the first study to detect biofilm with PNA-FISH and confocal laser scanning microscopy and to uncover a microbiota in the udders of dairy cows with bovine mastitis. The results from this cutting-edge study could lead to new and optimized treatments, decreased use of antibiotics, and improved animal welfare in the dairy industry.

Methodology:
Biopsies are collected from udders from dairy cows with chronic mastitis. Biopsies are collected at different locations in a healthy quarter and a quarter with mastitis. The biopsies are analyzed with cultivation and MALDI-TOF MS to determine the microbiota in the udders. To visualize the distribution and location of bacteria and biofilm in the udders, biopsies are stained with DAPI and PNA-FISH and investigated with confocal laser scanning microscopy.

Results:
Preliminary results show biofilm was detected in some quarters with mastitis. MALDI-TOF MS results showed the presence of bovine mastitis pathogens and possible commensals, indicating that the udders of dairy cows have an established microbiota.

Conclusion:
The preliminary results from these different investigations provide a deeper understanding of the pathogens responsible for chronic mastitis infections and the potential role of biofilm in chronic mastitis in dairy cows. The results indicate that biofilm formation could have a role in some cases of mastitis and that there is an established microbiota in the udders of dairy cows.
P93- Adding an extra-layer: how surface chemical modification of 3D printed objects changes their Chemistry and Biology

Anthony W. Coleman¹, Laurent Mollet¹, Lea Confort¹, Nolwenn Meyer¹, Mathieu Maillard¹, Beonjoon Kim², Didier Leonard³, Mickaël Desbrosses³, Eszter Roka⁴, Petra Arany⁴, Florent Perret⁵ and Ildiko Bácskay⁴

1 LMI CNRS UMR 5615, Université Lyon 1, Villeurbanne, 69622, France
2 LIMMS/CNRS-IIS UMI 2820, Institute of Industrial Science, The University of Tokyo, Tokyo, 153-8505, Japan
3 ISA, UMR 5280, Univ. Lyon 1, Villeurbanne F69100.
4 Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, H-4032, Debrecen, Nagyerdei körút 98. Hungary
5 ICBMS, UMR 5246, Université Lyon 1, Villeurbanne, F69622, France

3D printing has opened the way toward on-demand fabrication of custom devices, ranging from houses to pieces for the aero-space industry to jewelry, to bio-medical implants and to chemical reactionware. However almost all of such realisations rely on off-the-shelf polymers in the finished articles. Obviously, the introduction of chemical modifications into the pre-printed materials is both costly and wasteful as a very small proportion will be at the surface. In the case of bio-medical applications the question of surface colonisation and formation of biofilms which can protect pathological bacteria from treatment is at the current time of great interest.

Recognition that a number of widely used polymers, including Poly-Lactic Acid (PLA), Poly-Ethyl-Terpthalate (PET) and Poly-Methyl-Meth-Acrylate (PMMA) all contain reactive ester functions opens a simple route to surface modified 3D printed articles. We demonstrate that the use of the amidation reaction allows adjunction of sulphinic acid, phosphonic acid and oligo-amines at the surface of the above polymers. Moreover bio-compatibility tests show the modified systems does not possess cyto-toxicity. In further experiments it was possible to show that inhibition or acceleration of bio-film growth is controlled by the pKa of the surface function and that this control depends on the whether the ester function is along or orthogonal to the polymer backbone. Intriguingly, pH dependent measurement of surface energy suggests that there may be a novel form of "spectroscopic" information provided.

In conclusion, we have demonstrated that 3d printed structures may be surface modified using quite simple chemistry, that such surfaces are non-cytotoxic and finally that the growth of Candida biofilms on these surfaces can controlled by the nature of the chemical modification and also the chemical structure of the printed polymer.
P95- Serratiopeptidase affects adhesive features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients on biotic and abiotic substrates

Gianluca Vrenna, Rosanna Papa, Marco Artini, and Laura Selan

*Sapienza University of Rome* (Italy)

*Pseudomonas aeruginosa* is an opportunistic pathogen often involved in airway infections of cystic fibrosis (CF) patients. During CF infection, the lung environment is extremely hostile because of the very high concentrations of antibiotics, reduced nutrient availability, elevated osmotic stress and intermicrobial competition: conditions that force *P. aeruginosa* to adapt for survival. The virulence of *P. aeruginosa* isolate is strongly related to different factors such as the capability to form a biofilm, different types of cell/colonial motility, production of toxins and the invasion of pulmonary cells. The dynamic process of biofilm formation offers protection to bacterial cells and resistance to drugs and host immune attacks. The self-produced exopolysaccharide matrix (EPS) that can incorporate different bacterial communities ensures their survival and resistance to certain antibiotics, complicating bacterial eradication. Motility also contributes to biofilm formation and bacterial colonization of surfaces. Furthermore, the ability to adhere is the prelude for the internalization into lung cells, a common immune evasion mechanism used by most intracellular bacteria, such as *P. aeruginosa*. The impairment of bacterial cell adhesion and biofilm formation could represent a major target for the development of new therapeutic treatments for chronic infection control.

Previously reports evaluated the anti-infective properties of serratiopeptidase (SPEP), an extracellular metalloprotease produced by *Serratia marcescens*, in impairing virulence-related properties in bacteria, such as Staphylococci and *Listeria monocytogenes*. We demonstrated that SPEP was able to impair the attachment to inert surfaces and adhesion/invasion on eukaryotic cells with a mechanism independent by proteolytic mechanism. This work aims to investigate the effect of SPEP on some virulence factors produced by *P. aeruginosa* isolated from CF patients, such as biofilm formation and accumulation, pyocyanin and pyoverdine production, motility and invasion to alveolar basal epithelial cells (A549 cell lines).
P97- Combined anti-Biofilm activity of a dendritic dendron and amphotericin against *Candida* spp

Natalia Gómez-Casanova¹, José Luis Copa-Patiño¹, Juan Soliveri¹, Javier Sanchez Nieves¹, Jorge Pérez Serrano¹, F. Javier de la Mata²,³, Rafael Gómez²,³, Irene Heredero-Bermejo¹

1 Department of Biomedicine and Biotechnology, Faculty of Pharmacy. University of Alcalá, Madrid, Spain.
2 Department of Organic and Inorganic Chemistry, Research Institute in Chemistry "Andrés M. del Río" (IQAR), University of Alcalá, Madrid, Spain. Institute "Ramón y Cajal" for Health Research (IRYCIS), Spain.
3 Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN)

Strategies to overcome the problematic associated to biofilm-forming opportunistic *Candida* spp pathogens is of vital importance, especially in hospital environment. These pathogens are highly adaptable to different conditions and are able to develop resistance to clinically accepted drugs after prolonged exposure. In addition, the cytotoxicity of antifungals and the development of biofilms can even increase the probability to cause patient’s death. For this reason, studies focused on new and novel molecules are necessary to combat these pathogens and avoid *Candida* biofilm associated infections. In our study, a specific dendritic molecule was developed for this purpose. In vitro activity of this dendron, BDTL132, was tested against biofilm formation and established biofilms in *Candida albicans* CECT 1002 and *C. glabrata* CECT 1448 strains. The synergistic effect of this compound with amphotericin was determined. Candida cell viability was evaluated using the resazurin colorimetric assay and confirmed by plating on agar plates. Also, the cytotoxicity was tested on HeLa cell line. BDTL132 was active preventing biofilm formation, showing a minimum biofilm inhibitory concentration (MBIC) of 16 mg/L against *C. glabrata* and 32 mg/L against *C. albicans*. The minimum biofilm damage concentration (MBDC) against established biofilms was 32 mg/L in *C. glabrata* and 64 mg/L in *C. albicans*. Besides, one hundred percent of the cells were eradicated in established biofilms in both microorganisms. We noticed a significant reduction in these effective concentrations in synergy studies with the antifungal amphotericin. The compound presented low cytotoxicity at 64 mg/L. We conclude that dendritic compound may be an interesting new alternative to treat different species of *Candida*, individually or in combination with clinical antifungals.
P99- High-throughput screening for the identification of anti-biofilm compounds against polymicrobial communities.

Laura Barrientos-Moreno¹, Manuel Romero¹, Ruth Cornock², Fadi Soukarieh¹, Shaun N. Robertson¹, Miguel Cámara¹

¹ National Biofilms Innovation Centre, Biodiscovery Institute and School of Life Sciences, University of Nottingham, Nottingham, UK.
² Synthetic Biology Research Centre, Biodiscovery Institute and School of Life Sciences, University of Nottingham, Nottingham, UK.

Microbial biofilm infections represent a critical challenge in public health due to their high resistance to known antimicrobial treatments. Hence, there is an urgent need to discover new antimicrobial agents to face the antimicrobial resistance (AMR) crisis that threatens to become a major concern within the next decades. Repurposing approved drugs could provide an attractive avenue to help address the AMR challenge. Furthermore, as most infections are polymicrobial in nature, there is a need to test drugs in multispecies models. An automated high-throughput screening method to identify compounds that inhibit biofilm formation by a polymicrobial community was developed and tested with an FDA-approved library of 1591 compounds. Interkingdom biofilms we set up including the opportunistic pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* commonly associated with chronic biofilm-centred infections. Biofilm survival in the presence of 50 µM compounds was evaluated using a 2,3,5-triphenyltetrazolium chloride-based assay, which indicates the metabolic activity of the community. Additionally, replication on selective agar plates was used to determine the microbicidal or microstatic effect of the compounds on each species.

A total of 177 (11.1%) compounds were found to inhibit biofilm formation, including antimicrobial compounds currently used in the clinic, suggesting our screening approach is robust. Interestingly, 24 compounds comprising drugs used for neurological, metabolic or endocrine disease therapy reduced polymicrobial biofilm formation by ≥45% without affecting growth to less than 70% or displaying microbicidal activity, which is important to minimize the emergence of resistance. As these were not previously described as active against biofilms, they were studied further, and two compounds were found to inhibit both polymicrobial and single-species communities and therefore deserve future investigation as potential anti-biofilm compounds. The methodology described here is a powerful tool for rapid screening of antimicrobial compounds active against clinically-relevant polymicrobial communities allowing efficient selection of new therapeutic approaches.
P101- Anti-biofilm activities of a novel synthetic flavonoid against Candida sp.

Mihaela Savu 1, Cristina Veronica Moldovan 1, Lucian Birsa 2, Marius Stefan 1

1 Faculty of Biology, Biology Department, The Alexandru Ioan Cuza University of Iasi, Bld. Carol I, Nr. 11, 700506, Iasi, Romania
2 Faculty of Chemistry, The Alexandru Ioan Cuza University of Iasi, Bld. Carol I, Nr. 11, 700506, Iasi, Romania

Introduction
The increased Candida antifungal resistance due to biofilms is a matter of major concern in the medical community. Immunocompromised patients with Candida infections resistant to various drugs have very few treatment options. Therefore, identification of new efficient antifungals is considered a high priority goal of the scientific world.

Hypothesis
In this context, we evaluated the antifungal potential of a new synthetic flavonoid with bromide as halogen substituent at the benzopyran core (Br-Cl-flav) against Candida strains.

Methodology
Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) was used to evaluate the antifungal activity. The inhibition of biofilm formation was evaluated by crystal violet assay at different stages (initial attachment, biofilm formation and after biofilm development). Disruptive potential of the synthetic flavonoid on mature biofilms was also investigated. Biofilm metabolic activity was estimated using resazurine assay and biofilm cell viability was evaluated by CFU count. Fluorescence microscopy and SEM techniques were also employed to assess the biofilm formation in the presence of Br-Cl-flav.

Results
MIC and MFC values (15.62 μg/mL and 31.25 μg/mL respectively) indicate a strong antifungal effect of BrCl-flav against Candida strains. BrCl-flav showed important anti-attachment and anti-biofilm activities, as microscopy analyses revealed. Biofilm formation was inhibited at ¼ MIC, ½ MIC and MIC with more than 90% compared with control. The mature biofilm was 75% destroyed after 48 h exposure to BrCl-flav at a concentration equivalent to MIC and approx. 50 % at ½ MIC. No viable cells were evidenced in the biofilm after treatment with BrCl-flav at 2 x MIC and MIC.

Conclusion
BrCl-flav prevented biofilm formation and inactivated the fungal cells in mature biofilms. Based on its potent antibiofilm properties, BrCl-flav has a good potential to develop new effective antifungal agents as alternatives to overcome the menace of Candida biofilms.
Healthcare-associated infections with high mortality in hospitalized patients are caused by different pathogens, including yeasts. Among this group, Candida albicans is the most representative species and responsible for candidiasis, including superficial and invasive infection, a common cause of fungal infection worldwide. The main problem associated to Candida is the capacity to develop biofilms, which are very difficult to remove once established. In addition, few antifungals currently accepted for clinical use are capable of combating this pathogen. Therefore, the development of new molecules capable of eradicating biofilms, and especially at low concentrations, is essential. For this study, a specific new dendritic molecule was developed. In vitro activity of this dendrimer, BDNG001, was tested against C. albicans CECT 1002 strain on planktonic cells, on biofilm formation and on established biofilms. In the biofilm assay, the viability of C. albicans cells was assessed by a resazurin colorimetric assay and confirmed on agar plates. Then, cytotoxicity was tested on HeLacell line. Furthermore, cell damage in the C. albicans was visualized by scanning electron microscopy (SEM). BDNG001 showed an MIC of 2 mg/L in planktonic cells. In biofilm development, the compound showed a minimum biofilm inhibitory concentration (MBIC) of 4 mg/L against C. albicans and a minimum biofilm eradication concentration (MBEC) of 16 mg/L against established biofilm. Cell collapse caused by the dendrimer was observed by SEM. We conclude that the dendrimer BDNG001 has great potential for develop future in vivo studies that would allow us to better understand the scope of its activity.
P103- Transcriptional response of *Candida auris* biofilms to farnesol and tyrosol treatment.

Renátó Kovács, Noémi Balla, Fruzsina Kovács, László Majoros, Ágnes Jakab

Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Farnesol and tyrosol are two fungal quorum-sensing molecules with opposite effect in terms of *Candida* morphogenesis. Our group has reported the potential therapeutic benefit of these two compounds against various Candida species. However, little is known about biofilm-related molecular events induced by farnesol and tyrosol against *Candida auris*. We determined genome-wide gene transcription changes induced by these compounds using total transcriptome sequencing (RNA-Seq). For RNA extraction one-day-old biofilms were used where fungal cells were collected 1 hour following farnesol or tyrosol exposure. RNA-Seq libraries were prepared from total RNA using an Ultra II RNA Sample prep kit. The single read 75-bp sequencing reads were generated on an Illumina NextSeq500 instrument. Differences between treated and control groups were compared using a moderated t-test; the Benjamini-Hochberg false discovery rate was used for multiple-testing correction, and a corrected P value of 1.5-fold change or less than -1.5-FC. Our results revealed 587 and 1851 differentially expressed genes for farnesol and tyrosol, respectively (P1.5-fold change) or decreased (1.5-fold change) and 662 down-regulated (1.5-fold change) genes compared to control. Farnesol-induced genes involved in ribosomal small and large subunit biogenesis, RNA metabolic process and iron-sulfur cluster binding were up-regulated. Tyrosol resulted the up-regulation of genes involved in ribosome biogenesis, ribosomal small and large subunit biogenesis, RNA metabolic process, translation as well as iron-sulfur cluster binding. Moreover, tyrosol decreased the expression of beta-oxidation, carbohydrate catabolic process, response to endoplasmic reticulum stress, peroxisome, vacuole and cortical endoplasmic reticulum associated genes. Our study provides novel clues for future studies in terms of understanding of quorum-sensing molecules-related effect on *C. auris* biofilms.

This research was supported by the Hungarian National Research, Development and Innovation Office (NKFIH FK138462)
P104- Development and treatment of a novel in vitro biofilm model of bacterial vaginosis

William Johnston\textsuperscript{1,2}, Alicia Ware\textsuperscript{1,2}, Chris Delaney\textsuperscript{2,3}, Suzanne Hagen\textsuperscript{4}, Matthew Cummings\textsuperscript{5}, David Corcoran\textsuperscript{5}, Gordon Ramage\textsuperscript{2,3}, Ryan Kean\textsuperscript{1,2}

1 Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, United Kingdom, 2 Glasgow Biofilm Research Network, 3 Oral Sciences Research Group, University of Glasgow, Glasgow, United Kingdom, 4 Nursing, Midwifery and Allied Health Professions Research Unit, Glasgow Caledonian University, Glasgow, United Kingdom, 5 CC Biotech Ltd, London, United Kingdom.

Introduction: Bacterial vaginosis (BV) affects 30% of women of childbearing age in the western world and presents with 3-5 times increased risk of miscarriage and two-fold risk of pre-term birth. Currently, antibiotics such as metronidazole and clindamycin are employed to treat BV, however the success rate of these therapies is low due to formation of recalcitrance biofilms composed of obligate anaerobes including \textit{Gardnerella vaginalis}. Novel therapies are being investigated, although the ability to screen these compounds remains limited by a lack of clinically relevant in vitro models.

Hypothesis and aims: To develop and screen the efficacy of therapeutics against a polymicrobial biofilm model representative of BV.

Methodology: A 4-species biofilm model was developed consisting of \textit{Gardnerella vaginalis}, \textit{Fannyhessea vaginae}, \textit{Prevotella bivia} and \textit{Mobiluncus curtisi}. Biofilms were grown in NYC III broth over three days, and treated using antibiotics, a probiotic lactobacilli cocktail and a novel anti-\textit{Gardnerella} endolysin. Biofilm composition and treatment were assessed using live/dead qPCR and nanopore MinION sequencing.

Results: All species colonised biofilms to varying degrees, with \textit{G. vaginalis} being the most abundant. Despite this, biofilm composition remained largely unchanged by metronidazole and clindamycin (1-8x pMIC). Conversely, lactobacilli were able to incorporate into biofilms and significantly reduce the viable levels \textit{G. vaginalis}, \textit{F. vaginae} and \textit{M. curtisi} (p0.01 vs. untreated for all). Endolysin candidates showed good selectivity towards a range of Gardnerella species and significantly reduced viable \textit{G. vaginalis} within polymicrobial biofilms at 1-4x pMIC (1 log reduction for all, p0.01 vs. vehicle control). Although reduced, \textit{G. vaginalis} remained viable at $\approx$1x$10^6$ CFE/mL suggesting that biofilm penetration remains a potential hurdle for these compounds.

Conclusion: This study highlights the pitfalls of conventional BV therapies, and provides a polymicrobial model that allows for more effective pre-clinical screening for novel BV therapies.
P106- Targeting quorum sensing with monoclonal antibodies as a novel approach to tackle *Pseudomonas aeruginosa* biofilm-mediated infections

**Simone Lucanto¹, Shaun N. Robertson¹, Soumya Palliyil³, Andrew J. Porter³, Luisa Martinez-Pomares² and Miguel Cámara¹**

¹ National Biofilms Innovation Centre, Biodiscovery Institute, School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

² National Biofilms Innovation Centre, Queens Medical Centre, School of Life Sciences, University of Nottingham, Nottingham, NG72UH, United Kingdom

³ Scottish Biologics Facility, University of Aberdeen, Foresterhill, Aberdeen, AB24 3FX, United Kingdom

*Pseudomonas aeruginosa* has highly interlinked quorum sensing (QS) systems which represent a desirable anti-virulent target as they play a key role in controlling virulence determinants production and biofilm development in this opportunistic pathogen. The traditional approaches geared toward the QS inhibition (QSI) have focused on targeting the QS signal receptor using a range of antagonistic compounds. As an alternative approach, we developed sheep-mouse QSI-IgG2a monoclonal antibodies (mAbs) to tackle *P. aeruginosa* infections.

The mAbs have been produced in HEK293t eukaryotic cells and purified with protein A affinity chromatography. Binding was characterised by standard indirect and competitive ELISA. The QSI mAbs have been tested for their ability to inhibit QS-regulated phenotypes using *P. aeruginosa* biosensor and lab strains. Their ability to inhibit *P. aeruginosa* biofilms has been tested by growing biofilm in microtiter plates and evaluating their metabolic activity by 2,3,5-Triphenyltetrazolium chloride reduction assays.

Here, we successfully purified eight QSI-mAbs to high purity. Binding was characterised by ELISA, showing high sensitivity for their individual targets. When tested phenotypically, the mAbs delayed QS activation in *P. aeruginosa* using biosensor-mediated assay and inhibited the production of pyocyanin in PAO1-L. Preliminary data shows that in the presence of QSI-mAbs, *P. aeruginosa* biofilms are more susceptible to antimicrobial treatment.

Overall, our data support the potential of using anti-QSI mAbs as anti-virulent agents against *P. aeruginosa*. Further work will focus on investigating the ability of the mAbs to penetrate deep into *P. aeruginosa* biofilms.
**P107- Differences in antimicrobial potential of known and novel probiotics on in vitro biofilms containing oral pathobionts and commensals**

**Wannes Van Holm** (KU Leuven)  
Rita Carvalho (KU Leuven)  
Ingmar Claes (YUN probiotherapy)  
Naiera Zayed (KU Leuven)  
Sarah Lebeer (UAntwerp)  
Nico Boon (UGent)  
Kristel Bernaerts (KU Leuven)  
Wim Teughels (KU Leuven)

**Introduction:**
Several oral diseases are characterized by a shift within the oral microbiome towards a pathogenic, dysbiotic composition. Broad spectrum antimicrobials are often part of their treatment. However, with the rising antibiotic resistance, alternatives are increasingly desirable. Alternatively, supplying beneficial species through probiotics is increasingly showing beneficial results, however these probiotics are rarely evaluated comparatively. In this study, the in vitro effects of three known- and three novel lactobacillus strains, together with four novel *Streptococcus salivarius* strains was comparatively evaluated in vitro on a multispecies oral biofilm model.

**Methods:**
Using a multispecies biofilm model of both oral pathobionts and commensal bacteria, biofilms were formed on hydroxyapatite discs with- and without probiotics. After 24 h, biofilms were rinsed and cells were recovered before treatment with a viability dye (PMAxx) to specifically enumerate the DNA of living bacteria through v-qPCR.

**Results:**
Strain specific effects were observed as 1) differences between efficacy within genera and 2) genera differences: while some of the lactobacillus candidates were able to reduce the periodontal pathobiont *A. actinomycetemcomitans*, the *S. salivarius* strains were not. However, the *S. salivarius* strains were more effective against *P. intermedia*, *P. gingivalis* and *F. nucleatum*. Most of the lactobacillus strains also affected the prevalence commensal species within the biofilms, while this was lower for *S. salivarius* strains.

**Conclusion:**
While probiotics can aid in reducing pathobionts, differences between performances and targets of probiotics can limit their efficacy as well as potentially having unexpected effects on commensal species.
P108- An updated protocol for studying in vivo catheter biofilm infections using catheterized rats

Yutaka YOSHII, Ashwini CHAUHAN, David LEBEAUX, Jean-Marc GHIGO, Christophe BELOIN

1 Institut Pasteur, Université Paris Cité, UMR CNRS 6047, Genetics of Biofilms Laboratory, 75015 Paris, France.
2 Unité Mobile d’Infectiologie, Service de Microbiologie, AP-HP, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015, Paris, France.
3 Université Paris Cité, 75006, Paris, France.

Among central venous catheters, totally implantable venous access ports (TIVAP) are widely used for long-term administration of veinotoxic compound such as antineoplastic chemotherapy or parenteral nutrition. After TIVAP insertion, their use is associated with a risk of microbial contamination and subsequent catheter-related infection (CRI) possibly responsible for severe complications such as bloodstream infection or septic shock. This microbial contamination often leads to the formation of microbial communities on the catheter surface (i.e. biofilms), which impact on CRI has been increasingly recognized. Therefore, the development of in vivo biofilm models plays an essential role in validating in vitro results and is a critical step in investigating new strategies against CRIs before clinical application. Here, we present an updated version of our 2016 protocol (Chauhan A, et al., Nat Protoc) that provides a clinically relevant biofilm model by implanting clinically used TIVAP in Crl: CD(SD) male rats. Since the previous protocol has been accompanied by critical technical difficulties, such as maintaining anesthesia during surgery and inserting catheters into jugular veins, we have substantially changed the anesthetic and catheter insertion methods to make the protocol easily practiced by many researchers. As a result, the updated protocol brought a high successful catheterization rate of approximately 90%. The primary advantages of this model are the followings: 1) we can monitor within the TIVAP, in real-time, the increase or decrease of biofilm formation using bioluminescent bacteria (E. coli, P. aeruginosa, and S. aureus) and a bioluminescent imaging machine. 2) The efficacy of anti-microbial agents against biofilm infections on TIVAP can be evaluated by injecting those chemicals into the port. 3) A total hands-on time is ~3 h, including the TIVAP implantation followed by in situ luminescence monitoring and bacterial number counts. In conclusion, this updated protocol can be applied for studying CRIs, including antibiofilm strategies.
P225-Application of Immobilised Bacteriophage for Control of Biofilms.

Gordon Smith (1), Albulena Zhara (1), Laurence Rowan (1), Rajesh Odedra (2), Jason Clark (1)

1. Fixed Phage Ltd, West of Scotland Science Park, Glasgow, UK
2. Carus Animal Health, Castle Court, Surrey, UK.

Introduction
Bacteriophages (phages) are natural viruses of bacteria that can invade established biofilms leading to bacterial cell death and degradation of the biofilm structure [1]. Due to bacterial defences and phage particle instability, a natural equilibrium can eventually be formed within the biofilm structure with consistent bacterial and phage numbers and no further control of the biofilm [2].

Fixed Phage’s plasma mediated immobilisation technology allows irreversible attachment of phages to a range of substrates allowing the creation of antimicrobial material. This process also provides additional stability to phage particles and increased resistance to environmental stressors. The aim of this study was to assess the efficacy of phage solution and immobilised phage material at disrupting biofilms in vitro.

Hypothesis
Application of phage to an established biofilm disrupts the biofilm structure and causes bacterial cell death.

Methods
Phages were isolated targeting an oral pathogen and lytic phage activity was confirmed. Phages were then immobilised onto chitosan powder and incubated at 40°C for 30 days. The material was then applied to an in vitro biofilm model the impact of free and immobilised phage on the biofilm was assessed by measuring the biomass and cell counts.

Results
All phages showed lytic activity against planktonic cells but showed differing efficacies at reducing bacterial numbers and biomass in a biofilm. Phages were able to reduce the cell viability of a multispecies biofilm despite only targeting 1/10 bacteria present in the model. Phage immobilised onto chitosan powder survived incubation at 40°C and application resulted in a significant reduction in bacterial numbers.

Conclusions
Immobilised phage is a safe, natural, stable and effective antibiofilm agent that can prevent the formation of biofilms or control established biofilms in several environments. In the future, Fixed Phage plans to further assess the efficacy of phage control of biofilms in vivo.

Antimicrobial resistance (AMR) has become a global health problem. Bacteria are able to adapt to different environments, with the presence or absence of a host, forming colonies and biofilms [1]. One of the medical strategies used against biofilms is the therapy with drug delivery systems. In the present work three kind of nanomaterials were selected and synthetized for their biodegradability and bioavailability properties. Chitosan nanocapsules, alginate nanogels and non-ionic surfactant-based nanovesicles (niosomes) were chosen as nanocarriers to encapsulate four antibiotics belonging to the rifamycin family: rifamycin S, rifampicin, rifabutin and its analog, a spiropiperidyl rifabutin [2]. The rifamycin family, as known, is widely used against Mycobacterium tuberculosis, but recent studies have shown that they have also bactericidal activity on Staphylococcus epidermidis and Staphylococcus aureus [3,4].

All systems formulated were characterized in terms of size, zeta potential and their encapsulation efficiency and drug loading were calculated.

The nanocarriers were compared analyzing their cytotoxicity through MTT assay and their cellular internalization with flow cytometry and confocal microscopy. Calu-3 human airway epithelial cell line was used as a model cell, since S. aureus can develop nasal, mucosal surface and lung colonization. The activity of the nanocarriers against S. aureus and its biofilms were also tested. The strain selected was S.aureus 15981, a strong biofilm producer. It was performed a minimum inhibitory concentration (MIC) assay on the planktonic phase of the bacteria, and a minimum eradicate biofilm concentration (MBEC) assay using a Calgary Biofilm Device.
Acknowledgments
This work is part of a project that has received funding from the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No. 813439 (Break Biofilms) and also by the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 842652 and the CSIC’s MSCA IF extension.

References
Lichens, due to their symbiotic nature, constitute a chemical factory of original compounds. Depsides and depsidones are the constituents mainly biosynthesized by lichens. The aim of our study was to evaluate the activity of 11 compounds, isolated from selected lichens, against planktonic and sessile yeasts of Candida albicans. Among them, evernic acid (EA), particularly abundant in the oakmoss lichen, Evernia prunastri, is of increasing interest for its biological properties.

For anti-maturation and anti-biofilm tests, 2h and 24h old biofilms respectively were prepared in YNB-glucose medium at 37°C in microplates and were treated for 24 h or 48 h by compounds at concentrations ranging between 6.125 and 200 µg/mL. The minimal maturation inhibition concentration (MMIC) and minimal biofilm inhibitory concentration (MBIC) were determined using XTT method. Biofilms treated with EA at 50 or 100 µg/mL (150 or 300µM) for 48h and control biofilms were observed by Scanning Electron Microscopy (SEM) after cryofixation. The cytotoxicity was evaluated on HeLa and blood cells. Encapsulations of EA in cyclodextrine/cellulose nanocrystal complex were performed. Among 11 tested components, three depsides displayed promising activities. EA was the most active and displayed significant anti-biofilm and anti-maturation activity (MMIC50 and MBIC50=12.5 µM, p200 µg/mL). EA showed weak toxicity against HeLa cells (22%) at 18 µM and no hemolytic activity at 300µM. The observation by SEM of biofilms treated by EA at 50 µM, showed an alteration of the yeast cells’ morphology and the modification of the extracellular matrix. Encapsulated and vectorized EA displayed similar anti-biofilm activities with an increased solubility in aqueous media.

These experiments showed the EA ability to reduce preformed biofilms of C. albicans and to delay its maturation phase as well.
Frailty is an age-associated syndrome defined by an increased vulnerability due to a global decline in reserve and function. Changes in gut microbiota composition (dysbiosis) has been suspected to lead to frailty and dependence. Beside description of abnormalities in their taxonomic composition\(^1\), studies are now needed to investigate the physical and behavioral characteristics of aged-associated gut microbial community in its natural state: biofilms\(^2\).

**Methods:** Fecal samples (69 y/o, N=15 in both groups). Fecal samples were resuspended in anaerobic broth, and biofilms were generated after 72 hours on a mucin-coated polystyrene pegs, under anaerobic conditions as previously described\(^3\). Biofilm total biomass (Safranin O assay), metabolic activity (resazurin assay), matrix-associated saccharides (fluorescent lectins stain) and matrix-associated protein (Sypro Ruby stain) were evaluated in each biofilm (technical replicates of twenty biofilms per individual).

**Results:** The total biomass and metabolically viable bacteria within biofilms from elderly individuals were significantly lower compared to biofilms from younger individuals. The quantity of matrix-associated polysaccharides was highest in the group of intermediate compared to elderly or young donors. The highest quantity of matrix-associated proteins was detected in biofilms from frail elderly. Importantly, biofilms from frail individuals released more viable planktonic cells compared to biofilms from robust elderly, and younger individuals.

**Conclusions:** Gut microbiota from elderly is characterized by a reduced capacity to form biofilm ex vivo and by a modified composition of their matrix. Biofilms from frail individuals are dispersing more compared to biofilms from robust elderly. Studies are ongoing to determine whether dispersed bacteria have modified virulent properties and thereby potentially trigger local and systemic inflammation associated with age (inflammageing).
P113- Modulation of expression in genes involved in efflux and oxidative stress in biofilms formed by *Candida auris* and *Candida albicans* by photodynamic inactivation

**Stefanek Matus**, Cernakova Lucia, Dekkerova Jaroslava and Bujdakova Helena

1 Comenius University in Bratislava, Faculty of Natural Sciences, Department of Microbiology and Virology

**Introduction**: Employment of photodynamic inactivation (PDI) can effectively eradicate microorganisms, including biofilms of *Candida albicans* and *Candida auris*. Resistance does not affect PDI and its efficacy; however, some differences in susceptibility have been observed.

**Hypothesis**: The difference in the susceptibility of *C. albicans* and *C. auris* biofilms to PDI could be due to different levels of overregulation of stress response and not differences in efflux.

**Methodology**: PDI was tested against 24-h biofilms formed by 3 clinical isolates of *C. auris*, one standard strain, and one clinical isolate of *C. albicans* using methylene blue (250µM, 1mM) irradiated with a red laser (λ=660nm, 190 mW/cm²). Changes in the expression of efflux genes (CDR1, CDR2, MDR1) and genes involved in oxidative stress (CAP1, MRR1, SOD1-3, GPX2, GLR1) were determined before/after PDI by qPCR. Reactive oxygen species were measured by chemiluminescence.

**Results**: The highest inhibitory effect was achieved in all strains after irradiation for 300s. The irradiated group showed growth inhibition of more than 90% and 70% in *C. auris* and *C. albicans*, respectively. Relative changes in the expression of efflux pump genes were observed in all isolates. The genes CDR1 and CDR2 were overexpressed in all isolates (up to 4.8-fold, 6.6-fold, respectively), while strong up-regulation of MDR1 (up to 124.1-fold) occurred only in *C. auris*. Up-regulation of CAP1 (up to 2-fold) and SOD2 (up to 8.5-fold) occurred in *C. albicans*, while SOD3, GPX2 and GLR1 have been down-regulated compared to the control. The amount of detected hydrogen peroxide increased greatly after PDI.

**Conclusion**: PDI can be used effectively, even for highly resistant biofilms formed by *C. auris* and *C. albicans*. PDI modulates the expression of all efflux genes, CAP1 and SOD2, which implies how cells are trying to defend against the oxidative stress produced by PDI.
P114- A new PNA-FISH multiplex approach is capable of detecting bacterial vaginosis-associated species on vaginal samples

Lúcia G. V. Sousa1,2, Carina Almeida3, Jyoti Sharma4, Christina A. Muzny4, Nuno Cerca1,2

1 Centre of Biological Engineering (CEB), Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Braga, Portugal. 2 LABBELS –Associate Laboratory, Braga, Portugal. 3 INIAV, IP-National Institute for Agrarian and Veterinary Research, Vila do Conde, Portugal. 4 Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, United States.

Bacterial vaginosis (BV) is the most common vaginal infection in women of reproductive age, being characterized by a decrease in the number of Lactobacillus species and an increase in anaerobic bacteria including, but not limited to, Gardnerella spp., Fannyhessea vaginae, and Prevotella bivia. These bacteria interact and form a polymicrobial biofilm on the vaginal epithelium. The clinical diagnosis of BV is traditionally performed using the Amsel criteria, while research laboratories often use the Nugent score. However, both methods have limitations and misdiagnosis may have an impact on treatment, increasing the risk of complications.

We have developed a new peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) approach with three PNA probes able to detect key vaginal bacterial species associated with incident BV (iBV). Specificity and sensitivity of the probes were determined using pure cultures of +60 bacterial species/strains. Then, single-, dual-, and triple-species biofilms were grown in vitro, before visualization by confocal laser scanning microscopy coupled with PNA-FISH. Vaginal samples previously characterized for iBV, by 16S sequencing, were used to determine the feasibility of our protocol for the purpose of clinical diagnosis.

The new PNA probes were designed and all showed excellent ability to detect the target species with a sensitivity and specificity of 99.9%. Using the three probes it was possible to discriminate the bacterial spp. within the in vitro polymicrobial biofilms. Moreover, on vaginal samples, the probes were capable of detecting the three species, allowing a correct identification of key vaginal bacteria. This work provides a new method for the detection of key vaginal bacteria associated with BV and a possible improvement in the diagnosis of this infection. This new PNA multiplex approach will allow further study of the structure and composition of BV biofilm, including key interactions between vaginal bacterial species. A patent request has been filed.
P116- Plasma-activated water as an alternative for decontaminating *Salmonella* biofilms on egg farms

Andrea R. McWhorter¹, Adrian Abdo², Filbert Christone², Thomas Schmitt-John³, Katharina Richter³

1 School of Animal and Veterinary Sciences, University of Adelaide, South Australia
2 Department of Surgery, Basil Hetzel Institute for Translational Health Research and The Queen Elizabeth Hospital, University of Adelaide, South Australia
3 Plasmatreat GmbH, Steinhagen, Germany

**Introduction:** *Salmonella* establish persistent infection in layer hens which results in intermittent shedding of bacteria in faeces. This can increase contamination of the farm environment and risk of horizontal contamination of eggs. Thus, controlling Salmonella on-farm is important to public health. Disinfectants like quaternary ammonium chemicals are frequently used to decontaminate farm premises and equipment. While effective at reducing Salmonella, these chemicals are damaging to the environment. Additionally, there is evidence of emerging resistance, and formation of biofilms can allow these bacteria to persist after decontamination. Therefore, alternative decontamination strategies are needed. Plasma discharged in water (plasma-activated water; PAW), produces reactive oxygen and nitrogen species, resulting in increased conductivity, redox potential, and reduced pH. PAW has demonstrated bactericidal activity for both planktonic/free-floating Gram-positive and Gram-negative bacterial species.

**Hypothesis:** PAW is an effective disinfectant against *Salmonella typhimurium* biofilms.

**Methodology:** five PAW types were tested and four *S. typhimurium* strains (three isolates from different Australian hen farms and reference strain ATCC 14028) were used for each experiment. The bactericidal effect of each PAW was demonstrated with time-kill curves and AlamarBlue cell viability assay on 4-day old biofilms, while biofilm eradication was measured by crystal violet assay.

**Results:** The PAW with the highest redox potential and lowest pH had the highest efficacy eliminated culturable bacteria rapidly upon exposure. All PAW types significantly reduced biofilm biomass between 30-50% compared to regular growth conditions. Bacterial viability within biofilms showed that three PAW types resulted in a significant ≥95% reduction of bacterial cell viability compared to the growth control.

**Conclusion:** The bactericidal capacity of PAW on Salmonella highlights their potential use as an alternative control strategy on farms and in the food supply chain. Next, biofilm attachment to eggshells treated with PAW will be tested using the Amsterdam Active Attachment (AAA) model.
**P119- Examining the role of P. aeruginosa aminopeptidase AaaA in a synthetic chronic wound biofilm**

Claire Laxton¹, Ana da Silva¹, Robert Markus¹, Jack Leo², Jeni Luckett¹, Stephan Heeb¹, Kim Hardie¹

1 Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom
2 Department of Biosciences, Nottingham Trent University, Nottingham, United Kingdom

*Pseudomonas aeruginosa* is a leading cause of bacterial wound infections and is associated with disproportionately high mortality in burns patients. Because of its wide arsenal of virulence factors and biofilm formation ability, infections with *P. aeruginosa* are often chronic and difficult to treat. In this study, an in vitro synthetic wound model was used to examine the role of the Arginine-specific aminopeptidase of *P. aeruginosa* A (AaaA) in chronic wound biofilms. AaaA is a highly conserved, surface-tethered autotransporter which cleaves N-terminal arginine from peptides. In the oxygen and nutrient-limited environments of chronic wounds, this free arginine could act as a nutrient source or signalling molecule involved in biofilm regulation. AaaA has potential as an antimicrobial drug or vaccine target due to its accessibility and immunogenicity, and is known to be important for virulence in mouse chronic wounds, yet its mechanism is unclear. This study aims to probe the role of AaaA in the synthetic wound biofilm using the Spytag-Spycatcher protein tagging system, RT-qPCR and RNA-Seq to quantify and localise AaaA expression and identify co-regulated genes. We are also developing the use of ToF-SIMS with hybrid OrbiTrap™ (3D-OrbiSIMS) to examine the metabolic profile of *P. aeruginosa* within the synthetic wound biofilm, and gain insight into the impact of AaaA on its environment. So far, we’ve shown that in the synthetic wound, AaaA is present on the transcriptional and translational level and confers a small but significant survival advantage, as seen previously in vivo. We have also localised AaaA at the single cell level, to examine its distribution on the cell surface. Further work is underway to localise AaaA expression within the synthetic wound biofilm. Future work includes probing the role of AaaA in a polymicrobial wound, and the possible effects of increased local arginine on biofilm community structure.
**P120- Cyclic-di-GMP signaling controls metabolic activity in Pseudomonas aeruginosa**

Mads Lichtenberg¹, **Kasper Nørskov Kragh**¹,², Blaine Gabriel Fritz³, Julius B. Kirkegaard³, Tim Tolker-Nielsen¹,*, Thomas Bjarnsholt¹,²,*

¹ Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, 2200 Copenhagen, Denmark
² Department of Clinical Microbiology, Copenhagen University Hospital, 2100 Copenhagen, Denmark
³ Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark
*Corresponding authors

Bacteria in biofilms are embedded in an extracellular matrix and display low metabolic activity partly due to insufficient diffusive exchange of metabolic substrate. The extracellular matrix and low metabolic activity both contribute to the high antibiotic tolerance which is a hallmark of biofilm bacteria. Biofilm development in *Pseudomonas aeruginosa* is regulated by the second messenger molecule cyclic-di-GMP, where high internal levels lead to biofilm formation and low levels are associated with planktonic bacteria. Here, we show that c-di-GMP signalling is a major determinant of the metabolic activity of *P. aeruginosa*, both in planktonic culture and in two biofilm models. The high c-di-GMP content of bacteria in biofilms forces them to rapidly spend a large amount of energy on the production of exopolysaccharides, resulting in a subsequent low metabolic state. This suggests that the low metabolic state of the bacteria in mature biofilms to some extent is a consequence of a c-di-GMP-regulated survival strategy.
P123- An in vitro study of bacterial biofilm formation on a mucin-based biomimetic material

Mengqi Wu¹, Anthony Buckley¹, Mar Collado-González¹², Francisco M. Goycoolea¹.

1 University of Leeds, Leeds, UK
2 University of Murcia, Murcia, Spain

Mucin, the major component of mucus layer, can not only serve as a protection of epithelia, but also associates with the colonization of gut microorganisms. Species like Bacteroides and Akkermansia, also known as mucin degraders, can survive and colonize gut mucus by hydrolysing mucins from mucus layers. They can even modulate quorum sensing of gut microbiota by producing signal molecules to induce cell-to-cell communication in a multispecies environment.

In this study, mucin biomimetic gels (MBG) were prepared and used for coating glass coverslips to simulate gut mucus layers. We hypothesize that MBG-coated coverslips can serve as a biomimetic hydrogel platform for the in vitro study of bacterial biofilms. Three bacterial species, 1) Bacteroides thetaiotaomicron (Bt), a mucin degrader; 2) Lactobacillus paracasei (Lp), a negative control; and 3) Clostridioides difficile (Cd), which is believed to be chemottracted by mucin, were individually cultured with MBG-coated or uncoated coverslips. After anaerobic incubation for three days, coverslips were washed and stained with 1% (w/v) crystal violet. The amount of biofilm biomass on coverslips was determined spectrophotometrically from the OD600. Results indicate that all three strains can adhere on surface of both coated and plain coverslips. Extensive mucin-induced biofilm formation was also confirmed by SEM. However, the amount of biomass on mucin-coated ones in Bt and Cd culture was significantly larger than on uncoated coverslips (2.67-fold and 2.78-fold increase, respectively). Especially for those ones in Bt culture, they had the highest amount (with OD600 of 6.5 vs 0.4 and 2.6 in Bt, Lp, and Cd biofilms, respectively). From these preliminary results, we conclude that this MBG-coated biomimetic platform is amenable for in vitro bacterial biofilms studies, as well as for studies of mucin-bacteria interactions. Future studies will focus in analysing cell-cell communication between species including transcriptional studies of early-stage biofilm formation.
Uncontrolled biofilm growth is a major problem for many sectors across the globe. Food processing and related industries could be nominated as the most pathogen-affected ones, with severe economical and consumer health damages. There are many ongoing projects and several strategies worldwide targeting the control and inhibition of the biofilm development and growth. One of the most common foodborne pathogen with biofilm forming property is Salmonella enterica (SE). It can easily form biofilms on the common industrial surfaces like high-density polyethylene (HDPE) and stainless steel (SS), thus leading to a bacterial food contamination and resistance to traditional disinfectants and antimicrobials.

The goal of this work was to grow the SE biofilm on uncoated SS sample and study its morphology (by Scanning Electron Microscopy), ability to forms colonies after incubation at specific conditions (by culturing on selective agar) and measure the strength of the biofilm with the Crystal Violet assay (by measuring OD values). The very same protocol was performed on the coated SS samples: novel potential antimicrobial coating of 3 different nanocompositions was deposited and the influence on the biofilm growth was investigated. Study of the bacterial inhibition was also performed. In all the cases, we saw prevention of the biofilm formation and inhibition of the bacteria during the incubation due to the controlled release of antimicrobial species. Morphological changes, production of the extracellular matrix and investigation of the alive and dead bacteria on the surface of uncoated and coated coupons were assessed by means of Confocal Laser Scanning Microscopy. In this way it was possible to trick bacteria: the composition of the coating is similar to the one of Extracellular matrix (ECM), bacteria use it as a scaffold and die due to the cationic effect (direct contact) of the components of the coating and ion release (indirect contact).
INTRODUCTION
The complexity of mixed infections caused by Candida albicans and Staphylococcus aureus presents a substantial problem to manage and treat.

HYPOTHESIS
Presented work aimed to evaluate the antimicrobial effect of photodynamic therapy (PDT) on co-infection of S. aureus and C. albicans developed in an in vivo model - Galleria mellonella larvae (GML) in presence of photosensitizer methylene blue (MB) and quorum sensing molecule farnesol (FAR).

METHODOLOGY
GML were inoculated with different doses of pathogens to monitor the survival of single/co-species infection. The proliferation of S. aureus and C. albicans during co-infection was determined after 24 h using colony-forming units (CFU) estimated per larva. The survival of GML was evaluated and compared between infected/co-infected groups and the groups of individuals treated with either MB, MB irradiated with a red laser (PDT), or with the combination of MB-FAR and PDT.

RESULTS
The optimal inocula for C. albicans and S. aureus were determined for 2.5x10^5 and 1x10^6 cells/larva, respectively. In co-infection, 5x10^4 and 6x10^5 cells/larva were tested for C. albicans and S. aureus, respectively. Additionally, 24 h after co-infection, C. albicans and S. aureus CFU/larva increased from 5x10^4 to 1.5x10^5 and 6x10^5 to 3x10^7, respectively. The highest therapeutic effect was achieved in the group of GML infected with S. aureus after application of PDT in combination with 0.5 mM MB and 150 μM FAR resulting in a 50% difference in survival between treated and untreated GML.

CONCLUSION
Preliminary results showed a different response of GML to treatment depending on whether single or co-infection was tested, with the best response of the single-species S. aureus infection. Co-infected GML did not respond appropriately to the therapy. Further research is needed to optimize the tested concentrations of MB and FAR, or modify the PDT approach.
P129- Pre-clinical validation of cold-plasma technology for the treatment of biofilm infections

Adrian Abdo 1, Thomas Schmitt-John 2, Katharina Richter 1,3

1 Richter Lab, Department of Surgery, Basil Hetzel Institute for Translational Health Research & The Queen Elizabeth Hospital, University of Adelaide, Adelaide, Australia
2 Plasmatreat GmbH, Queller Straße 76-80, 3803 Steinhagen, Germany
3 Institute for Photonics and Advanced Sensing, University of Adelaide, Adelaide, Australia

Introduction: The frequent failure of best medical care with antibiotics to control biofilm infections necessitates discovery of innovative, more effective treatments, such as cold-plasma technology; an ionised gas that enhances physico-chemical properties of materials. Cold-plasma discharged in water produces a plethora of reactive oxygen and nitrogen species, resulting in lower pH and increased redox potential, creating a potent antimicrobial environment to treat infections without antibiotics.

Hypothesis: Plasma-activated water (PAW) effectively kills ESKAPE bacteria in biofilms.

Methods: The minimum inhibitory concentration and the antibiofilm activity of PAW was assessed in ESKAPE pathogens using the AlamarBlue viability assay in vitro. Focussing on methicillin-resistant Staphylococcus aureus (MRSA), the antibiofilm activity of PAW was determined in a 3D-biofilm model with real-time imaging (bioflux flow system), in a wound infection model using an artificial dermis and in an in vivo infection model using Galleria mellonella larvae. Cytotoxicity of PAW was evaluated in human keratinocytes (HaCaT) by the lactate dehydrogenase assay. Statistical analysis: one-way ANOVA.

Results: PAW inhibited the growth of ESKAPE pathogens at 25% concentration (in media) and killed 99% of biofilm for all bacteria tested (p0.0001). These results were further confirmed by real-time imaging, which showed substantial killing and partial removal of 3D-MRSA biofilms under flow conditions. In a wound infection model using an artificial dermis, PAW treatment of MRSA biofilms resulted in a 4 log10 CFU reduction of MRSA compared to untreated controls (equal to 99.99% biofilm eradication). Galleria mellonella larvae infected with MRSA and treated with PAW survived the deadly infection compared to infected, untreated controls. Human cells were tolerant to PAW, showing no toxicity in keratinocytes over 48 hours PAW exposure.

Conclusion: PAW was highly effective against multidrug-resistant bacterial biofilms. Cold-plasma technology has the potential to produce effective antimicrobial treatments, for example, PAW as an antibiotic-free lavage for surgical wounds.
P130- Genetic Screening Methods Reveal Vulnerabilities in Extracellular DNA and Cellulose Production in Mycobacterial Biofilms

Saara Lehmusvaara, Alina Sillanpää, Milan Wouters, Ndeqwa Maina, Milka Hammaren, Kirsi Savijoki, Mataleena Parikka

1Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
2Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Antwerp, Belgium
3Department of Food and Nutrition Sciences, University of Helsinki, Helsinki, Finland
4Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Mycobacteria, including *Mycobacterium tuberculosis*, are highly tolerant to antibiotic treatments, which leads to prolonged treatments and possibilities to antibiotic resistance development. One crucial reason for the high antibiotic tolerance is development of bacterial biofilms, which has recently been shown to occur also in human tuberculosis. By targeting of mycobacterial biofilms, antibiotic sensitivity could be enhanced. We have created a MycoMarT7 transposon based randomly mutated library of *Mycobacterium marinum* and developed screening methods to reveal genetic networks associated with deposition of two main components of mycobacterial biofilm matrix: cellulose and extracellular DNA (eDNA). Here, mutant clones were cultured in biofilm forming conditions, followed by staining with eDNA or cellulose binding dyes. To confirm altered cellulose abundance in the identified mutant clones, we developed an HPLC based semi-quantitative method. Whole genome sequencing of the three best hits from the eDNA screen revealed a deletion in a gene encoding a glycosyl transferase. Interestingly, this deletion enhanced eDNA levels in early biofilm formation, and surprisingly, sensitized biofilms to rifampicin, a first line antibiotic against tuberculosis. Taken together, we have developed efficient screening methods to identify genes involved in eDNA and cellulose synthesis/deposition to mycobacterial biofilms. Inhibition of these key mechanisms in biofilm development would enhance antibiotic sensitivity and thus is a promising target for novel antimicrobe drug development.
P131- A comparative study on the antimicrobial activity of three cold plasma jets against Staphylococcus aureus biofilms

Thomas P. Thompson (1), Ross Duncan (1), Aled Morton (1), Jordanne-Amee Maybin (1), Carla McDonnell (2), David Riley (2), Paula Bourke (3), Noreen J. Hickok (4), Theresa A. Freeman (4), Brendan F. Gilmore (1)

(1) Biofilm Research Group, School of Pharmacy, Queen’s University Belfast, Belfast, UK
(2) School of Mathematics and Physics, Queen’s University Belfast, Belfast, UK
(3) College of Science and Health, Dublin Institute of Technology, Dublin, Ireland
(4) Department of Orthopaedic Surgery, Thomas Jefferson University

Soft-tissue infections and surgical site infections such as those related to indwelling medical devices represent a potential site for biofilm formation. Biofilm infections related to implants are extremely difficult to treat, and this issue is further complicated by the increased prevalence of antimicrobial resistance microbes [1]. Of particular concern is methicillin-resistant S. aureus (MRSA) [2], its presence significantly reduces the possibility of successfully eradicating joint infection.

Modern medicine requires new therapies such as cold atmospheric plasma (CAP) to overcome this challenge. CAP treatment has consistently demonstrated antimicrobial effects against clinically relevant pathogens such as Pseudomonas aeruginosa biofilms [3,4] and has real promise as a new non-antibiotic candidate for treating infections. Furthermore, the approval of FDA and EU plasma devices such as J-Plasma®, kINPen® MED, and Canady Hybrid Plasma® Scalpel suggests that the CAP could be quickly adapted for medical application for treatment/prevention of surgical site infections.

In this study, we investigated CAP as a novel technology to combat S. aureus and MRSA using in vitro biofilm models. To undertake this, we used three different plasma devices - a kHz-driven, in-house–designed cold plasma jet, the Bovie Medical J-Plasma®, and the kINPen® MED. Each plasma device was characterised using optical emission spectroscopy and reactive species determination (nitrate, nitrite, and hydrogen peroxide). Each device was revealed to be extremely effective at reducing the bioburden of MRSA biofilms with some producing up to a 4-fold reduction in 120-sec plasma treatment. Furthermore, our results demonstrated that while the quantity and presence of specific reactive species are important contributors to the anti-biofilm activity other factors including treatment surface, manipulation of the device, and carrier gas are important.
P133- Mechanistic studies of probiotic bacteria tackling pathogenic biofilms


[1] Laboratory for Biointerfaces, Empa, the Swiss Federal Laboratories for Materials Science and Technology, Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

It is estimated, that at least 2 in 100 people in developed countries like the United States will experience a chronic wound once in their lifetime, which strongly negatively affects life quality of patients, and has a significant economic impact on health care systems [1, 2]. The prevalence of chronic wounds is expected to rise due to an aging population with increasing obesity and diabetes rates [3, 4]. The treatment of chronic wounds is very challenging, because they tend to become infected with pathogenic biofilm forming bacteria [5] that are highly tolerant to conventional antibiotics and antimicrobials [6].

Research for alternative and adjuvant therapies is urgently needed. We found the application of certain Lactobacillus bacteria such as L. plantarum and their secreted compounds have antimicrobial and anti-biofilm properties against clinically relevant pathogens such as Pseudomonas aeruginosa and Staphylococcus aureus. Furthermore, we investigate the underlying mode of actions of the probiotics against pathogens via both analytical chemistry and omics approaches.

P134- The biochemical characterization of a novel cysteine protease from the oral microbiota

Leo F (1,2), Wickström C (2), Lood R (1,3), Svensäter G (2)

1 Genovis AB, Scheelevägen 2, 223 63 Lund, Sweden.
2 Department of Oral Biology, Faculty of Odontology, Malmö University, Malmö, Sweden.
3 Department of Clinical Sciences Lund, Division of Infection Medicine, Lund University, Lund, Sweden.
Corresponding author: Fredrik Leo, fredrik.leo@mau.se or fredrik.leo@genovis.com

It was recently showed that presence of MUC5B can specifically upregulate enzymes from oral microbial biofilm communities. The aim of this study is to biochemically characterize one of these enzymes, herein called enzyme X, in terms of substrate specificity, pH- and ion preference, glycan dependence and enzyme classification. Enzyme X is in silico annotated as a glycan-dependent protease and hence is expected to hydrolyse glycoproteins in biologically relevant conditions. To facilitate analysis, we used the substrate TNFR-IgG1 Fc fusion protein etanercept, resembling MUC5B in terms of its high abundance of both N- and O-glycans. Etanercept was used to determine enzyme X’s biochemical properties, optimal enzymatic conditions, as well as the protease sensitivity to inhibitors. Finally, we determined the enzyme’s dependence of glycans for activity, by sequential removal of O-glycan monosaccharides. The highest activity was seen in pH 5.5-7.0 in temperatures ranging from 29 to 45°C. The enzyme activity was slightly increased with Ca2+, Mg2+ and EDTA, whereas Zn2+ ions completely abolished the activity. Sodium chloride does not have a major impact on activity, whereas the reducing agent L-cysteine gives a vast improvement. Seven protease inhibitors out of a panel of twelve, had a negative effect on the activity suggesting the enzyme being classified either as a serine protease, but more probably as a cysteine protease. The O-glycoform preference test indicated the enzyme was hydrolysing etanercept independently on the glycan, hence being a more general protease. The biochemical data is biologically logical and relevant, and unexpectedly it is not dependent on any glycans enabling hydrolysis. Further investigations on the substrate preference, digestion pattern and localization within or outside the cell are needed to clarify the enzyme’s potential influence on oral biofilms.
P135- Identification and Antimicrobial Resistance Profiling of Respiratory Pathogens Using Multi-excitation Raman Spectroscopy

Dr Callum Highmore, Dr Adam Lister, Dr Niall Hanrahan, Professor Saul Faust, Professor Sumeet Mahajan, Professor Jeremy Webb

School of Biological Sciences, University of Southampton, National Biofilms Innovation Centre

Currently, antibiotic sensitivity testing relies on culture-based techniques, delaying diagnosis of antimicrobial resistant (AMR) infections. In addition, detection of biofilms, a mode of bacterial growth that facilitates AMR, relies on complex methods which are time, labour and cost intensive. Raman spectroscopy is a rapid, selective, and label-free method of fingerprinting a sample through interrogation of vibrational modes of molecules and offers the potential for real-time, close-to-patient diagnostics for biofilms and AMR. We have developed a multi-excitation Raman spectroscopy methodology that enhances Raman capacity for pathogen identification and characterisation. By analysing bacterial samples at wavelengths 532nm and 785nm, we can achieve rapid strain-level differentiation of respiratory pathogens Pseudomonas aeruginosa and Staphylococcus aureus. By combining multi-excitation Raman spectra with support vector machine (SVM) analysis, identification of both species following inoculation into artificial sputum medium was achieved with 99.75% accuracy, including 100% accuracy for drug-sensitive and drug-resistant S. aureus. Analysis of Raman peaks associated with P. aeruginosa and S. aureus were applied to Raman images of mono- and dual-species biofilms, to work towards label-free identification of biofilm constituents.

AMR sensitivity profiles (for tobramycin, ceftazidime, ciprofloxacin, and imipenem) were generated for 21 P. aeruginosa strains and compared with their Raman spectral signatures using the multi-excitation methodology. Spectra could be correctly categorised by their antibiotic sensitivity profile using SVM, with >98% accuracy. Respiratory pathogens with unknown antibiotic sensitivity profiles will undergo similar analysis to determine the capability of the multi-excitation Raman methodology to predict AMR and recommend antibiotic treatments for bacterial respiratory infection. Together these data provide the basis for a potential new clinical diagnostic platform. With a rapid methodology that requires little sample preparation, it could have the transformative capability to save lives at scale and reduce the spread of AMR.
P137- Development of ad hoc methods to evaluate drug efficacy against *Haemophilus influenzae* biofilms reveals cinnamaldehyde analogs as a promising alternative to counteract infection

María Lázaro-Díez¹, Javier Asensio-López¹,², Ioritz Sorzábal³, María Monteserín-Leiva², Saioa Burgui², Eva Arroyo-Urea⁴, Ana González-Paredes⁴, Carlos Ortiz de Solórzano³,⁵, Fernando Herranz⁴,⁶, Junkal Garmendia¹,⁶

1 Instituto de Agrobiotecnología, Consejo Superior de Investigaciones Científicas (IdAB-CSIC)-Gobierno de Navarra, Mutixa, Spain; 2 Asociación de la Industria Navarra (AIN), Navarra, Spain; 3 Centro de Investigación Médica Aplicada (CIMA), Navarra, Spain; 4 Instituto de Química Médica, Consejo Superior de Investigaciones Científicas (IQM-CSIC), Madrid, Spain; 5Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain; 6 Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain

**Introduction.** Biofilms in nontypeable *Haemophilus influenzae* (NTHi) are directly associated with otitis media development and, moreover, this bacterial life style is believed to have a major role in NTHi chronicity and survival in the lower airways of COPD patients. Importantly, the density dependent sensor quorum sensing network conducted by Al-2 has been shown to have an important function in NTHi biofilm formation and maturation. Considering the lack of standardized protocols to test the efficacy of anti-biofilm compounds, we propose here a methodology for this purpose, further validated by using two Al-2 inhibitors against NTHi biofilms.

**Hypothesis.** Cinnamaldehyde analogs have an anti-biofilm effect on NTHi.

**Methodology.** Three clinical NTHi strains were used in this study. Two cinnamaldehyde analogs and ampicillin were evaluated as anti-biofilm compounds. Biofilm formation inhibition experiments were performed in 96 well plates. After 24 h incubation at 37°C, inhibition of biofilm formation was determined by crystal violet staining and CLSM. Moreover, minimal biofilm eradication concentration (MBEC) was determined after addition of different concentrations of each drug during 6 h on preformed biofilm. Biofilm CFUs were plated and MBEC was determined as the lowest concentration where CFUs were not observed.

**Results.** (1) Biofilm formation inhibition experiments showed dose-dependent decrease of biofilm formation by the isolates tested when using subinhibitory concentrations (under MIC) of both cinnamaldehyde analogs. (2) MBEC experiments showed total eradication of preformed NTHi biofilms when using Al-2 inhibitors at lower concentrations (between 100-400 μg/mL, depending on strain and drug) than those needed when using ampicillin (>6,400 μg/mL).

**Conclusions.** Two assay types to assess drug anti-biofilm efficacy were developed and validated for NTHi across genomic-phenotypic heterogeneous clinical isolates. Molecules used in this study showed effect in both inhibition of biofilm formation and preformed biofilm eradication. Cinnamaldehyde analogs are shown as a promising alternative for combating biofilm NTHi infections.
P138- In vitro anti-bacterial, anti-biofilm, and anti-adhesive effects of Lactobacillus strains on Pseudomonas aeruginosa isolated from cystic fibrosis patients

1 Esingül Kaya, 1 Giuseppantonio Maisetta, 1 Elisa Catelli, 1 Sofia Mazzantini, 2 Veronica Lupetti, 2 Arianna Pompilio, 2 Giovanni Di Bonaventura, 1 Semih Esin, 1 Giovanna Batoni

Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy 1; Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy 2

Introduction: Chronic lung infections caused by Pseudomonas aeruginosa (PA) play a significant role in the mortality and morbidity of cystic fibrosis (CF) patients. The widespread bacterial resistance to conventional antimicrobials demands the identification of new strategies to complement or replace current antibiotic therapies.

Hypothesis/aims: To test the hypothesis that selected probiotics strains, administered by aerosol, could directly interfere with the growth of PA strains, in this study we aimed at screening several commercial Lactobacillus strains for their ability to: i) grow in experimental conditions resembling CF environment; ii) inhibit planktonic or biofilm growth of PA strains; iii) adhere and inhibit PA adhesion to lung epithelial cells (A549).

Methodology: Lactobacilli were grown in an artificial sputum medium (ASM) and their CFU numbers were evaluated at 24h as compared to the corresponding inoculum. Clinical PA strains were co-cultured with lactobacilli in ASM and their CFU number compared to that of monocultures. Antibiofilm effects of lactobacilli were evaluated via crystal violet staining. Adhesion assays were performed by pre-incubating Lactobacillus strains with A549 cells for 2h followed by washes and incubation with PA strains for an additional 1h.

Results: L. rhamnosus and L. plantarum showed significant growth capacity in ASM. L. plantarum was also able to inhibit the growth of two clinical PA strains and to cause a statistically significant reduction of both biofilm formation and preformed biofilms of the same PA strains. Most of the lactobacilli tested showed adherence capability on A549 cells, although at different extent. L. acidophilus showed the highest adherence capacity and ability to diminish PA adherence up to 65%.

Conclusions: Overall, L. rhamnosus, L. plantarum and L. acidophilus seem promising candidates to survive in the CF-lung environment and compete with CF PA strains. The study received support from the Italian Cystic fibrosis research foundation, Project FFC#13/2021.
P140- Investigating the antimicrobial efficacy of plasma activated water against food spoilage organisms

Laura McClenaghan, Thomas P. Thompson, Brendan F. Gilmore

Biofilm Research Group, School of Pharmacy, Queen’s University Belfast, BT9 7BL, UK

Spoilage organisms are a major concern for the meat industry. Their ability to survive and grow under processing and storage conditions followed by continuous adaptation and increasing tolerance of microbial population to antibiotics and disinfectants has made it even more difficult to control. Although they rarely cause human disease, they can affect the quality of the final product and cause premature spoilage during storage resulting in food waste. They often persist in food processing environments as biofilms which can result in the recurrent contamination of food products. Cold atmospheric plasma is a novel and promising alternative technology for microbial inhibition in food processing environments where the antibacterial effects are a result of the low pH and the reactive oxygen and nitrogen species.

This study aims to identify the spoilage organisms in vacuum packaged meat using Nanopore DNA sequencing and to determine the antibiofilm efficacy of plasma activated water (PAW) generated using a novel plasma bubble reactor. The microbial consortium will also be analysed pre and post plasma treatment, with a sensory analysis carried out on the quality of the meat samples following PAW exposure.

Sequencing results have shown that lactic acid bacteria including *Carnobacterium divergens*, *Lactococcus piscium*, *Lactococcus cremoris* and *Leuconostoc gelidium* were the dominant species. Complete inactivation with PAW was obtained in under 60 seconds for all the isolated spoilage organisms. Chemical scavengers were used to specifically quench reactive species generated in the PAW to investigate their roles in antimicrobial activity and showed the short-lived species superoxide and singlet oxygen are responsible for the bacterial inactivation. The impact of PAW on the quality of meat samples during storage was also assessed with no negative sensory changes. Further studies will include biofilm susceptibility with preliminary results showing a high antimicrobial effect against biofilms after a short exposure time.
P141- Biofilm disruption through controlled antimicrobial release from ultrasound-responsive silica nanoparticles

Sarah A. Kuehne (1), Menisha Manhota (1, 2), Maria Odyniec (2), Rachel L. Sammons (1), Damien A. Walmsley (1), Zoe Pikramenou (2)

(1) School of Dentistry, The University of Birmingham, Birmingham, United Kingdom.
(2) School of Chemistry, The University of Birmingham, Birmingham, United Kingdom.

Introduction
Root canal infections are notoriously difficult to treat, as bacteria colonise the microscopic network of dental tubules within the tooth, forming biofilms. Considering the ever-increasing antimicrobial resistance, it is imperative to utilise methods that are targeted, allowing for treatment at the site of infection, ideally without disrupting the surrounding healthy microbiome. Silica nanoparticles can encapsulate suitable antimicrobials which are only released upon a trigger.

Here we have created such particles, containing cetylpyridinium chloride (CPC) or ciprofloxacin (CPX), and treated oral biofilms in vitro.

Hypothesis
A drug delivery system equipped with triggered release allows for enhanced, controlled killing of biofilms.

Methodology
Silica nanoparticles have been synthesised in a one-pot method encapsulating CPC. Release upon cavitation has been monitored and measured by the absorbance of CPC with UV-Vis spectroscopy.
Biofilms of oral Streptococci were formed on coverslips. These were treated with ultrasound (US) and particles. Viability was measured using live/dead staining, confocal microscopy and colony counting.

Results
Streptococcus sanguinis biofilms treated with US and particles showed significantly more dead bacteria, than biofilms treated with the drug alone, US alone or plain particles, showing a synergistic effect of US (cavitation) and targeted drug release from nanoparticles. Nanoparticles encapsulating CPX showed enhanced killing of Streptococcus mutans biofilms compared to S. sanguinis biofilms, highlighting potential for selectively inhibiting pathogenic species.

Conclusion
Antimicrobials can be released from silica nanoparticles, using ultrasound as the trigger, which leads to targeted killing of biofilms.
**P142- The role of cyclic-di-AMP in co-aggregation and biofilm formation of *Fusobacterium nucleatum***

Penelope McLaney (1), Maria Muchova (1), Daniel J. Slade (2), **Sarah A. Kuehne** (1)

(1) School of Dentistry, The University of Birmingham, Birmingham, United Kingdom.
(2) Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.

**Introduction**

*Fusobacterium nucleatum* is pivotal in the formation of oral biofilms and in particular the shift from a healthy oral microbiome to a disease-associated state. Despite this recognition, little is known about the molecular mechanisms of *F. nucleatum*, including its interaction with other oral bacteria. Signalling molecules like cyclic dimeric adenosine monophosphate (c-di-AMP) have been studied in many bacteria and shown to be involved in virulence and communication.

**Hypothesis**

C-di-AMP is involved in co-aggregation and biofilm formation of *F. nucleatum*.

**Methodology**

The wildtype strain *F. nucleatum* ATCC23726, a c-di-AMP synthesis mutant and a c-di-AMP hydrolysis mutant were used in the experiments. Co-aggregation was measured using photo-spectrometry and included two further oral species: *Streptococcus oralis* and *Porphyromonas gingivalis*. Mono- and multi-species biofilm biomass was determined using a crystal violet assay.

**Results**

The synthesis mutant aggregated significantly less with *P. gingivalis*, which was corroborated with multi-species biofilm growth, where the mean biomass of the mutant (OD600 = 3.67) was significantly lower than wildtype (OD600 = 7.54). In contrast the hydrolysis mutant showed significantly higher biomass than the wildtype in mono-species biofilms.

**Conclusion**

Results suggest a role for c-di-AMP in auto-aggregation, as well as in co-aggregation with *P. gingivalis*, an important oral pathogen.
P143- Spatiotemporal monitoring demonstrates prebiotic treatment responses and reversibility in a periodontal multispecies biofilm model

Justien Ghesquière, Department of Chemical Engineering, KU Leuven, Leuven, Belgium
Wannes Van Holm, Periodontology and Oral Microbiology, KU Leuven, Leuven, Belgium
Kenneth Simoens, Department of Chemical Engineering, KU Leuven, Leuven, Belgium
Erin Koos, Department of Chemical Engineering, KU Leuven, Leuven, Belgium
Nico Boon, Center for Microbial Ecology and Technology (CMET), UGent, Gent, Belgium
Wim Teughels, Periodontology and Oral Microbiology, KU Leuven, Leuven, Belgium
Kristel Bernaerts, Department of Chemical Engineering, KU Leuven, Leuven, Belgium

The onset of periodontitis is initiated by a complex interplay between bacterial interactions in the oral community, its environment and the host. Development of successful treatment strategies highly benefit from in vitro periodontal model systems which allow characterization of largely unknown modes of bacterial (inter)actions. This study uses a 700 mm² drip flow biofilm reactor to investigate spatial and temporal effects of L-arginine (a known oral prebiotic) treatment on a periodontal multispecies biofilm model. The drip flow biofilm reactor is inoculated with a periodontal community for a short anaerobic recirculation phase, followed by a continuous aerobic feeding phase with half-strength medium. Five key species are present, of which four are fluorescently labelled. Daily metabolic analysis and confocal imaging combined with end-point qPCR measurements return metabolite consumption and production rates, and structural and compositional biofilm features, respectively.

Over a time course of three days, the biofilm reaches metabolic, structural, and compositional steady-state conditions. Subsequent exposure to L-arginine (1.5% in the medium flow) destabilizes the oral biofilm in terms of biofilm volume, thickness and composition already after the first day of treatment. The biofilm becomes thinner and shows shifts in local occupancies towards higher abundancies of streptococcal strains that metabolize L-arginine. After a 4-day treatment, the biofilm developed into a less pathogenic biofilm, mainly caused by a reduction in bacterial numbers of *Fusobacterium nucleatum* with more than 2 log values. Interestingly, if after initial exposure L-arginine is again left out of the medium, bacterial numbers of *F. nucleatum* as well as structural biofilm features shift again to values similar to the untreated control biofilm.

This study demonstrates the strength of a drip flow periodontal biofilm model in studying dynamic treatment responses. In ongoing work, this setup is used to investigate the effects of a synbiotic (prebiotic + probiotic combination) for periodontitis.
**P145- Phytochemical analysis and anti-biofilm effect of Hopea ferrea against multi-species cariogenic consortia biofilms**

**Nurul Izzah Mohd Sarmin** (1), Oscar Allan Furlong Lopez (2), David Moyes (3), Avijit Banerjee (2), Khulood Al-Mansour (2), Fatin N adiah Thaqifah Shahriman (1) And Fatimah Salim (1)

(1) Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns), Universiti Teknologi MARA (UiTM), Selangor, Malaysia  
(2) Centre of Oral, Clinical and Translational Science, King’s College London, London, United Kingdom  
(3) Host-Microbiome Interactions, King’s College London, London, United Kingdom

**Introduction:** Dental caries is a chronic disease that progresses slowly in most people which the biofilm formation is the key virulence factor. Hypothesis This study hypothesised that an in vitro 6-species oral biofilm can be disrupted by a plant extract from Hopea ferrea, resulting in reduced cariogenicity.

**Methodology:** Minimum inhibitory concentrations (MIC) and minimal bactericidal concentration (MBC) were determined to assess antimicrobial effects of the extract against planktonic Streptococcus mutans. A cross-kingdom representative cariogenic consortia (S. mutans, Streptococcus oralis, Actinomyces oris, Actinomyces viscosus, Lactobacillus acidophilus and Candida albicans) were grown anaerobically in artificial saliva where gastric mucin is the main carbon source for biofilm formation. The biofilm biomass and metabolic activity at different concentrations of H. ferrea extract and incubation periods were assessed using crystal violet and XTT assays. Real-time polymerase chain reaction (qPCR) was used to determine microbial composition of biofilms post-treatment. Furthermore, LDH assay was used to evaluate the cytotoxic activity of the extract. The phytochemical profiles of H. ferrea was determined by ultra-high performance liquid chromatography-high-resolution tandem mass spectroscopy (UHPLC-HRMS/MS) analysis.

**Results:** H. ferrea showed antibacterial activity against S. mutans at MIC and MBC of 0.3125 mg/mL. Following exposure to 1.25 mg/mL of H. ferrea extract, multi-species cariogenic biofilms showed a significant reduction (pp<0.05) in metabolic activity and total biomass. However, the extract was less active against maturated biofilms. More importantly, H. ferrea significantly suppressed L. acidophilus and enriched S. mutans abundance in the multi-species biofilm. The phytochemical analyses carried out on H. ferrea extract showed the major constituent was stilbenoids. In addition, the extract demonstrated no obvious cytotoxicity in human fibroblasts.

**Conclusions:** These findings demonstrate the biofilm-modifying activity of H. ferrea in early biofilm formation emphasizing the therapeutic capacity of phytochemicals as an effective tool to inhibiting pathological activity of oral biofilms.
Biofilm formation in multi-drug-resistant *Klebsiella pneumoniae* strains of food and human origin: effect of culture conditions and Quorum Sensing

Sergio Silva-Bea¹, Ana Parga¹, Isidro García-Meniño²,³, Javier Fernández⁴, Azucena Mora², Ana Otero¹

¹ Department of Microbiology and Parasitology, Faculty of Biology, University of Santiago de Compostela, Santiago de Compostela, Spain
² Laboratorio de Referencia de Escherichia coli (LREC), Department of Microbiology and Parasitology, University of Santiago de Compostela, Lugo, Spain.
³ Present address: National Reference Laboratory for Antimicrobial Resistances, Department for Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany.
⁴ Microbiology Service, Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain.

Background

Biofilm-formation is a crucial virulence factor of the multiresistant pathogen *Klebsiella pneumoniae*. Thus, there is an increasing interest in developing anti-biofilm strategies for the treatment of this pathogen, specifically by interfering with the bacterial communication system known as Quorum Sensing (QS). The lack of a standard method for biofilm study in this pathogen, plus the discrepancies found in the bibliography about the QS signals in *K. pneumoniae*, prompted us to study more deeply this phenotype for the future development of anti-biofilm applications.

Methods

Biofilms from 24 *K. pneumoniae* multi-drug resistant strains of different food and human origin were studied using the Active Attachment method under aerobic/microaerobic conditions in LB and LB+0.4% glucose. The effect of DNase I and alpha-amylase and the presence of the QS signal AI-2 and AHL production was studied in the supernatants.

Results

The Active Attachment method is a suitable method for biofilm quantification in *K. pneumoniae* with proven repeatability. Culture conditions strongly affect biofilm formation. LB+0.4% glucose in microaerobiosis (LB+G uAE) is the best condition for strain discrimination. Greater variability between strains and higher biofilm formation were found in human-origin strains, all of them positive for the carbapenemase OXA-48. Most biofilm hyperformers were associated with ST-307 and ST-45. An inverse correlation between biofilm-formation, and antibiotic resistance and bioluminescence production by JAF548 strain, was found in 4 biofilm-hyperformer strains in LB+G uAE condition. AHLs could not be detected in the conditions tested. AI-2 production was detected in all strains and was higher with glucose supplementation. DNase I and alpha-amylase showed high anti-biofilm activity in a hypervirulent strain.

Conclusion

Substantial variability was found among strains regarding biofilm-forming capacity depending on culture conditions. More studies are necessary in *K. pneumoniae* to understand the process of biofilm-formation and the role of QS for the future development of anti-biofilm strategies.
P149- Multiple holins contribute to extracellular DNA release in *Pseudomonas aeruginosa* biofilms

Amelia L. Hynen, James J. Lazenby, George M. Savva, Laura C. McCaughey, Lynne Turnbull, Laura M. Nolan* and Cynthia B. Whitchurch*.

ALH, JJL, LCM, LT, CBW: The three institute, University of Technology Sydney, Ultimo, New South Wales, 2007, Australia.
GS, CBW: Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UQ, UK.
LCM: Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.
LMN: National Heart and Lung Institute, Imperial College London, London, SW3 6LR, UK.
CBW: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK.
* These authors contributed equally.

Bacterial biofilms are comprised of aggregates of cells encased within a matrix of extracellular polymeric substances (EPS). One key EPS component is extracellular DNA (eDNA), which acts as a glue, facilitating cell-cell and cell-substratum interactions. The role of eDNA in maintaining structural integrity of submerged (hydrated) biofilms has been well documented in many bacteria, including *Pseudomonas aeruginosa*. We have previously demonstrated that eDNA is produced in *P. aeruginosa* submerged and actively expanding (interstitial) biofilms via explosive cell lysis. This phenomenon involves a subset of the bacterial population explosively lysing, due to peptidoglycan degradation by the endolysin Lys. Here we use time-lapse phase and fluorescence microscopy, in combination with quantitative spatio-temporal image analyses, to demonstrate that in *P. aeruginosa* three holins, AlpB, CidA and Hol, are involved in Lys-mediated eDNA release within both submerged and actively expanding biofilms. These holins are involved in eDNA release to different extents, depending upon the type of biofilm and the stage of biofilm development. We also demonstrate that eDNA release events determine the sites at which cells begin to cluster to initiate microcolony formation during the early stages of submerged biofilm development. Furthermore, our results show that sustained release of eDNA is required for cell cluster consolidation and subsequent microcolony development in submerged biofilms. Overall, this study adds to our understanding of how eDNA release is controlled temporally and spatially within *P. aeruginosa* biofilms.
P151- Skin dysbiosis in inflammatory acne lesions and the role of Cutibacterium acnes biofilm

Ilaria Cavallo 1, Francesca Sivori 1, Aldo Morrone 2, Fulvia Pimpinelli 1, Enea Gino Di Domenico 3

Microbiology and Virology, IRCCS San Gallicano Institute, Rome, Italy 1;
Scientific Direction, IRCCS San Gallicano Institute, Rome, Italy 2;
Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Rome, Italy 3.

Background
Acne vulgaris is a common inflammatory disorder affecting more than 80% of young adolescents. Cutibacterium acnes plays a role in the pathogenesis of acne lesions, although the mechanisms are poorly understood.

Objectives
To characterize the microbiome at different skin sites in acne patients and the putative role of biofilm production in promoting the growth and persistence of C. acnes isolates.

Methods
Swabs from non-inflammatory (NI) and inflammatory lesions (LA) of 10 acne patients and the skin of 10 healthy subjects (HS) were analyzed using both 16S rRNA sequencing, whole-genome sequencing, and traditional culture methods.

Results
Microbiota analysis showed a significantly lower alpha diversity in LA than in NI and HS. At the species level, C. acnes was significantly more abundant in LA than in NI and HS. Notably, the whole-genome sequencing analysis showed that five genes (rcsB, dppB, clpS, acsA, ytpA) were univocally associated with the IA1 phylotype. In particular, acsA and rcsB are correlated with increased biofilm formation in different bacterial species, while ytpA, a lysophospholipase, is a virulence factor in host-tissue degradation and inflammation. Notably, IA1 isolates were more efficient in early adhesion and biomass production than other phylotypes, exhibiting a significant increase in antibiotic tolerance for ampicillin, benzylpenicillin, clindamycin, and doxycycline compared to the same cells in planktonic state.

Conclusions
The skin microbiome dysbiosis observed in the LA and the correlation with virulent C. acnes phylotypes may shed new light on the pathogenesis of acne, providing novel diagnostic and therapeutic approaches.
**P152- *P. aeruginosa* is capable of natural transformation in biofilms**

Laura M. Nolan, Lynne Turnbull, Marilyn Katrib, Sarah R. Osvath, Davide Losa, James J. Lazenby and Cynthia B. Whitchurch

LMN, LT, MK, SRO, DL, CBW: The three institute, University of Technology Sydney, Ultimo, New South Wales, 2007, Australia.

LMN: National Heart and Lung Institute, Imperial College London, London, SW3 6LR, UK.
JJL, CBW: Microbes in the Food Chain Programme, Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UQ, UK
CBW: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

Natural transformation is a mechanism of horizontal gene transfer that enables bacteria to acquire naked DNA from the environment. Extracellular DNA (eDNA) in biofilms provides a vast reservoir of genetic material that can be sampled by bacteria that are competent for natural transformation. Whilst Pseudomonas aeruginosa is known to produce large quantities of eDNA that is required for biofilm formation and has a remarkable level of genome plasticity, it has long been thought to be incapable of natural transformation. *P. aeruginosa* possesses homologs of all proteins known to be involved in natural transformation in other bacterial species, including type IV pili (T4P). We hypothesised that *P. aeruginosa* may be competent for natural transformation in biofilms where eDNA production and T4P are produced. We found that *P. aeruginosa* in biofilms is competent for natural transformation of both genomic and plasmid DNA and that T4P facilitate but are not essential for natural transformation of plasmids. Surprisingly, we have found that other genes thought to be essential for natural transformation are not required for plasmid natural transformation by *P. aeruginosa*. This suggests that plasmid uptake by natural transformation may occur through a novel mechanism that is distinct from the well characterised pathways used for natural transformation of genomic DNA. Plasmids underpin much of bacterial evolution and contribute to the spread of antimicrobial resistance. Surprisingly, half of all known plasmids have no recognisable conjugation or mobilisation systems. Therefore, we currently have no understanding of how half of the global plasmid content is transmitted through bacterial populations. Natural transformation of non-mobilisable plasmids in biofilms may be an important and under-explored process that provides genetic diversity and transmission of traits including genes conferring bioremediation capabilities, virulence and antimicrobial resistance.
P153- Effect of endocrine disruptors on *Pseudomonas aeruginosa* biofilm formation

Maëliss Manac’h¹, Marie Le Calvé¹, Audrey David², Mélissande Louis², Audrey Thiroux³, Ali Tahrioui², Magalie Barreau², Julien Verdon³, Alexandre Crépin³, Jean-Marc Berjeaud³, Jocelyne Caillon⁴, Olivier Lesouhaitier², Alexis Bazire¹, Alain Dufour¹, Sylvie Chevalier², Sophie Rodrigues¹

1 Laboratoire Biotechnologie et Chimie Marines, Université Bretagne Sud, UR3884, LBCM, IUEM, Lorient, France
2 Bacterial Communication and Anti-infectious Strategies laboratory (CBSA), UR4312, Université de Rouen Normandie, Université de Caen Normandie, Normandie University, Evreux, France.
3 Laboratoire Ecologie & Biologie des Interactions, UMR CNRS 7267, Université de Poitiers, Poitiers, France
4 IRS 2 Laboratoire de Thérapeutique Expérimentale et Clinique des Infections, EA3826, University of Nantes, Nantes, France

**Introduction:** Phthalates constitute an important class of chemical compounds commonly used as plasticizers. They are included in the composition of a wide variety of products (e.g. paints, adhesives, toys, cosmetics and medical device), from which they can be released into the environment while some of them are endocrine disruptors. Due to their widespread utilization, pathogen bacteria are likely to be exposed to these molecules. Endocrine disruptors can exacerbate infectious diseases and increase their severity, but the underlying mechanisms have not yet been deciphered.

**Hypothesis and aims:** *P. aeruginosa* is an opportunistic pathogen and its biofilm formation ability is closely related to chronic infections. The aim of this study was to evaluate the impact of endocrine disruptors on *P. aeruginosa* biofilm formation.

**Methodology:** The effects of DBP and its substitute TXIB (chosen according to a previous screening) were examined. Briefly, *P. aeruginosa* was exposed to DBP or TXIB during biofilm culture, then biofilm structures, biovolumes and matrix composition were monitored using confocal laser scanning microscopy.

**Results:** When exposed to TXIB, biofilm biovolumes were increased, whereas biofilm matrixes were altered since proteins and extracellular DNA (eDNA) abundances were reduced. Furthermore, *P. aeruginosa* exposure to TXIB seemed to favour pellicle formation. By contrast, DBP did not affect biofilm formation, as no significant changes were measured compared to controls without endocrine disruptor.

**Conclusion:** In our conditions, *P. aeruginosa* responded to one of the two tested endocrine disruptors. Our results suggest that the presence of some endocrine disruptors in the environment could promote biofilm formation, which could in turn modify its infectious properties.
Biofilm formation is considered a major cause of therapeutic failure because bacteria in biofilms have higher protection against antimicrobials. Thus, biofilm-related infections are extremely challenging to treat and pose major concerns for public health, along with huge economic impacts. *Pseudomonas aeruginosa*, in particular, is a “critical priority” pathogen, responsible for severe infections, especially in cystic fibrosis patients because of its capacity to form resistant biofilms. Therefore, new therapeutic approaches are needed to complete the pipeline of molecules offering new targets and modes of action. Biofilm formation is mainly controlled by Quorum Sensing (QS), a communication system based on signaling molecules. In the present study, we employed a molecular docking approach (Autodock Vina) to assess two series of chromones-based compounds as possible ligands for PqsR, a LuxR-type receptor. Most compounds showed good predicted affinities for PqsR, higher than the PQS native ligand. Encouraged by these docking results, we synthesized a library of 34 direct and 25 retro chromone carboxamides using two optimized routes from 2-chromone carboxylic acid as starting material for both series. We evaluated the synthesized carboxamides for their ability to inhibit the biofilm formation of *P. aeruginosa* in vitro. Overall, results showed several chromone 2-carboxamides of the retro series are potent inhibitors of the formation of *P. aeruginosa* biofilms (16/25 compound with % inhibition ≥ 50% at 50 μM), without cytotoxicity on Vero cells (IC50 > 1.0 mM). The 2,4-dinitro-N-(4-oxo-4H-chromen-2-yl) benzamide (6n) was the most promising antibiofilm compound, with potential for hit to lead optimization.
P157- Contribution of genetic variations in the regulatory region of surface adhesin-encoding genes to colonization of prosthetic implants by Staphylococcus aureus

Liliana Laverde¹, Maite Echeverz¹, Margarita Trobos², Cristina Solano¹, Iñigo Lasa¹.

1. Navarrabiomed-Universidad Pública de Navarra-HUN, IDISNA. 31008-Pamplona, Navarra, Spain.
2. Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, 40530 Gothenburg, Sweden.

Introduction
A major challenge faced by microbiologists in the postgenomic era is to identify those changes in bacterial genomes that are most likely related with phenotypic abilities to cause disease. When these changes (SNPs) occur at intergenic regions, the causal link between the SNPs and host-adaptive traits is less recognizable and functional validation assays become necessary. Staphylococcus aureus is the leading cause of Periprosthetic Joint infections (PJI). Surface adhesins play an important role in the primary attachment to the plasma proteins that cover the surface of prosthetic devices after implantation.

Hypothesis
Differences in the level of expression of surface adhesins modulate the capacity of S. aureus to colonize implanted prostheses.

Methodology
Sequence alignment identified genetic variations in the promoter region of genes encoding surface adhesins in a collection of clinical S. aureus strains isolated from PJI (n=45) and infected wounds (n=26). The region encompassing 200 nucleotides upstream the first codon of each adhesin-encoding gene was fused with a reporter gene. The effect of genetic variations on the expression level of each adhesin was analyzed by western blot.

Results
The results revealed that the strength of the promoters controlling the expression of surface adhesins is highly variable among strains. Genetic variations in the promoter region of genes encoding orthologous adhesins are characteristic of each lineage and correlate with differences in expression levels of each surface adhesin. Consequently, isolates from the same lineage showed a distinctive profile of surface adhesins expression. Coinfection experiments with a representative isolate of the four most abundant sequence types confirmed differences in the capacity of strains to colonize implanted prostheses.

Conclusion
Genetic variations in the regulatory regions of genes encoding surface adhesins lead to differences in their expression profiles and also, in the colonization capacity of S. aureus lineages.

Acknowledgement: This project has received funding from the European Union’s H2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No 801586 and 754412.
The misuse of antimicrobials is threatening the welfare of future generations. The lack of innovative antimicrobial compounds to replace those that become ineffective compels us to search for new ones.

We therefore tested the antimicrobial activity of 108 novel compounds (pure and crudes) from plants belonging to the Annonaceae and Euphorbiaceae families, against different Gram positive and negative bacteria. We performed a hit finding assay using Escherichia coli MG1655 and Bacillus subtilis YB866 as reference strains. For 30 candidate compounds we made titration curves to estimate EC50, EC90 and MIC against Pseudomonas putida KT2440, Pectobacterium carotovorum NBRC#3380, Staphylococcus epidermidis ATCC35984, and Enterococcus raffinosus NBRC#100492. We explored the biofilm-forming capacity of these strains in different media. A robust protocol using resazurin was developed to evaluate MIC in biofilm for S. epidermidis and P. putida using commercial antibiotics. Cytotoxicity in human cell lines was also tested for the selected compounds.

Two crudes and a pure compound showed antimicrobial activity against all the strains. EC50 values for both crudes were in the range from 69.4 µg/ml to 222.8 µg/ml, while for the pure compound it was from 0.9 µg/ml to 480 µg/ml; their cytotoxicity was also within the same ranges. Regarding biofilm formation, P. putida and S. epidermidis formed a robust biofilm in Mueller-Hinton (MH) plus 2% of glucose (G), while B. subtilis formed a moderate biofilm in MH + 2% G plus 3% casein hydrolysate (CH), E. coli in LB, and E. raffinosus in M63 + 0.2% G + 0.5% CH + 0.4% arginine. Preliminary results with high reproducibility, using kanamycin for P. putida and doxycycline for S. epidermidis, showed an increment of the EC50 and EC90 with respect to the values in planktonic and biofilm. We will estimate these parameters for our compounds.
P160- A new BiofilmChip device for testing biofilm formation and antibiotic susceptibility

Núria Blanco-Cabra 1,7, Maria José López-Martinez 2,3,4,7, Betsy Verónica Arévalo-Jaimes 1, María Teresa Martin-Gómez 5, Josep Samitier 2,3,4 and Eduard Torrents 1,6

1. Bacterial Infections and Antimicrobial Therapies Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.
2. Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.
3. Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain.
4. Department of Electronics and Biomedical Engineering, University of Barcelona, Barcelona, Spain.
5. Microbiology Department, Hospital Universitari Vall d’Hebron, Barcelona, Spain.
6. Microbiology Section, Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain.

Currently, three major circumstances threaten the management of bacterial infections: increasing antimicrobial resistance, expansion of chronic biofilm-associated infections, and lack of an appropriate approach to treat them. To date, the development of accelerated drug susceptibility testing of biofilms and of new antibiofouling systems has not been achieved despite the availability of different methodologies. There is a need for easy-to-use methods of testing the antibiotic susceptibility of bacteria that form biofilms and for screening new possible antibiofilm strategies. Herein, we present a microfluidic platform with an integrated interdigitated sensor (BiofilmChip). This new device allows an irreversible and homogeneous attachment of bacterial cells of clinical origin, even directly from clinical specimens, and the biofilms grown can be monitored by confocal microscopy or electrical impedance spectroscopy. The device proved to be suitable to study polymicrobial communities, as well as to measure the effect of antimicrobials on biofilms without introducing disturbances due to manipulation, thus better mimicking real-life clinical situations. Our results demonstrate that BiofilmChip is a straightforward tool for antimicrobial biofilm susceptibility testing that could be easily implemented in routine clinical laboratories.
P161- Quorum quenching against N-acylhomoserine lactones modifies in vitro periodontal biofilm formation

Ana Parga 1, Andrea Muras 1†, Paz Otero-Casal 2,3, Alexandre Arredondo 4, Agnès Soler-Ollé 4, Gerard Àlvarez 4, Vanessa Blanc 4, Ana Otero 1

1 Department of Microbiology and Parasitology, CIBUS-Faculty of Biology, University of Santiago de Compostela, Santiago de Compostela, Spain.
2 Department of Surgery and Medical-Surgical Specialties, Faculty of Medicine and Odontology, University of Santiago de Compostela, Santiago de Compostela, Spain.
3 Unit of Oral Health, CS Santa Comba-Negreira, SERGAS, Spain.
4 Department of Microbiology, Dentaid Research Center, Cerdanyola Del Vallès, Spain.
† Current address: Laboratory of Microbial Pathogenesis, Navarrabiomed, Public University of Navarre (UPNA), University Hospital of Navarra, IdisNa, Pamplona, Spain.

Recent studies have revealed the presence of N-acylhomoserine lactones (AHLs), typical Gram-negative quorum sensing signals, in the oral environment. These pieces of evidence open the possibility of using quorum quenching (QQ) strategies to control oral biofilms. As AHLs may play a role in oral biofilm formation in certain conditions of oral health, disruption of these molecules could help in the prevention or treatment of oral diseases. We have studied the response of in vitro multispecies biofilms to the addition of the broad-spectrum QQ enzyme Aii20J. Biofilms were generated from saliva and gingival crevicular fluid samples taken from healthy donors (n=11) and patients with periodontal disease (PD; n=29). Quantification of the generated biofilm biomass via crystal violet staining and, in parallel, analyses of the microbial composition using 16S rRNA sequencing (Illumina) were performed.

Disruption of AHLs with the QQ enzyme Aii20J resulted in significant reductions of biofilm biomass in the majority of conditions tested, including Gram-positive predominated biofilms. These reductions were even more significant in biofilms generated from PD samples. Interestingly, bacterial relative abundance at the genus level did not significantly change when biofilms were treated with Aii20J. However, the differential abundance of some odontopathogens was significantly lower in the presence of the QQ enzyme. Findings in this work reinforce the hypothesis of the importance of AHLs in oral biofilm formation. We report that in vitro biofilms of both healthy and PD origin respond to AHL disruption by significantly modifying their phenotypical behaviour without altering their microbial relative abundance. Further studies on the key molecular mechanisms underlying the observed changes in the presence of the QQ enzyme are required.
P162- Evaluation of the effect of Quorum Quenching on in vitro, mixed-species biofilm formation from poultry meat samples

González-Pinto, L. (1), Parga, A. (1), Mora, A. (2), Otero A. (1)

(1) Departament of Microbiology and Parasitology, Faculty of Biology-CIBUS, Universidade de Santiago de Compostela, Santiago de Compostela, 15782 A Coruña, Spain
(2) Laboratorio de Referencia de Escherichia coli (LREC), Department of Microbiology and Parasitology, Faculty of Veterinary, Universidade de Santiago de Compostela, 27002 Lugo, Spain

Poultry meat is a worrying source of multi-resistant bacteria. Evidence points to quorum sensing (QS) as an important mechanism in meat contamination and spoilage. Thus, biofilm inhibition via quorum quenching (QQ) is a feasible strategy to prevent pathogen transmission.

This work aimed to study AHL-mediated QS processes and the effect of the QQ enzyme Aii20J on in vitro, mixed-species biofilm formation from chicken (n=10) and turkey (n=10) samples packaged under different conditions.

Biofilms were cultured in the active attachment model (AA-model) using glass coverslips and on polystyrene cell culture plates at 25 °C for 24 hours. Chicken samples formed significantly more biofilm than turkey samples on polystyrene. Butchery samples formed more biofilm than modified atmosphere (MA) packaged samples in both systems.

_Pseudomonas_ spp. concentration was higher in chicken- than in turkey samples, and it was significantly higher in butchery- than in MA samples. Also, a significant correlation between biofilm formation and _Pseudomonas_ spp. concentration in the initial samples was observed, indicating the relevance of this bacterium in biofilm formation.

AHLs were detected in initial samples and untreated biofilm supernatants. Aii20J reduced biofilm formation in 50% of chicken- and 50% of turkey samples in the AA-model. Inhibition was higher in polystyrene, with reductions in 0% of chicken samples. In the AA-model, biofilm formation was reduced in 67% of butchery samples, whereas in polystyrene, it was reduced in 64% of MA samples.

Aii20J decreased _Escherichia coli_ presence in 47% of the biofilms in which this bacterium was detected (n=15). _E. coli_ removal was more effective in MA- than in butchery samples. Results indicate that QQ is a promising strategy to prevent meat spoilage and pathogen transmission. Further genomic analyses are needed to investigate changes in bacterial populations under the effect of Aii20J and to assess its effect on meat samples.
Atopic dermatitis (AD) is a common, chronic inflammatory skin condition characterized by recurring, itchy lesions. Patients with atopic dermatitis are at increased risk for both acute and chronic bacterial skin infections, particularly by *Staphylococcus aureus*. The complex interplay between host inflammation, microbial community composition, and microbial physiology in lesion development and persistence is not clear. This study utilized dual-RNA and 16S rDNA amplicon sequencing to examine the microbial/host gene-expression and microbial community composition in skin biopsies and swabs from lesional and non-lesional AD skin from 40 AD patients over 14 days, following stoppage of topical treatments. Additionally, skin biopsies were collected from 40 healthy volunteers. The specific aim of this study is to examine whether temporal changes in the skin inflammation during flares lead to microbiome alterations, or vice versa. Preliminary transcriptomic analyses demonstrated a unique transcriptome signature for lesional and non-lesional AD skin. This study is currently ongoing and we anticipate that these physiological changes exert a significant pressure on the microbial community.
P166- Biofilm phenotyping of patients chronically infected with Pseudomonas aeruginosa reveals a putative biomarker for biofilm infection in cystic fibrosis

Declan Power 1,4, Odel Soren 1, 4, Thomas Garfield 5, Robert Howlin 1, 2, 4, Raymond Allan 1, 2, 4, Caroline Duigan 1, 2, 4, Paul Skipp 1,4, Jane Davies 6, Gary Connett 3, Saul Faust 2,3, Jeremy Webb 1, 2, 4.

1 National Biofilms Innovation Centre, Centre for Biological Sciences, University of Southampton, Southampton SO17 1BJ, UK,
2 NIHR Southampton Respiratory Biomedical Research Centre, Southampton SO16 6YD, UK,
3 University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK,
4 Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, UK,
5 Faculty of Medicine, University of Southampton, Southampton SO17 1BJ, UK,
6 National Heart and Lung Institute, Imperial College London, London, United Kingdom

Pseudomonas aeruginosa (PA) in the cystic fibrosis (CF) airways is associated with persistent morbidity, and increased mortality. Structured biofilm aggregates offer an increased tolerance to antimicrobials, and aggressive antibiotic (Abx) regimes are rarely capable of clearing the underlying chronic infection. Biofilm identification requires complex techniques and is unable to be diagnosed with clinical bacterial culture. A lack of biomarkers escalates this diagnostic issue.

This study aims to combine phenotypic and proteomic data to identify potential biomarkers for biofilm infection.

Expectorated sputum samples were fluorescently labelled via in-situ hybridisation (FISH) for PA and assessed microscopically for biofilm status; the PA phenotype was determined for 62 patients undergoing an antibiotic-requiring exacerbation, and further assessed for alterations to the biofilm status following Abx treatment. Sputum from 22 patients was processed for proteomic analysis.

FISH analysis across the entire cohort revealed the changes in the total biofilm biomass following the treatment course were non-significant (p1.5 (p 0.00167) of protein abundance within the sputum following Abx treatment. Human histone H4 was observed to track with disease state, with decreased abundance post Abx. The change in histone H4 negatively correlates to that of the total biofilm biomass.

Biofilm phenotyping was successful in characterising patient biofilm status and stressed the importance for personalised approaches to CF treatments. A potential biomarker, histone H4, for the CF disease state and PA biofilm phenotype has been identified as being of interest for further analysis.
Objective: To construct a reliable method for cultivation of surface microbes from chronic wounds maintaining the geographical 2D organization.

Background: Current gold standard for cultivation from chronic wounds involves sampling by swabbing a 1cm² surface area (the Levine technique). Research has found that bacterial biofilms are not uniformly distributed within chronic wounds and that the distribution varies in relation to surface and depth of the tissue. Alternative swabbing techniques (e.g. Z-technique) has the possibility of covering a greater surface area but suffer the same pitfall as all swabbing techniques; little to no information about the 2D organization of the bacteria in the wound.

We aimed to develop an alternative method for sampling using a filter paper. The method is called “Imprint” and was developed to potentially increase the number of identified bacterial species and reflect the 2D organization of bacteria in the wound.

Methods: Two filter types were tested in vitro for 1) their ability to transfer bacteria from a culture plate and 2) their ability to maintain 2D geography by transferring colonies in distinct patterns. The filter type that performed the best was tested on 12 patients with chronic ulcers. Consecutive Imprints were performed plus a swab covering the same surface area. Bacteria were identified by MALDI-TOF.

Results: In vitro testing concluded that the nylon 5 μm filter (GVS North America) was able to transfer bacteria between culture plates maintaining 2D organization. In vivo testing concluded that Imprint was a fast and feasible method. Analysis showed that Imprint was able to identify the same microbes as E-swab and that consecutive Imprints produced similar results in terms of 2D organization.

Conclusion: Imprint is a novel method for culturing bacteria from soft tissue infection. Imprint identifies the same microbes as E-swab and provides reproducible results in terms of 2D organization.
P168- Effect of Sex Steroid Hormones on an Oral Microcosm in vitro

Pilar Cornejo Ulloa, Berndt W. Brandt, Mark J. Buijs, Monique H. van der Veen, Bastiaan P. Krom

Department of Preventive Dentistry, Academic Centre for Dentistry
Amsterdam (ACTA), University of Amsterdam and VU University
Amsterdam, G. Mahlerlaan 3004, 1081 LA Amsterdam, The Netherlands

**Introduction**: Sex steroid hormones (SSH) such as estrogen, progesterone and testosterone are cholesterol derived molecules that regulate various physiological processes. They are present in both blood and saliva, where they come in contact with oral tissues and oral microorganisms. Several studies have confirmed the effect of these hormones on different periodontal-disease-associated bacteria, using single-species models. It has been observed that bacteria can metabolize SSH, use them as replacement for vitamin K and as enhancers of virulence factors. However, it is still unclear what the effects of SSH are on the oral microbiome.

**Hypothesis**: SSH can promote the growth of bacteria associated to periodontal disease in an oral-microcosm-biofilm model.

**Methodology**: saliva-derived oral microcosms were grown using the Amsterdam Active-Attachment model. Mc Bain medium without added serum nor menadione was used and after a period of initial attachment, the biofilms were exposed to either estradiol, estriol, progesterone or testosterone at a 100-fold physiological concentration. Menadione was used as positive control. After 12 days, biofilm viability (colony forming units), biofilm fluorescence and microbiome profiles by 16S rRNA gene sequencing were determined. The supernatants were tested for proteolytic activity using the Fluorescence Resonance Energy Transfer analysis (FRET).

**Results**: No significant differences were found in biofilm viability, fluorescence or microbiome profiles. FRET analysis revealed an increased and non-specific proteolytic activity on the samples exposed to progesterone and estradiol, comparable to that exhibited by the biofilms supplemented with menadione. Samples supplemented with testosterone and estriol showed a decreased proteolytic activity.

**Conclusion**: None of the tested SSH had clear effects on the oral biofilms that could suggest clinical implications in vivo. However, differences in bioavailability compared to in vivo could explain why little to no significance was observed. Differences between proteolytic activity could indicate a slight shift in the biofilm’s metabolism that should be studied further.
PhD candidate, Oscar Allan Furlong Lopez  
Centre for Oral, Clinical & Translational Sciences (King’s College London)  
Prof. Avijit Banerjee  
Centre for Oral, Clinical & Translational Sciences (King’s College London)  
Dr David Moyes  
Centre for Host-Microbiome Interactions (King’s College London)  
Dr Prasanna Neelakantan  
Restorative Dental Sciences (The University of Hong Kong)

Introduction: Dental caries remains the most prevalent biofilm-mediated oral condition worldwide. Therefore, innovative strategies are needed to manage this multi-factorial disease. Evidence has shown that antiseptics and antibiotics can cause undesirable side effects such as microbial ecology disbalance with the host (dysbiosis) and antimicrobial resistance development. Thus, other approaches are gaining more attention. Natural anti-virulent agents can impact biofilm integrity, formation, inter-microbial signalling, fermentation of sugars and acid environmental adaptation without microbicidal effects.

Hypothesis: The tested hypothesis of this study is that exo-polymeric substance disrupting enzymes (dextranase mixed with glucoamylase) in combination with plant-derived molecules (trans-cinnamaldehyde or vanillin) can reduce caries-related virulent factors with a non-microbicidal effect.

Methodology: Developing and pre-formed in-vitro cariogenic poly-bacterial (5 species) and cross-kingdom (5 bacterial species + Candida albicans) biofilms were cultured anaerobically in 24-well polystyrene plates and used for anti-virulent assays. Mixtures of enzymes (8 U/ml glucoamylase and 8 U/ml dextranase) combined with vanillin (5 mg/ml) or trans-cinnamaldehyde (450 ug/ml) were diluted and delivered in artificial saliva + 0.2% glucose. Microbiological assays of biomass (crystal violet assay), metabolic activity (XTT assay), microbial growth (O.D. 600 assay) and microecology (PMA-qPCR) were performed. The experiments were repeated at least three independent times, and the data generated were analysed with one-way ANOVA and P≤0.05 was considered significant.

Results: Assessments of cariogenic biofilms showed that this integrated approach could significantly reduce biomass, metabolic activity, and acid environment (p<0.05). The microecology of treated biofilms was shifted to a less cariogenic profile compared to the no treated group.

Conclusion: Non-microbicidal therapies would represent a safer alternative in the clinical setting to minimise adverse effects such as dysbiosis and antimicrobial resistance.
P170- Monitoring diabetic foot ulcer microbiome dynamics via bench-top sequencing

B. Short¹, J. L. Brown¹, C. Delaney¹, C. Williams², R. D. Short³ and G. Ramage¹

1 University of Glasgow, 2 Lancaster Royal Infirmary, 3 Lancaster University

With approximately 10% of the 4.5 million people suffering from diabetes in the United Kingdom alone suffering from a foot ulcer at some point in their lives, these ulcers are prone to infection, making them a frequent cause of hospitalisation both in the UK and worldwide. The microbiome of these tenacious infections was defined by collecting two swabs from patients entering clinics for routine treatment using the Levine method. One swab was utilised for baseline microbiome sequencing using the Oxford Nanopore MinIONTM (ONM). This was partnered with an in silico meta-analysis of the chronic wound microbiome. The second swab was used as a starting inoculum for ‘real-world’ biofilms. Biofilms were subjected to antibiotic treatment and the composition of these biofilms was defined pre- and post-treatment using ONM. We show the DFU microbiome is dominated by Staphylococcus, Pseudomonas and Corynebacterium species by combining ONM and in silico meta-analysis findings. Biofilm composition before exposure to antibiotics exhibited a similar composition to that of un-altered swabs. Microscopic analysis of these biofilms revealed highly complex microbial networks. Following antibiotic therapies, biofilms exhibited a decrease in biomass and viability. Notably, the relative abundance of Gram-negative organisms such as Pseudomonas were particularly sensitive to treatments. Despite the anti-biofilm effects of selected treatments, a considerable proportion remained intact. Herein, we describe an in-depth analysis of the microbiome of the DFU using OMN which can be used to monitor highly complex, yet defined, biofilm consortia and document their response to treatment. This highlights a current inability to effectively eradicate elaborate biofilm networks within DFUs. We report a group of organisms that are highly representative of the DFU microbiome, these organisms can be introduced into a multi-species biofilm model whose accuracy far exceeds that of current models, providing more appropriate platforms for recapitulating the chronic wound environment.
Conjoint Induction of Small Colony Variants and Biofilm Formation of *Staphylococcus aureus* under Gentamicin Stress

Marius COLIN, Maëliss LAURENT, Charlène COQUISART, Fabien LAMRET, Fany REFFUVEILLE, Sophie C. GANGLOFF

University of Reims Champagne-Ardenne, Biomaterials and Inflammation in Bone Site (EA4691 BIOS), Faculty of Pharmacy, Reims, France

Despite care involving antibiotic therapy, infected tissues debridement and replacement of prosthesis, 10% to 50% of prosthetic joint infection still lead to relapses. *Staphylococcus aureus* is the most prevalent pathogen involved and can persist at bone/prosthesis site through biofilm formation. Furthermore, relapsing infections are also linked to the presence of Small Colony Variants (SCVs), slow-growing colonies with higher tolerance toward antibiotics. The link between SCVs and biofilm formation has not been clearly identified yet. The aim of this study was to investigate on this relation by following both these mechanisms in a challenged *S. aureus*.

Two *S. aureus* strains were cultured in well plates in presence of enriched tryptic-soy agar and a range of gentamicin concentrations. Biofilm biomass in wells was revealed by crystal violet staining. Bacteria from planktonic phase (PP) and biofilm phase (BP) were plated on agar. Colonies were enumerated and SCVs were discriminated from WT. Both strains responded similarly to gentamicin, with Minimum Inhibitory Concentration at 32 µg/mL. Surviving bacteria were recovered and cultured from both PP and BP at 64 and 128 µg/mL. Biofilm biomass was observed in wells with 32 µg/mL and 64 µg/mL. Growing gentamicin concentrations induced a progressive predominance of BP and an increase of SCVs percentage in both phases. At above-MIC concentration, SCVs percentages consistently reached 99-100% of total colonies in PP, while highly variable in BP with a global decrease at 128 µg/mL. Bacteria derived from SCVs are currently tested for their abilities to tolerate gentamicin and to form biofilm, in comparison to wild type. This study highlights that both biofilm and SCVs are triggered by gentamicin stress. The proportion of SCV related bacteria in biofilm seems driven by factors that will be investigated in future work.
P174- pHenotype: *Candida albicans* biofilm capacity reveals a commensal niche in oral caries

Mark C. Butcher¹, Christopher Delaney¹, Bryn Short¹, Dave Bradshaw², Jonathan R. Pratten and Gordon Ramage¹

1 - Oral Sciences Research Group, Glasgow Dental School, School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G2 3JZ.

2 - Oral Healthcare R&D, GlaxoSmithKline Consumer Healthcare, Weybridge KT13 0DE, UK

Introduction
The presence of *Candida albicans* has commonly been reported as a potential indicator or risk factor in the development of caries in children and adults. Typically commensal in nature, it has been established that the development of pathogenicity in *Candida albicans* is driven by environmental factors which lead to its overabundance or phenotypic switching to a more pathogenic hyphal morphology. Additionally, this change has been found to aid in adhesion to oral surfaces and in providing a substrate and environmental niche for other microorganisms.

Hypothesis
We posit that alteration of *Candida* phenotype directly influences the microbial and cariogenic profile of a multi-species biofilm.

Methods
10 *C. albicans* isolates with high and low biofilm phenotype were selected for co-aggregation assessment with other organisms from a published "caries" biofilm model profiled based on biomass, metabolism, drug response, response to environmental stimuli, as well as pH and lactate production. Meta-analysis of published oral microbiome papers was carried out to derive and test a functionally distinct biofilm model representative of dental caries. Nanopore® sequencing was employed to examine the microbiome of saliva from healthy donors to assess these previous environmental stimuli on "true-to-life" biofilm models. These isolates were examined as part of a multi-species biofilm model grown on bovine enamel and assessed for shifting microbial profile, acidogenesis and alteration of substrate when exposed to environmental stimuli and conventional and novel treatments.

Results
*C. albicans* was found to buffer pH profile of "caries" organisms in both dual and multi-species formats. Using sucrose as an environmental stimulus of multi-species biofilms resulted in both a shift in microbial distribution and a higher erosion profile in bovine enamel.

Conclusion
*Candida* provides a key role in the aggregation of non-commensal micro-organisms while also providing an obvious impact on the cariogenic profile of a multi-species biofilm model.
P176- Synergistic interactions in multispecies biofilms by dairy strains recovered from the surface of a pasteurizer

Faizan Ahmed Sadiq
Marc Heyndrickx
Koen de Reu
Affiliation: Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium.

Introduction
Diverse bacteria on food contract surfaces exist as a result of their intricate interactions on food contact surfaces in the form of biofilms. We aimed to determine the most detrimental combinations of bacteria that thrive following cleaning and disinfection (C&D) on the surface of pasteurizer in the dairy industry because of their cooperative biofilms. We hypothesized that some bacterial species will stimulate more biofilm formation in combination with other species due to some unknown interactions.

Methodology
A total of 26 strains belonging to 11 species and 12 genera, recovered from the surface of dairy pasteurizers following C&D were subjected to single and mixed-species biofilm trials. All bacteria were divided into groups of 7 species each (3 groups) and members of each group were mixed in all possible four-species combinations (99 combinations) to find out the most problematic combinations of species. Biofilm forming abilities were determined using 96-well polystyrene surfaces using Brain-Heart Infusion medium and later confirmed on stainless steel surfaces using 6-well microtiter plates.

Results
Only one strain proved to be a good biofilm former (Microbacterium lacticum: OD595 = 3.72) in single culture. Out of the 3 groups, 33 four-species combinations in one group showed synergy in mixed biofilms. The highest synergy was observed among Bacillus licheniformis, M. lacticum, S. rhizophila, and Calidifontibacter indicus. Of the four strains, M. lacticum was the only one capable of forming abundant biofilm in isolation under the in vitro conditions investigated. There was a ~ 4-fold increase in the biofilm mass when all strains developed a biofilm together. Study involving cell free supernatant of the cooperating strains showed that cell viability as well as physical presence of cooperating strains were required for the observed synergy in biofilms.

Conclusion
We conclude that certain species combinations are more prevalent due to cooperative interactions.
P177- Homocysteine and inflammatory cytokines in the clinical assessment of patients with chronic venous leg ulcers infected by biofilm-producing bacteria

Cavallo Ilaria¹, Sivori Francesca¹, Aldo Morrone², Fulvia Pimpinelli¹, Di Domenico Enea Gino³

1 Microbiology and Virology, IRCCS San Gallicano Institute, Rome, Italy
2 Scientific Direction, IRCCS San Gallicano Institute, Rome, Italy
3 Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, Rome, Italy

Introduction: Inflammation and infection play a key role in developing chronic venous leg ulcers (VU), causing deep pain and severe complications.

Hypothesis: This study aimed to explore host and microbial factors associated with the clinical outcome of infected (IVU) and non-infected VUs (NVU).

Methodology: 101 patients with IVU and NVU were included in this study. Clinical data, including ulcer duration, depth, and size, were collected in both groups. The levels of inflammatory cytokines were measured from the wound fluid.

Results: IVU were fifty-six (55.4%) while NVU were forty-five (44.5%). IVUs showed a most severe clinical outcome, with a significant increase in duration (P=0.02), size (P=0.0001), and depth (P=0.0008) of the wound as compared to NVUs. In addition, significantly higher levels of interleukin (IL)-6, IL-10, IL17A, IL22, and TNF-alpha were found in patients with IVUs compared to those with NVUs. Notably, homocysteinemia (HHcy) was significantly (P=0.0001) higher in patients with IVUs than NVUs. Among IVUs, Gram-negative bacteria were 51.7%, while the Gram-positives were 48.3%. At the species level, Staphylococcus aureus was the most common isolate (43.8%), followed by Pseudomonas aeruginosa (18.0%). Strong biofilm-producers (SBPs) (73.0%) were significantly (P=0.0001) more abundant than weak biofilm-producers (WBP) (27.0%) in IVUs. SBPs were present in 97.7% of the IVUs as single or multispecies infections. Specifically, SBPs were 94.9% for S. aureus, 87.5% for P. aeruginosa, and 28.6% for Escherichia coli.

Conclusion: In infected wounds, the tissue environment and biofilm production can support chronic microbial persistence and a most severe clinical outcome even in the presence of an intense and persistent immune response, as shown by the high levels of inflammatory molecules. The measurement of HHcy levels may offer a novel biomarker for the clinical assessment of IVUs caused by biofilm-producing bacteria and, possibly, for therapeutic monitoring.
P178- Cold plasma therapy for combatting skin-related biofilm infections

Jason L Brown(1,2), Ahmed Bakri(1,2), Abdullah Baz(1,2), Bryn Short(1,2), Toby Jenkins(3), Robert D Short(4), Craig Williams(2,5), Gordon Ramage(1,2)

(1) Oral Sciences Research Group, Glasgow Dental School, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8TA, UK
(2) Glasgow Biofilm Research Network, 378 Sauchiehall Street, Glasgow, G2 3JZ, UK
(3) Department of Chemistry, University of Bath, Bath BA2 7AY, UK
(4) Department of Chemistry and Material Science Institute, University of Lancaster, Lancaster, LA1 4YB, UK
(5) Microbiology Department, Lancaster Royal Infirmary, University of Lancaster, Lancaster, LA1 4YW, UK

Introduction. Wound infections can be highly problematic to treat by clinicians. These can encompass diseases such as diabetic foot ulcers which are interkingdom in nature, but also nosocomial infections that are often driven by fungal microorganisms. Given the ever-increasing pressures associated with antimicrobial resistance, there remains a need to move away from conventional antibiotic therapy for combatting such infections. One possible avenue involves the use of cold-activated plasma (CAP) which has been shown to exhibit antimicrobial attributes, whilst also contributing to skin healing and repair. Therefore, the purpose of this study was to test the killing efficacy and inhibitory effects of CAP on clinically relevant skin biofilms and assess the cytotoxicity of the treatment against host cells in vitro.

Methods. Biofilms grown in a hydrogel matrix system were treated with CAP for a range of short-exposure times (< 5 minutes). Biofilm viability was determined using a range of conventional microbiological methodologies including live/dead quantitative PCR, whilst treated and untreated biofilms visualized using scanning electron microscopy. The cytotoxicity effects of the treatment were further assessed against skin-relevant host cells in vitro, utilising a range of immunological and molecular techniques.

Results. CAP treatment of the biofilms resulted in reductions in viability and disruption to the cellular structure in cells embedded in the microbial community at a microscopic level. Assessment of the host response in two- and three-dimensional tissue models revealed CAP treatment possessed immunomodulatory effects against the host cells, whilst eliciting certain levels of cytotoxicity, all when compared to untreated controls.

Conclusion. CAP treatment may provide a useful localized alternative to conventional antibiotics in targeting polymicrobial and/or fungal biofilms associated with chronic wounds or nosocomial-associated infections. However, further studies are required to assess whether CAP treatment is safe for use in humans, as not to be detrimental to the host microenvironment.
P180- Cold plasma treatment of biofilms affects their interactions with free antibiotics and antibiotic-loaded liposomes

Ross Duncan¹, Thomas P. Thompson¹, Aled Morton¹, Hussein Kenaan¹, Vicky Kett¹ and Brendan Gilmore¹

1 School of Pharmacy, Queen’s University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland

Cold Atmospheric Plasma (CAP) has become a key area of interest in clinical microbiology because of its ability to significantly reduce the viability of biofilms through the generation of Reactive Oxygen and nitrogen Species (RONS) [1]. The recovery of biofilms after a wide range of treatments arises from persister cells which exhibit very low metabolic activity [2]. Recent studies have shown that the abundance of EPS produced along with catalase production plays a key role in reducing the lethal affects of CAP within biofilms by sequestering the RONS responsible for DNA damage and disruption of the bacterial cell membrane [3]. However, the use of liposomes to carry antimicrobials into biofilms has become increasingly popular and recent research suggests eradication can be achieved with a lower concentration than free drug by itself [4].

Here we propose that treatment of biofilms with CAP along with antibiotic or liposomal administration can aid penetration and achieve eradication at lower concentrations. Preliminary analysis of MRSA biofilm tolerance to cold plasma, free antibiotics and loaded liposomes was achieved by assessing characteristics including quantification of total living bacterial cells, metabolic activity and biomass. Live/dead staining along with confocal microscopy was used to image biofilms to further assess how these conditions affected cell survival within the biofilm. Liposomes loaded with fluorescent dye and fluorescent antibiotics were used to show interactions with the biofilm and how this is altered with plasma exposure. Finally, Liposome stability after plasma treatment was determined by measuring particle size, PDI and charge along with measuring drug and fluorescent dye leakage.

Initial results show liposomes increase antibiotic potency within biofilms and this is further enhanced for both free-drug and liposomes with cold plasma treatment. Live/dead stain imaging of biofilm conforms with this preliminary data. Liposome stability study suggests limited oxidation occurs in lipid bilayer.

P181- Inhibition of *E. coli* Growth and Biofilm Formation by ZnCl2 and ZnO Nanoparticles – a comparative mechanistic study

Toni Vitasovic, Marcel Cecatto and Elena Ferapontova

Affiliation: Interdisciplinary Nanoscience Center (iNANO) Natural Science, Aarhus University
Address: Gustav Wieds Vej 1590-14, DK-8000 Aarhus C, Denmark

Metal oxide nanoparticles (NPs), particularly zinc oxide (ZnO), returned into the spotlight as one of the most potent non-conventional antibacterials due to their wide spectrum of antimicrobial activity, unique physicochemical properties, and low cost of production. Surprisingly, the antibacterial mechanism of ZnO still remains controversial, with metal ion release and lipid peroxidation being debated over as the main modes of action.

In this work, we scrutinized the antibacterial mechanistic pathways of ZnO NPs (100 nm) and zinc chloride (ZnCl2) as a source of Zn2+, in dark conditions against *E. coli* DH5alpha – a model microorganism commonly used in biocide action research. The antibacterial activity tests of ZnO and ZnCl2 showed great inhibitory effects on *E. coli* DH5alpha proliferation, with minimal inhibitory concentrations of 5 and 68 μg ml⁻¹, respectively. Electrochemical and microscopic investigations suggest that the antibacterial activity of ZnO NPs can be ascribed to ZnO interactions with bacterial cell walls, accumulation in the cytoplasm, and generation of reactive oxygen species (ROS). Whereas, the antibacterial activity of ZnCl2 is associated with the disruption of Zn2+ homeostasis in the bacterial cytoplasm and the formation of insoluble ZnO/OH nanocomposite. Our current efforts are focused towards optimizing antifouling polymeric coating containing ZnO NPs and treatment of *E. coli* biofilms with ZnO NPs and ZnCl2.
P183- Adaptive evolution of phenotypic switching during environmental change

Tim Holm Jakobsen (I), Anne Kristine Servais Iversen (I), Mads Lichtenberg (I), Blaine Mads Hansen (I), Hans Gottlieb (II), Klaus Kirketerp-Møller (III), Thomas Bjarnsholt (I)

I, Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, Denmark
II, Department of Clinical Medicine, Herlev and Gentofte Hospital, Denmark
III, Copenhagen Wound Healing Center, Bispebjerg University Hospital, Copenhagen, Denmark

Introduction
Chronic wounds have a negative impact on patients’ quality-of-life and constitute an increasing healthcare issue worldwide due to a rise in the elderly population. Although the chronic wound microbiome has been well investigated, knowledge regarding factors underlying microbial colonization affecting wound progression and healing are underreported.

Objective
We investigate the role of bacterial biofilms in chronic wounds to address changes in the local microenvironment that have the potential to affect treatment and healing processes.

Methods
This longitudinal study investigates two groups of patients with chronic low extremity wounds. Group 1 consists of 110 ambulatory patients from whom we collected swabs for bacterial identification, and debridement for dual RNA-sequencing for computational modelling of host and pathogen markers of infection. Group 2 consists of 10 patients undergoing split-thickness skin graft. During operation, we collected swabs and biopsies from the wound before and after debridement, the surrounding skin, and the donor site. The biopsies are used for visualization and omics studies to investigate host-pathogen interaction and to link phenotypic traits to genomic sequences. A biobank has been established with collected clinical isolates for in vitro characterization studies and for whole genome sequencing to study adaptation.

Results
In average Group 1 contain 2-5 bacterial species ranging from 1 to 10 with Staphylococcus aureus and Enterobacter cloacae being the most prevalent. Almost 50% of the identified species are gut microbiota. Studies of Group 2 show that Pseudomonas aeruginosa and Enterococcus faecalis are not removed by operation. Some P. aeruginosa and S. aureus clinical isolates show decreased virulence and increased tolerance to antibiotics. Presently, we are analyzing sequencing and WGS data.

Conclusion
To our knowledge this is the most comprehensive study of clinical chronic wound samples to date. The study will provide empirical evidence and analytical discussions of the role of biofilms in chronic wounds.
P184- Effect of disinfectants on biofilm-associated wild type *Salmonella*

Ane M. Osland¹, Claire Oastler², Emma Brook², Becky Gosling², Mardjan Arvand³, Katharina Konrat³, Lene K.Vestby³

1 Norwegian Veterinary Institute (NVI) 2 Animal and Plant Health Agency (APHA) 3 Robert Koch Institute (RKI)

Introduction: Salmonellosis was the second most reported zoonosis in the EU in 2020, affecting more than 50,000 people. Salmonellosis is caused by *Salmonella*, a bacterium frequently found in intestines of healthy animals. *Salmonella* are known to form biofilms and in this state, the bacteria are more tolerant to disinfectant and cleaning routines. Therefore, they may survive in the environment and cause infection in animals and humans and contamination of food products.

Hypothesis: There is no ISO standard method that takes into account *Salmonella* biofilms when assessing the efficacy of disinfectants for use in the porcine industry. We aim to evaluate the competency of current test methods and the differences in disinfectant susceptibility between *Salmonella* strains.

Methodology: We are testing *Salmonella* biofilms tolerance to Glutaraldehyde, a commonly used active ingredient in the porcine industry. Six wild type and two reference strains of S. enterica from finishers and sow/gilt faeces with various levels of known biofilm production are studied. Testing is by three established methods with biofilm formed at 20°C for 48 hours on glass beads, stainless steel coupons and PVC coupons before they are treated for 30 minutes with Glutaraldehyde at various concentrations.

Results: Preliminary results show that greater than 15 times the concentration of Glutaraldehyde is needed to reach ≥ 5 log10 reduction on wild type and reference strains in biofilm compared to planktonic reference strains. Differences in the concentration are observed within the isolate panel and between the methods. The results will be discussed further in the presentation.

Conclusion: Our results demonstrate that disinfection procedures should be reconsidered to ensure that sufficient concentrations of Glutaraldehyde are used to cause ≥ 5 log10 reduction of bacteria in biofilm, on different materials. They also show the need for the development of a standard method for testing disinfection of *Salmonella* biofilms.

This work was supported by funding from the European Union’s Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme
The polysaccharide extracted from the biofilm produced by *Burkholderia multivorans* C1576 (Epol C1576) contains Rha residues with 25% O-methyl substitution, thus rendering the polymer backbone less polar than other polysaccharides. We already demonstrated that Rha makes the polysaccharide able to interact with hydrophobic molecules as well as to exhibit self-aggregation tendency [1]. These two properties might have a particular relevance for the biofilm forming process and for the matrix maintenance.

To better disclose the role of Epol C1576 in biofilm matrix formation, morphological investigations were carried out by means of Atomic Force Microscopy (AFM), both in solid and liquid state, as well as by Transmission Electron Microscopy (TEM).

AFM experiments both in solid and liquid state showed that Epol C1576 forms compact structures with globular morphology. The average diameter of the globules, obtained spray-drying polysaccharide solutions on freshly cleaved mica surfaces, was 16 nm. In liquid experiments the diameter was larger, obviously due to hydration of the polymer. The Epol C1576 ability to self-aggregate was demonstrated both in solid and in liquid experiments.

TEM experiments gave independent confirmation of both the presence of a globular morphology of the polysaccharidic chain and the ability to self-aggregate, thus excluding artifacts in AFM sample preparation.

Parallel to these studies, the molecular dynamic simulation carried out in vacuum on a segment of 100 monosaccharides showed that after 270 ns the polymer chain passes from the starting extended chain to a coiled conformation.

In conclusion, the ability of Epol C1576 to assume a compact conformation and to self-aggregate was demonstrated. These properties can be traced back to the presence of Rha residues and their O-methylation.

This work was supported by the Prime Agreement R01GM123283 from the National Institute of General Medical Sciences of the NIH.

P186- Characterization of a new anti-biofilm molecule from *Psychrobacter* sp. TAE2020

Caterina D’Angelo, Angela Casillo, Maria Michela Corsaro, Andrea Carpentieri, Maria Luisa Tutino, Ermenegilda Parrilli

Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy

Introduction: Microbial biofilm formation is a problem in a wide range of environmental, industrial, and medical areas. In medical settings, biofilms are the cause of chronic, nosocomial, and medical device-related infections. *Staphylococcus epidermidis* is a successful nosocomial pathogen for its intrinsic ability to form biofilms on the surface of indwelling medical devices. The use of antibiotics alone is ineffective for treating biofilm-related infections, consequently, new control strategies are emerging consisting of the use of biological anti-biofilm compounds as their main objective. In this contest, the Polar marine bacteria, represent an untapped reservoir of biodiversity since they can synthesize a broad range of bioactive compounds, including anti-biofilm molecules.

Hypothesis and aims: This study aimed to evaluate the ability of the Antarctic bacterium, *Psychrobacter* sp. TAE2020, to produce anti-biofilm compounds active against *S. epidermidis* strains.

Methodology: Cell-free supernatant was tested against *S. epidermidis* biofilm. The active sample was subjected to suitable purification strategies to identify the molecules responsible for the biological activity.

Results: TAE2020 cell-free supernatant was able to inhibit the biofilm formation and disaggregate the mature biofilm of two *S. epidermidis* strains. Moreover, our study showed that TAE2020 cell-free supernatant is also able to impair the initial attachment of the bacterial cells to the surfaces, by molecules acting as anti-adhesive surfactant agents.

The anti-adhesive activity was associated with a new complex of proteins and polysaccharides, that we named Catasan. An enriched sample allowed the identification of Omp38, a 40kDa outer membrane protein participating to the Catasan complex. *Psychrobacter* sp. TAE2020 Omp38 shares 46% of sequence identity with AlnA, a 45 kDa protein belonging to the Alasan complex, a good bioemulsifier produced by *Acinetobacter radioresistens* KA53.

Conclusion: In consideration of the novel results obtained, Polar marine bacteria might represent potential candidates for the discovery of new compounds limiting pathogen biofilm formation.
P188- Self-assembling multimeric protein scaffold particles exposing IHF as vaccine antigen for prevention of *P. aeruginosa* biofilm lung infection

Daria A. Egorova#, Ksenya V. Danilova#, Tatyana M. Grunina#*, Alexandr M. Lyaschuk#, Andrey I. Solovyev#, Ivan N. Kravtsov#, Maria A. Dmitrieva#, Valentina S. Rykova#, Irina L. Tutykhina#, Yulia M. Romanova#, Vladimir G. Lunin#*

# Gamaleya National Research Center of Epidemiology and Microbiology, Ministry of Health of the Russian Federation, 123098 Moscow, Russia
*
* All-Russia Research Institute of Agricultural Biotechnology, 127550 Moscow, Russia

**INTRODUCTION**

There are still no approved vaccines for prevention of biofilm related infections caused by *P. aeruginosa* (as well as against many other biofilm-forming pathogens). Integration host factor (IHF) proteins are perspective targets for antibiofilm treatments, including vaccine development. IHFs stabilize eDNA in matrix and makes biofilm stable and tolerant to therapeutics. Moreover, IHFs are highly conserved among broad range of bacterial species, that rise opportunity to create «pan biofilm» vaccine. Self-assembling multimeric protein scaffold particles (MPSPs) is perspective platform for recombinant vaccine development due to multimeric antigen presentation, ability to induce strong immune response, and robust manufacturing process.

**HYPOTHESIS**

We hypothesized that effective targeting of IHFs could be reached through utilizing MPSP with exposed DNA-binding domains of IHFs.

**METHODS**

To create vaccine antigen targeting IHF we applied aldolase based MPSP in fusion with DNA binding domains of IHFs from *P. aeruginosa*. Recombinant MPSP-IHFs were produced in E. coli and purified on Heparin Sepharose. For immunogenicity evaluation on mice, we have tested schedules with different routes of immunization and adjuvants. For protectivity studies we used mice model of chronic lung infection caused by *P. aeruginosa* entrapped in agar-alginate beads.

**RESULTS**

MPSP-IHFs were highly immunogenic. Combination with Alum and Freund’s adjuvants induced comparable titers of specific antibodies while in the absence of adjuvant MPSP-IHFs were able to induce humoral immune response, but it had vanished rapidly. Seven days post *P. aeruginosa* challenging there were no *P. aeruginosa* CFU in 4 out of 6 lung samples of immunized animals while in nonimmunized group 6 out of 7 samples were positive.

**CONCLUSION**

MPSP with exposed IHFs might be considered as a potential vaccine antigen for prevention of biofilm-associated infection. Further research will be devoted to deeper characterization of immune response, expanding infection models and pathogens, as well as safety evaluation.
The increasing resistance of bacteria to antibiotics is one of WHO concerns, the development of new technologies and therapies that provide effective treatments are mandatory. One of the strategies of bacterial resistance is the formation of biofilms, these structures are bacterial sessile communities irreversibly attached to a biotic or abiotic surface, and embedded in an extracellular polymeric substances matrix of its own production. Bacterial biofilms are very difficult to eradicate with conventional antibiotics therapies.

An alternative solution is the use of metallic nanoparticles, such as gold nanoparticles (Au-NPs).

The hypothesis of this work is that Au-NPs have antibiofilm activity against *S. aureus* ATCC 6538, *A. baumanii* ATCC 19606 and *P. aeruginosa* ATCC 902, *E. coli* 144 and *P. mirabilis* 2921 (Montevideo, Uruguay).

Au-Nps were synthesized by a modified Turkevich/Frens seed growth method with tetrachloroauric (III) acid trihydrate and sodium citrate dihydrate. The nanoparticles were characterized using UV-Visible spectroscopy, the hydrodynamic radius and zeta potential was determined on a Zetasizer ZS (Malvern Instruments). The Nps activity over the biofilm was tested at different concentrations, the biofilm biomass was semi-quantified by violet crystal assay on a 96 microtiter well.

The synthesized Au-NPs had a hydrodynamic radius of ~37 nm, with a polydispersity index of 0.24, and a Z-potential of -27 mV. Au-Nps were capable of inhibiting the formation of *A. baumanii*, *P. aeruginosa*, *E. coli*, and *P. mirabilis* biofilms compared to the control. Furthermore, the Au-Nps eradicate *A. baumanii* and *P. mirabilis* mature biofilm. However, this effect was not observed in *E. coli*, *P. aeruginosa* and *S. aureus*. These results suggest that the synthesized Au-NPs has potential use as treatments for biofilm-related infections caused by pathogenic bacteria.
Inherited genetic resistance traits and innate tolerance to traditional antibiotic therapies, have made bacterial biofilms a major and ongoing concern for public-health. There is a growing need for novel methods of drug delivery, to increase the efficacy of antimicrobial agents. This research evaluated the anti-biofilm and bactericidal effects of ultrasound-responsive gas-microbubbles (MBs) with either air or nitric oxide, using an in vitro Pseudomonas aeruginosa biofilm model grown in artificial wound medium. Four lipid-based MB formulations were evaluated: room-air MBs (RAMBs) and nitric oxide MBs (NOMBs) with no electrical charge, and cationic (+) RAMBs+ and NOMBs+. Two principal treatment conditions were used: i) ultrasound-stimulated MBs only, and ii) ultrasound-stimulated MBs with sub-inhibitory (4 µg/mL) gentamicin. Total treatment time: 60 seconds passive MB-biofilm interaction, and 40 seconds ultrasound exposure; each formulation was tested in triplicate. Cationic microbubbles were engineered to promote binding of MBs to negatively charged biofilms; they also demonstrated intrinsic bactericidal properties. Ultrasound-stimulated RAMBs and NOMBs without antibiotic achieved reductions in biofilm biomass of 93.3% and 94.0%, respectively. Their bactericidal efficacy was limited, reducing culturable cells by 26.9% and 65.3%, respectively. NOMBs with sub-inhibitory antibiotic produced the most significant reduction in biofilm biomass (99.9% SD ± 5.21%), and reduction in culturable bacterial cells (99.9% SD ± 0.07%). Without antibiotic, the bactericidal efficacy of RAMB+ and NOMB+ was greater than their uncharged counterparts, reducing culturable cells by 84.7% and 86.1% respectively; increasing to 99.8% with antibiotic. This study demonstrates the anti-biofilm and bactericidal utility of ultrasound-stimulated MBs, and is the first to demonstrate the efficacy of a NOMB for the dispersal and potentiation of antibiotics against bacterial biofilms in vitro. The biofilm system and complex growth-medium recapitulated key morphological features of in vivo biofilms. The results offer new insight for the development of new clinical treatments, for example, in chronic wounds.
P192- Characterization of virulence factors and biofilm production of *Pseudomonas aeruginosa* strains isolated from diabetic foot infections (DFIs) and exploration of phages activity as a new therapy

Hanane Raqoui, Anna Bertoncelli, Chiara Milan, Greta Tartaglione, Alice Cantachin, Alice Casaroli, Annarita Mazzariol

Department of Diagnostics and Public Health, University of Verona, Verona, Italy

**Introduction.** Diabetes is a chronic disease characterized by a hyperglycemia in the patient, which can lead to the development of diabetic foot infections (DFIs), leading to serious complications that can ends with amputation of the limbs. DFIs are one of the most important chronic diabetic complications of which *Pseudomonas aeruginosa* is one of the major causes.

The aim of the study is characterize genetically some *P. aeruginosa* isolated from DFIs by searching the most relevant virulence factors genes responsible for their virulence and adaptation to cause DFIs.

**Materials and methods** This analysis included 45 *P. aeruginosa* associated with DFIs. In order to find any relation between the virulence factors and particular clinical manifestation of *P. aeruginosa* infections, were detected toxA, exoS and exoU virulence factors genes among these isolates by PCRs. Then, the biofilm formation on plastic surface was evaluated by crystal-violet assay. Finally, phages screening tests were carried out to evaluate the lytic activity of some environmental phages against those strains.

**Results** The PCR for the 3 toxins showed that 35 out of 45 isolates carried toxA, 32 out of 45 exoS and 6 out of 45 esoU. Among the isolates tested positive for toxA, 30 are also positive for esoS and 4 for esoU. 5 out of 45 strains did not reveal the presence of any virulence gene. The biofilm quantification showed that 25 out of 45 isolates were classified as strong biofilm producers, 13 out of 45 were moderate biofilm producers, 7 out of 45 were weak biofilm producers. The efficacy of phages proved to be variable depending on the strain and the phages.

**Conclusions.** *P. aeruginosa* isolates generally express cytotoxicity or invasion phenotypes which is correlated with presence of ExoU or ExoS respectively. The presence of exoU was found to be extremely less recurrent than the other exotoxins, with a frequency of 13.3%. Biofilm production also seem to play an important role as virulence factor in DFI and phage use could contribute to eradicate infections.
P193- The role of *Salmonella* biofilm formation during gut colonisation


(1)KU Leuven – Department of Microbial and Molecular Systems, Centre of Microbial and Plant Genetics (CMPG), Leuven, Belgium

Despite an increasingly strict regulatory framework in the food sector, *Salmonella Typhimurium* remains a significant public health concern. For *Salmonella* to cause infection, it must withstand a wide array of hurdles both inside and outside the host. It has been described extensively that biofilm formation is important for the survival of *Salmonella* outside the host. However, its exact role during gut colonisation and infection remains to be elucidated.

The main aim of this study is to unravel the ambiguous role of biofilm formation during *Salmonella* infection by employing *Caenorhabditis elegans* as a model system for in vivo colonisation. Hereto, worms were infected with either biofilm-producers, biofilm non-producers, or a mixture of both strains. The interplay between biofilm components and the immune system was validated by infecting both immuno-competent and immuno-deficient worms.

Our results showed that in monoculture, biofilm non-producers can colonise the intestine to a greater extent than biofilm producers. It was hypothesised that the biofilm-deficient strain could suppress the worm’s immune reaction, possibly by increasing the expression of the SPI-1 effector protein SptP, which is known to interfere with the MAPK-pathway in *C. elegans*. In support of this hypothesis, biofilm producing *Salmonella* showed increased proliferation in immuno-deficient worms, whereas the colonisation profile of biofilm-deficient *Salmonella* remained the same. Infection with a mixture of producers and non-producers resulted in similar overall colonisation as infection with non-producing monocultures, both in immuno-competent and immuno-deficient worms. In these duocultures, the biofilm non-producers significantly outperformed the biofilm-producers. Possibly, this could be attributed to the lower growth rate of biofilm producers due to the cost associated with biofilm production. Overall, our work suggests that Salmonella biofilm inhibition leads to immunosuppression in *C. elegans*, resulting in an increased proliferation of the pathogen in the intestinal lumen.
**P194- Modulating the tongue microbial community with nitrate**

Ioanna Chatzigiannidou 1, Josefien Van Landuyt 1, Wim Teughels 2, Tom Van de Wiele 1, Nico Boon 1*

1 Center for Microbial Ecology and Technology (CMET), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
2 Department of Oral Health Sciences, KU Leuven, Leuven, Belgium

The human tongue is coated by a taxonomically and metabolically diverse biofilm. Some members of this biofilm can reduce dietary nitrate to nitrite, an important process for human systemic health and particularly, good cardiovascular function. At the same time, the tongue hosts microorganisms that cause oral malodor through the production of volatile sulfur compounds (VSCs). In this study, we aim to investigate the effect of nitrate rich food consumption on the tongue biofilm community and whether nitrate supplementation can benefit nitrate-reducing microorganisms and influence the growth and metabolism of the VSCs-producing microorganisms. Healthy individuals were asked to consume beetroot juice, which is rich in nitrate, for a week and their tongue microbial community was studied by 16S rRNA gene amplicon sequencing. None of the known VSCs-producing microorganisms differed after one week of beetroot juice consumption, but *Actinomyces*, a nitrate-reducing genus, increased in abundance. To further evaluate if *Actinomyces*, in the presence of nitrate can influence the growth and activity of VSCs-producing microorganisms, we performed in vitro co-cultures of *Actinomyces viscosus* with two known VSCs-producing species, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. Although the growth of the two species was not affected by the presence of *A. viscosus* and nitrate, changes were observed in the metabolic activity of *P. gingivalis*, such as lower butyrate production, as well as a lower pH reduction. Overall, it appears that increased nitrate consumption can benefit certain taxa of the tongue biofilm that are able to reduce nitrate and one of these taxa, *Actinomyces*, can affect VSCs production by modulating local pH and the metabolic activity of VSCs-producing microorganisms.
Background: A major problem in various sectors, including the medical and industrial sector, are bacterial biofilms. Bacteria in biofilms are difficult to treat since the biofilm matrix of extracellular polymeric substances (EPS) can increase the tolerance against antibiotics, disinfectants and other stresses up to a 1,000 times, as such heavily impeding antimicrobial treatments. This has led to the need for novel strategies to treat these infections and contaminations. A promising biofilm prevention strategy is the use of biofilm EPS inhibitors such as the 5-aryl-2-aminoimidazoles derivatives developed at KU Leuven (Belgium), which can be finetuned for activity by substitution of the side chains. To develop the compounds towards industrial and medical applications, we here aimed to further investigate the effect of the substitution patterns on toxic and mutagenic traits.

Methods: The biofilm inhibiting activity of the compounds was determined using crystal violet staining in a Calgary Biofilm Device (CBD) against a large selection of biofilm forming bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The toxicity of the compounds was investigated with a *Caenorhabditis elegans* based agar assay and the mutagenicity with a standardized Ames test.

Results: Combining the results of the different assays has led to the optimal substitution pattern and thus the most suited biofilm inhibitor to use in both medical and industrial applications. The selected compound has a broad activity spectrum even at very low concentrations, shows no toxicity in the *C. elegans* assay and has no mutagenic effects to a gradient of concentrations.

Conclusions: The selected biofilm inhibitor shows great potential as a safe strategy to treat biofilm infections, both in applications where the compound would be released or covalently attached to surfaces.
P201- Determination of the elution capacity of dalbavancin in bone cements: New alternative for the treatment of biofilm-related prosthetic joint infections

Marta Díaz-Navarro, Clinical Microbiology and Infectious Diseases Department; Pablo Sanz-Ruiz, Orthopedic Surgery and Traumatology Department; Mar Sánchez-Somolinos, Clinical Microbiology and Infectious Diseases Department; Marta Tormo, Universidad Complutense de Madrid; Antonio Benjumea, Orthopedic Surgery and Traumatology Department; José Matas, Orthopedic Surgery and Traumatology Department; Patricia Muñoz, Clinical Microbiology and Infectious Diseases Department; Javier Vaquero, Orthopedic Surgery and Traumatology Department; Maria Guembe, Clinical Microbiology and Infectious Diseases Department.

Introduction: The use of local antibiotics loaded in bone cement is the most widely used procedure for the treatment of biofilm-septic sequelae in orthopedic surgery (in addition to surgical debridement and systemic antibiotics). Dalbavancin (D) is a lipo-glycopeptide approved for the treatment of skin and soft tissue infections that has a long half-life and can be administered twice a week.

Hypothesis: We consider that D could have a high elution capacity in bone cement similar to that of vancomycin (V).

Methodology: Palacos® R bone cements were manually mixed with V and D at 2.5% and 5%. Three cylinders of same size were obtained from each of the mixtures and incubated in 5 ml PBS at 37°C under agitation for 14d. Aliquots were obtained at different incubation times and were analyzed by HPLC (V) and mass cytometry (D).

Results: V release at 14 days was higher than dalbavancin at each concentration tested (p=0.05, both). However, 5%D showed similar cumulative release to 2.5%V (p=0.513).

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Cumulative concentration (µg/ml, Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5%V</td>
</tr>
<tr>
<td>1</td>
<td>16.13 ± 1.88</td>
</tr>
<tr>
<td>2</td>
<td>31.49 ± 2.44</td>
</tr>
<tr>
<td>4</td>
<td>49.08 ± 3.56</td>
</tr>
<tr>
<td>8</td>
<td>68.60 ± 4.15</td>
</tr>
<tr>
<td>24</td>
<td>89.31 ± 3.25</td>
</tr>
<tr>
<td>48</td>
<td>110.55 ± 1.07</td>
</tr>
<tr>
<td>168</td>
<td>132.29 ± 2.65</td>
</tr>
<tr>
<td>336</td>
<td>158.49 ± 3.09</td>
</tr>
</tbody>
</table>

Conclusions: 5%D showed a good elution capacity in bone cement, that reached similar cumulative concentration than 2.5%V. Moreover, considering that MIC90 of D and V is 0.06 mg/L and 2 mg/L, respectively, and due to D long half-life, it may be a new alternative for the treatment of biofilm-related periprosthetic infections loaded in bone cement.
P202- Vulvovaginal candidiasis: Modulation of *Candida* biofilm by *Escherichia coli* in clinical samples


Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Spain

**Introduction**: The pathogenesis of VVC is due to biofilm formation and epithelial invasion which induce local inflammatory responses and histopathological changes in the vaginal epithelium and promote the formation of antifungal-tolerant persister cells. In addition, it has been reported that *Escherichia coli* (EC) can establish a vaginal intracellular reservoir which modulates *Candida* (CA) biofilm formation.

**Hypothesis**: The behavior of CA biofilm can be modulated by the simultaneous presence of EC. In vitro co-culture models may explain differences between biofilm production of strains in solitary or in co-infection.

**Methodology**: During 6 months we prospectively detected CA and EC in vaginal swabs obtained from patients attended at our institution. We selected those patients having positive vaginal cultures with both CA and EC (cases) and a randomly selected comparator group (same size) of patients with either CA or EC (controls). We tested biomass and metabolic activity by crystal violet and XTT assays, respectively, using an in vitro model of a 24-hour-mature biofilm.

**Results**: We collected a total of 229 vaginal swabs, 5 (2.2%) of which had CA and EC (cases), 29 (12.7%) had only CA, 48 (21.0%) had only EC, and 147 (64.2%) were negative. Overall, biomass production was significantly higher in mixed cultures. Median (IQR) absorbance of CV was as follows: mixed culture, 0.238 (0.135-0.389); CA, 0.157 (0.119-0.198), p=0.01; EC, 0.173 (0.084-0.277), p=0.039. No differences were observed regarding metabolic activity.

**Conclusions**: We observed that vaginal co-infection of CA and EC is unusual (2.2%). Ours is the first study assessing CA and EC biofilms in co-culture in a large collection of clinical specimens. Biofilm production is the same whether strains were isolated from patients with co-infection or in solitary culture. Ongoing studies are being performed to assess cell density of each microorganism alone or combined in co-cultures by CLSM.
INTRODUCTION AND OBJECTIVES

*Staphylococcus haemolyticus* is a common cause of sepsis in premature babies and children with cancer. *S. haemolyticus* is part of our normal microbiome and has only a few virulence factors. However, clinical *S. haemolyticus* isolates are highly antibiotic-resistant and form biofilms on invasive catheters and medical implants. Such infections are difficult to treat; the biofilm matrix prevents sufficient diffusion of antimicrobial substances into the biofilm, cells are dormant in addition to a high degree of antibiotic resistance in clinical *S. haemolyticus* isolates. Biofilm is an important virulence factor that also reduces phagocytosis. Little is known about the strategies *S. haemolyticus* uses to establish an infection or evade the host immune system. Recently, four different capsule types were found in *S. haemolyticus*. Expression of capsule in *S. aureus* inhibits expression of several adhesion proteins, and factors involved in biofilm formation. Since biofilm is a known virulence factor in *S. haemolyticus*, we want to investigate the interaction between capsule production and biofilm and how this is regulated under different growth conditions for clinical isolates versus commensal isolators.

METHODS

We will investigate whether the different capsule types are associated with different biofilm-producing abilities and if capsule expression will contribute to reduced biofilm formation. This will be examined by a combination of molecular manipulation (such as knockouts and heterologous expression), negative staining techniques followed by microscopy, biofilm assays and RT-PCR to examine capsule expression during different growth conditions. Depending on the findings, animal models may also be included.

Results:

In progress:

We hope these results and knowledge will help identify new therapeutic targets and better treatment strategies for sepsis with *Staphylococcus haemolyticus* in immune compromised patients.
Introduction: Antibiotic treatment regularly fails to cure patients suffering from infections caused by adaptively resistant biofilm communities. Even though at least two thirds of all clinical infections are associated with biofilms, there are no approved biofilm-specific therapies on the market or in clinical trials.

Hypothesis and aims: This project aims to identify biofilm regulators in the WHO-priority pathogen Pseudomonas aeruginosa to provide novel targets for the design of biofilm-specific therapies.

Methodology: Using a transposon insertion sequencing (TnSeq) approach, the genome of P. aeruginosa PA14 was screened for genes functionally contributing to biofilm growth. Mutant composition and frequency of the TnSeq pool after biofilm growth on human skin organoids were compared to those in planktonic cultures to identify regulatory genes playing a specific role in the biofilm growth state.

Results: TnSeq identified around 350 genes functionally contributed to biofilm growth in the human skin organoid model including nearly 50 regulatory genes. Many well-established biofilm regulators belonging, for example, to the Gac-Rsm and the alginate regulatory pathway as well as several novel regulators were detected. The requirement of a selection of these regulators in skin biofilms was confirmed using a competition assay. Furthermore, comparison with genes previously identified with TnSeq to be involved in biofilm growth on hydroxy apatite, the major ingredient in bones, showed little overlap suggesting that the gene network underlying biofilm growth is at least in parts shaped by the environment.

Conclusion: TnSeq is a powerful tool us to systematically investigate the regulatory gene network driving biofilm growth.
P206- Testing antibiotic susceptibility of complex polymicrobial biofilms through impedance measurements: an application in dentistry

Miglė Žiemytė, Andrés López-Roldán, Ana Rodriguez, Marta Reglero Santaolaya, Mariam Ferrer and Alex Mira

1- Genomics & Health Department, FISABIO Foundation, Valencia, Spain
2- Department of Stomatology, Faculty of Medicine and Dentistry, University of Valencia, Valencia, Spain

Treatment of polymicrobial biofilms caused by oral bacteria in periodontitis is normally selected empirically or by the use of qPCR or DNA hybridization methods. These molecular approaches are directed toward establishing the levels of different periodontal pathogens in samples from periodontal pockets and using those to infer the antibiotic treatment with potentially best efficacy. However, current methods are costly and do not consider the antibiotic susceptibility of the whole biofilm.

Thus, in our study, we have developed a method to culture subgingival samples ex vivo in a fast, label-free impedance-based system where biofilm growth is monitored in real-time under exposure to different antibiotics, producing whole biofilm susceptibility results in only four hours. We have also performed a double-blind randomized clinical study where periodontal patients were treated using antibiotics selected by the standard qPCR method or the one indicated by the impedance system. The antibiotic selection did not coincide in both methods in more than 50% of the cases. In addition, 16s rRNA gene sequencing and evaluation of clinical parameters showed that the impedance method provided better results than qPCR in more than 40% of the cases. We hypothesize that the disagreements with DNA-based molecular methods stem from the polymicrobial nature of periodontal disease giving rise to multiple interactions and unpredictable antibiotic susceptibility, as well as from intra-specific variability in antibiotic susceptibility. The analysis of clinical outcomes and microbiological features together with the reduced cost and low analysis time is of great clinical relevance and supports the use of impedance measurements for improved individualized antibiotic selection.
P207- Disclosing the biofilmome of *Escherichia coli*

Miriam Kristine Nilsen 1
Anna Kaarina Pöntinen 2
João A Gama1 Vidar Sørum 1
Elizabeth G Aarag Fredheim 1

1 Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway Tromsø, Norway
2 Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Norway

The relationship between biofilm formation in clinical infections and the genetic background of *Escherichia coli* is unknown. *E. coli* biofilms are associated with osteomyelitis, urinary tract- and device-associated infections, all of which may cause systemic disease (sepsis). Consequentially, such clinical biofilms can result in difficult-to-treat recurring infections and increased mortality.

We aim to disclose the biofilmome of *E. coli*, resolving its biofilm forming potential by analysing 3254 isolates from human blood cultures. First, we will identify all genes coding for products excreted from the cytosol by genomic analyses and a structured literature search. Second, the presence/absence of known and putative biofilm-associated genes will be correlated with the biofilm formation (determined through a panel of different phenotypic methods).

Initial results suggest that the ability to produce curli and cellulose, previously described matrix-components of *E. coli* biofilms, is widespread across the collection, but two central genes responsible for cellulose synthesis are absent in parts of the study population. PGA is also highly prevalent, but completely missing from ST69. Fimbria are important for initial adhesion, and certain fimbria-associated genes such as sfa and fimH are highly prevalent whereas others vary, being a potential foundation for biofilm-associated genetic profiles. This research can reveal determinants of biofilm formation, providing a deeper understanding the role of this important mode of growth in shaping the virulence of clinical *E. coli*. Additionally, it may reveal potential novel targets for antimicrobial treatments focused on the non-essential process of biofilm formation, thus imposing less selective pressure for resistance development compared to traditional drugs.
P208-Magnesium doped zinc oxide nanoparticles with activity against pathogenic bacterial biofilms

Nicolás Navarro Martínez 1,3,5, Erlen Y. Cruz Jorge 1, María José González 1, Sofía Sánchez 2,3,5, Javier Morales 2,3,5, Luciana Robino 4, Paola Scavone 1.

1 Laboratorio de Biofilms Microbianos, Depto. de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable.
2 Departamento de Ciencias y Tecnología Farmacéuticas, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.
3 Advanced Center for Chronic Diseases, Universidad de Chile.
4 Departamento de Bacteriología y Virología, Instituto de Higiene, Universidad de la República.
5 Center of New Drugs for Hypertension (CENDHY), Universidad de Chile.

The increasing resistance of bacteria to antibiotics is one of WHO concerns, the development of new technologies and therapies that provide effective treatments are mandatory. One of the strategies of bacterial resistance is the formation of biofilms, these structures are bacterial sessile communities irreversibly attached to a biotic or abiotic surface, and embedded in an extracellular polymeric substances matrix of its own production. Bacterial biofilms are very difficult to eradicate with conventional antibiotics therapies.

An alternative solution is the use of metallic nanoparticles, such as zinc oxide nanoparticles (ZnO:MgO-Nps).

The hypothesis of this work is that ZnO:MgO-Nps have antibiofilm activity against S. aureus ATCC 6538, A. baumanii ATCC 19606 and P. aeruginosa ATCC 902, E. coli 144 and P. mirabilis 2921 (Montevideo, Uruguay).

ZnO:MgO-Nps were synthesized by precipitation method with zinc acetate, magnesium acetate and potassium hydroxide. The nanoparticles were characterized using UV-Visible spectroscopy, the hydrodynamic radius and zeta potential was determined on a Zetasizer ZS (Malvern Instruments). The Nps activity over the biofilm was tested at 3/2, 1, and 1/2 concentrations, the biofilm biomass was determined by violet crystal assay on a 96 microtiter well.

The synthesized ZnO:MgO-NPs had a hydrodynamic radius of ~210 nm, with a polydispersity index of 0.45, and a Z-potential of ~15 mV. These nanoparticles were capable of inhibiting the formation of A. baumanii, P. mirabilis 2921 and E. coli, and biofilms compared to the control. Otherwise, the antibiofilm activity of Au-Nps over the mature biofilm was observed in A. baumanii and E. coli. The reported results suggest that the synthesized ZnO:MgO-NPs has potential use as treatments for biofilm-related infections caused by pathogenic bacteria.
P209- Effect of temperature exposure on the ability of an endodontic sealer with QAC BJM Root Canal Sealer to inhibit biofilm growth.

Stanislav Voroshilov (1,2),
Michael Solomonov (3),
Marina Jurina (2),
Baghish Harutyunyan (1),
Vigen Goginyan (1),
Yury Nikolaev (2).

( 1 - Armbiotechnology NAS RA, Yerevan, Armenia. 2 - Winogradsky institute of microbiology RAS, Moscow, Russia. 3 - Department of endodontics, Sheba Medical Center, Tel-Hashomer, Israel.).

Introduction
We chose the topic because of its novelty and significance for dental practice. There are studies on the effect of temperature on the filling material during the root canal filling. But they mainly focused on its chemical and physical properties. However, our work was focused on antibiofilm properties.

Hypothesis
Heating the sealer after it has been kneaded, which is common in clinical practice, could impair the ability of the QAC biocide molecules to inhibit biofilm growth.

Methodology
We used samples made out of endodontic sealers heated at various temperatures to grow biofilm on them. The biofilm cell viability was assessed by MTT assay.

Results
1. The obtained CFU values of the biofilm of the filling material with biocide QAC BJM RCS were significantly lower than those of the control material AH plus.
2. There was no correlation between samples materials heated at different temperatures and biofilm CFU.

Conclusion
The results make it possible to refute the working hypothesis by postulating the absence of the effect of the temperature regimes of heating the test material with the QAC BJM RCS biocide on its ability to resist biofilm growth.
P210- The bepA-L gene cluster in *Burkholderia cenocepacia* H111 produces a water-insoluble polysaccharide essential for biofilm formation

Paola Cescutti¹, Barbara Bellich¹, Lucrecia C. Terán¹, Magnus M. Fazli², Francesco Berti³, Roberto Rizzo¹, Tim Tolker-Nielsen²

¹ Department of Life Sciences, University of Trieste, via L. Giorgieri 1, Bdg. C11, 34127 Trieste, Italy
² Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen, Denmark
³ GSK, Via Fiorentina 1, 53100 Siena, Italy

The *Burkholderia cepacia* complex (Bcc) is a group of more than 20 closely related bacterial species which colonize humans, where they are associated with chronic granulomatous disease and cystic fibrosis (CF). *B. cenocepacia* and *B. multivorans* are the two most commonly isolated Bcc species from CF patients where they cause infections that are extremely difficult to treat with sometimes fatal outcome. Biofilm formation is a virulence trait of Bcc strains, and has been associated with persistence of infections and increased tolerance to antibiotics. In the biofilm matrix, exopolysaccharides are of importance for mechanical stability and antibiotic tolerance. The bepA-L gene cluster of *B. cenocepacia* H111 encodes for an exopolysaccharide which was shown to provide structural stability to biofilms and to be essential for biofilm formation in flow-chambers. Expression of the bepA-L gene cluster is regulated both by c-di-GMP and the transcriptional regulator BerA. The aim of our investigation was to identify the product of the bepA-L gene cluster. Two different exopolysaccharides were isolated from *B. cenocepacia* H111 biofilms and characterized: H111-SOL, a water-soluble polysaccharide containing rhamnose and mannose residues, and H111-INS, a water-insoluble polymer made of glucose, galactose and mannose. In order to establish which of these two polymers is the product of the bepA-L gene cluster, exopolysaccharides were isolated from biofilms produced by mutant strains, and investigated by NMR spectroscopy and composition analysis. The results showed that the product encoded by the bepA-L gene cluster is the water-insoluble H111-INS polymer which was therefore named Bep polysaccharide. We also demonstrated that the wild type *B. cenocepacia* H111 strain produces the Bep polysaccharide, thus underlining its potential importance in “in vivo” infections.

This work was supported in part by an agreement with Cornell University, under Prime Agreement R01GM123283 from the National Institute of General Medical Sciences of the NIH.
**P211- Application of Expansion Microscopy for Bacterial Biofilms**

Dante Castagnini 1,2; Karina Palma 1,2,3; Jorge Jara-Wilde 1,2,3; Nicolás Navarro M. 5,6; Maria José González 6, Nicole Canales 1,2; **Paola Scavone** 6, Steffen Härtel * 1,2,3,4,

1 Laboratory for Scientific Image Analysis SCIAN-Lab, Integrative Biology Program, Institute of Biomedical Sciences ICBM, Faculty of Medicine, University of Chile, Santiago de Chile, Chile
2 Biomedical Neuroscience Institute BNI, Faculty of Medicine, University of Chile, Santiago de Chile, Chile
3 Centro de Informática Médica y Telemedicina CIMT, Faculty of Medicine, University of Chile, Santiago de Chile, Chile
4 National Center for Health Information Systems CENS, Santiago de Chile, Chile
5 Advanced Center for Chronic Diseases (ACCDiS), Santiago, Chile
6 Laboratorio de Biofilms Microbianos, Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

Bacterial biofilms are complex and dynamic structures composed by one or more microbial species, irreversibly attached to a biotic or abiotic surface, and embedded in an extracellular polymeric matrix of its own production. Biofilms play an essential role in nature, but they also have a very significant impact in the healthcare system, where they confer resistance to antimicrobials and the immune system. For their study, confocal microscopy (CSLM) has been widely used because it allows observing the 3D architecture through optical sectioning, and spatial-temporal structural dynamics through live-imaging. However, CSLM is bound to the optical resolution and bacterial size is almost at the limit of diffraction, which precludes resolving morphological and structural features in biofilms, and subcellular structures cannot be revealed. Expansion Microscopy (ExM) is based on the polymerization of a swellable ionic gel throughout the sample, which preserves positions and distances of the targets of interest, allowing super resolution visualization of fixed biological samples by physically augmenting their volume ～60-fold (～4-fold linear augment). Combining CSLM and ExM considerably improves resolution by increasing the physical distance between objects. In the present work, we developed an ExM technique for planktonic cells and biofilm of Proteus mirabilis. The standard ExM protocol was modified to treat the bacteria with mutanolysin, proteinase K, α-amylase, and cellulase. After expansion, CSLM images were acquired, and image processing and analysis were applied to quantify bacterial morphology and estimate the expansion factor. Results show that expansion was achieved with a 4.8 factor in the planktonic cells, and 5.1 in biofilms. This first biofilm ExM opens new options in the study and comprehension of biofilms and bacterial subcellular structures.
**P212- ACTIVITY OF S- (3,4-DICHLOROBENZYL) ISOTIOUREIA (A22)**

Pauline Cordenonsi Bonez, pHD  
Grazielle Guidolin Rossi, pHD  
Roberto Christ Vianna Santos, pHD  
Marli Matiko Anraku de Campos, pHD

*Pseudomonas aeruginosa* are able to form biofilms in medical devices and living tissues, can cause severe chronic infections in humans. Thus, it is important to search effective alternatives against the formation of biofilms. A22 inhibits MreB protein from the bacterial cytoskeleton altering the microbial cells shape, which can affect many properties, including motility and biofilm formation. In this context, this work aimed to evaluate, for the first time, the antibiofilm action of A22 on *P. aeruginosa*, as well as their potential cyto and genotoxic effects. The cyto and genotoxicity tests were performed by MTT test and Comet assay, respectively. The manuscript measures the adhesion capacity of clinical isolates and standard strain in polystyrene plates. Essential factors to the biofilm physiology of *P. aeruginosa*, such as swimming, swarming ant twitching motility, as well as adhesion to HeLa cells and adhesion to High Density Polyethylene (HDPE) were evaluated in the presence and absence of A22. Atomic Force Microscopy (MFA) was used to visualize adhesion on HDPE substrate. This study confirmed the excellent antimicrobial activity of A22, especially in relation to multiresistant isolates. Likewise, it was shown that A22 maintains the cell viability of human Peripheral Blood Mononuclear Cells (PBMCs) and did not demonstrate genotoxic effects on the cells. The manuscript results showed a high adhesion pattern in 14 multiresistant clinical isolates, with inhibiting the adhesion of 9 of these microorganisms. A22 was able to decrease adhesion and biofilm formation of the *P. aeruginosa* PAO1 on polystyrene plates, HeLa cells and HDPE. Moreover, the swarming and twitching motilities were significantly decreased by A22, in subinhibitory concentrations. The impact and scientific contribution of this work are based on the discovery of a potencial new therapeutic possibility against infections associated with biofilms. The A22 presents as an useful and promising tool to decrease microbial adhesion in both living and inert surfaces, given its low toxic effects. However, this results stimulate the deepening in methodologies that aim the insertion of A22 as a new antibacterial or coating agent on medical materials.
P213-Assessment of antimicrobial technologies for future medical devices

Sofia Wareham Mathiassen 1,2
Mohammed Nateqi 2,3,
Kasper Nørskov Kragh 1
Thomas Bjarnsholt 1, 4

1. Costerton Biofilm Center, Immunology and Microbiology, Copenhagen University
2. Front End Innovation, Novo Nordisk
3. Department of Biotechnology and Biomedicine, DTU
4. Department of Clinical Microbiology, Rigshospitalet

Antimicrobial resistance represents one of the major threats to public health worldwide, causing 700,000 deaths annually, and estimated to rise to 10 million in 2050, according to the WHO. Medical devices contribute to a substantial part of nosocomial infections, especially in patients with compromised immune systems. To combat this, antimicrobial materials for biomedical use are being explored to reduce hospital- and device-acquired infections. However, standardized test methods vary, and laboratory test conditions make results difficult to transfer to end-use settings. This study evaluates commercially available antimicrobial materials for medical devices to mitigate microbial proliferation and biofilm formation in clinically relevant settings. Various technologies are tested through a modified ISO 22196 protocol, with relevant temperature, humidity, and increased contact times. A novel colorimetric assay is developed to gain a deeper assessment of the efficacy and mode of action of the materials. These findings will serve as a testing toolbox for the employment of these materials in next-generation medical devices.
Anti-biofilm activity of fluorocytosine (5-FC) in *Escherichia coli* requires its conversion to a nucleotide and involves penicillin-binding protein 1B

Srikanth Ravishankar (*), Karen Leth Nielsen (*), Paolo Landini (*) and Elio Rossi (*)

(*) Department of Biosciences, University of Milan, Italy

(*) Department of Clinical Microbiology, Rigshospitalet, Denmark

Acute and chronic infections caused by opportunistic pathogens resistant to antibiotics are on the rise. Anti-virulence and anti-biofilm agents, i.e. drugs that selectively “disarm” pathogenic bacteria are considered a promising strategy. Several antimetabolites, like 5-fluorocytosine (5-FC) have been shown to possess anti-virulence activity in Gram negative bacteria, yet their mechanism of action is not understood.

In this work, we show that, at concentrations subinhibitory for growth, 5-FC impairs biofilm formation by *Escherichia coli*, both in the laboratory strain MG1655 and clinical isolates, by reducing the expression of the csgBAC operon involved in curli fimbriae production. 5-FC activity requires its conversion into fluoronucleotides, as inactivation of the upp gene, which encodes the enzyme that synthetises UMP from uracil, prevented the 5-FC-dependent inhibition of biofilm formation and curli gene expression. To identify molecular targets of 5-FC, we screened an *E. coli* genomic overexpression library and observed that a short fragment of the mrcB gene, encoding penicillin-binding protein 1B, involved in peptidoglycan biosynthesis, was able to restore curli production in the presence of 5-FC. In contrast, overexpression of full-length mrcB led to downregulation of curli expression. Interestingly, we observed a 3-fold increase in mrcB transcript level upon 5-FC treatment in *E. coli*, suggesting that inhibition of curli fibers might be linked to the increased expression of the mrcB gene. Inactivation of the mrcA gene, another bifunctional peptidoglycan synthase whose activity overlap with the one of mrcB, showed no effect on curli expression suggesting a specific role of mrcB in controlling curli production. Overall, our results suggest that 5-FC anti-biofilm activity requires its conversion into a nucleotide, and that it might potentially be mediated by the increased expression of the mrcB, suggesting a direct link connecting peptidoglycan biosynthesis with curli production and biofilm formation.
Methods for bacterial detection are needed to advance the infection research and diagnostics. Based on conformation-sensitive fluorescent tracer molecules, optotracing was recently established for dynamic detection and visualization of structural amyloids and polysaccharides in the biofilm matrix of gram-negative bacteria. Here, we extend the use of optotracing for detection of gram-positive bacteria, focusing on the clinically relevant opportunistic human pathogen Staphylococcus aureus. We identify a donor-acceptor-donor-type optotracer, whose binding-induced fluorescence enables real-time detection, quantification, and visualization of S. aureus in monoculture and when mixed with gram-negative Salmonella Enteritidis. An algorithm-based automated high-throughput screen of 1920 S. aureus transposon mutants recognized the cell envelope as the binding target, which was corroborated by super-resolution microscopy of bacterial cells and spectroscopic analysis of purified cell wall components. The binding event was essentially governed by hydrophobic interactions, which permitted custom-designed tuning of the binding selectivity towards S. aureus versus Enterococcus faecalis by appropriate selection of buffer conditions. Collectively this work demonstrates optotracing as an enabling technology relevant for any field of basic and applied research, where visualization and detection of S. aureus is needed.
P216- Evaluation of Silver Nanoparticles as a potential treatment for pathogenic bacterial biofilms

María José González1, Erlen Y. Cruz Jorge1, Nicolás Navarro Martínez1,2,3,5, Sofía Sánchez2,3,5, Javier Morales2,3,5, Luciana Robino4, Paola Scavone1.

1 Laboratorio de Biofilms Microbianos, Depto. de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable.  
2 Departamento de Ciencias y Tecnología Farmacéuticas, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile.  
3 Advanced Center for Chronic Diseases, Universidad de Chile.  
4 Departamento de Bacteriología y Virología, Instituto de Higiene, Universidad de la República.  
5 Center of New Drugs for Hypertension (CENDHY), Universidad de Chile.

The increasing resistance of bacteria to antibiotics is one of WHO concerns, the development of new technologies and therapies that provide effective treatments are mandatory. One of the strategies of bacterial resistance is the formation of biofilms, these structures are bacterial sessile communities irreversibly attached to a biotic or abiotic surface, and embedded in an extracellular polymeric substances matrix of its own production. Bacterial biofilms are very difficult to eradicate with conventional antibiotics therapies.

An alternative solution is the use of metallic nanoparticles, such as silver nanoparticles (Ag-Nps). The hypothesis of this work is that Ag-NPs have antibiofilm activity against *S. aureus* ATCC 6538, *A. baumanii* ATCC 19606 and *P. aeruginosa* ATCC 902, *E. coli* 144 and *P. mirabilis* 2921 (Montevideo, Uruguay). Ag-Nps were synthesized by citrate reduction method using silver nitrate as precursor silver salt and tannic acid as stabilizer. The nanoparticles were characterized using UV-Visible spectroscopy, the hydrodynamic radius and zeta potential was determined on a Zetasizer ZS (Malverrn Instruments). The Nps activity over the biofilm was tested at different concentrations, the biofilm biomass was semi-quantified by violet crystal assay on a 96 microtiter well.

The synthesized Ag-NPs had a hydrodynamic radius of ~20 nm, with a polydispersity index of 0.32, and a Z-potential of -35 mV. Ag-Nps were capable of inhibiting the formation of *A. baumanii, E. coli*, and *P. mirabilis* biofilms compared to the control. Also, the Ag-Nps were able to eradicate *A. baumanii, E. coli*, and *P. mirabilis* mature biofilm. However, this effect was not observed in *P. aeruginosa* and *S. aureus*. These results suggest that the synthesized Ag-NPs has potential use as treatments for biofilm-related infections caused by pathogenic bacteria.
The need for more effective strategies aimed at prevention and eradication of microbial biofilms in chronic and device-associated infections is well understood. Biofilms are known to exhibit elevated tolerance to antibiotic treatment compared to their planktonic counterparts, so clinical alternatives are urgently required. Incidences of biofilm-related infections complicate primary joint replacement at rates of 1-2%, or 3-5% in revision surgeries (Fernandes et al. 2013). Causative pathogens of such infections include bacteria of the genus: Staphylococcus, Streptococcus and Pseudomonas.

Plasma, a partially or wholly ionised gas, is often referred to as the fourth state of matter which, in nature, are often thermal in nature. The ability to generate plasmas at temperatures below 40°C, referred to as non-thermal or ‘cold’ atmospheric-pressure plasma (CAP) has given rise to the nascent field of plasma medicine. CAP comprises a complex mixture of ions, metastables, reactive oxygen and nitrogen species (RONS), free electrons and ultraviolet radiation at tissue tolerable temperatures. Antimicrobial applications of atmospheric pressure non-thermal plasma have been gaining increasing interest from a wide range of scientific disciplines, including promotion of wound healing, selective targeting cancer cells and activation of immune cells. CAP-produced RONS have been shown to cause oxidative stress, membrane peroxidation, and degradation of DNA including extracellular DNA (eDNA) a major component of the biofilm extracellular matrix, indicating the cold plasmas may target both the matrix and the cells within.

This study will aim to elucidate the efficacy of a commercially available, FDA-approved helium plasma jet on MRSA biofilms in vitro, which will be correlated with quantification of reactive oxygen and nitrogen species generated in the plasma. Extracellular matrix degrading enzymes will be incorporated into CAP treatment as putative synergistic approach to facilitate biofilm eradication.
P220- Development of an *in vitro* model for studying biofilms in broiler drinking water systems

C. Oastler 1,2; R. La Ragione 1; M. Chambers 3; R. Gosling 1; F. Martelli 1; R. Davies 1

1. Department of Bacteriology, Animal and Plant Health Agency, Weybridge, United Kingdom ● 2. Department of Pathology and Infectious Diseases, University of Surrey, Guildford, UK ● 3. Department of Microbial Sciences, School of Biosciences and Medicine, Guildford, UK.

**Introduction**: Drinking water systems (DWS) in commercial poultry housing are only cleaned between flocks and are susceptible to the development of internal biofilms. Contamination of DWS by bacteria, such as *Salmonella*, can perpetuate flock infections.

**Hypothesis**: Current *in vitro* models for studying biofilms in DWS are limited as they only simulate individual DWS components/materials. The aim was to develop and assess biofilm formation within an *in vitro* model of a poultry DWS.

**Methodology**: A realistic scaled model of a poultry DWS (pipeline and drinker nipples) was constructed with polyvinylchloride (PVC) and stainless-steel coupons also placed within the DWS to aid biofilm sampling. WHO hard water inoculated with $10^3$ CFU/ml of live *Salmonella* vaccine (Salmovac 440) was circulated in the DWS as a closed continuous flow system. After 4-days incubation at room temperature, drinker nipples and coupons were removed, sonicated, and *Salmonella* in biofilms enumerated.

**Results**: Biofilm formation by the vaccine strain on PVC and stainless steel was confirmed with an average 4.6 and 5.0 log CFU/cm$^2$, respectively, recovered after 4-days incubation, shaking at 50rpm, in hard water. *Salmonella* was recovered from biofilms formed within the DWS (drinker nipples and coupons) even with a relatively low bacterial load.

**Conclusion**: Development of an *in vitro* DWS model facilitates the more realistic study of biofilm formation, and has the potential to be used to assess the effectiveness of biofilm inhibition or removal treatments.
P221- NaCl Triggers the Sessile-to-Motile Transition of Bacillus subtilis

Prem Anand Murugan, Manish Kumar Gupta, T. Sabari Sankar, Sivasurender Chandran and Saravanan Matheshwaran

1 Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India
2 Department of Physics, Indian Institute of Technology, Kanpur, India
3 School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, India
4 Centre for Environmental Sciences and Engineering, Indian Institute of Technology, Kanpur, India
5 Mehta Family Centre for Engineering in Medicine, Indian Institute of Technology, Kanpur, India

Various chemical cues are known to alter the motile and sessile states of bacteria differentially and, in turn, the formation of biofilms. However, the underlying mechanisms at the cellular and molecular level remain less understood, which severely limits our ability to control biofilms. Here, we systematically studied the effects of NaCl on the dynamics of biofilm formation across various length scales and the associated changes in the regulation of gene expression in an undomesticated natural isolate of Bacillus subtilis. Interestingly, NaCl induced significant changes in the architecture of pellicles and yielded systematic increase in lateral expansion rates of biofilms when grown on an agar surface. At the microscopic level, both in the presence and absence of NaCl, bacteria displayed super-diffusive motion at times lesser than a second. However, at larger delay times, we observed an intriguing NaCl-induced transition from sub-diffusion behavior of individual bacterial cells to rapid diffusion behavior. In addition, NaCl reduced the dynamical heterogeneity of the bacterial cells within the biofilm. The reduced heterogeneity and the increased flagellation in a subpopulation of cells in the presence of NaCl corroborates well with the observed higher motility of the cells. Further, the cellular uptake of NaCl resulted in the downregulation of several genes underlying the formation of biofilms, revealing the role of chemical cues like NaCl in controlling the gene regulatory circuit underlying the sessile to motile transition. Our study opens a new avenue to decipher the competitive advantage provided to the subcellular populations by NaCl due to lifestyle switch in Bacillus subtilis.
P222- Co-culture of *Pseudomonas aeruginosa* and *Staphylococcus aureus* Triggers Lipid A Modifications in an *in vitro* Biofilm Flow Reactor

**Kristen J. Brao,¹,² Matthew E. Sherman,¹,³ Francesca Gardner,¹ Richard Smith,¹,⁴ Hyojik Yang,¹ Mark E. Shirtliff,¹,² Robert K. Ernst,¹,² Janette M. Harro¹**

¹Department of Microbial Pathogenesis, University of Maryland School of Dentistry, Baltimore MD, USA  
²Department of Molecular Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA  
³Department of Biochemistry, University of Maryland School of Medicine, Baltimore, MD, USA  
⁴Department of Epidemiology, University of Maryland School of Medicine, Baltimore, MD, USA

*Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are the most frequently observed organisms co-infecting the lungs of patients with cystic fibrosis (CF). To model the interactions of PA and SA over time, we introduced PA to an established SA biofilm in a continuous flow biofilm reactor. Similar to CF patients’ airways, PA quickly became the dominant species (p<0.05, two-tailed unpaired t-tests), although SA was not eliminated. To characterize the interactions between SA and PA, we performed RNAseq on polymicrobial biofilm samples at 12, 24, and 72 hours after the addition of PA. Single-species biofilms served as reference samples. Transcriptomic analysis identified the PA *arn* operon and its transcriptional regulators among the most up-regulated genes at 12 and 24 hours. The *arn* operon is involved in the addition of 4-amino-4-deoxy-L-arabinose (Ara4N) residues to the terminal phosphates of lipid A, the membrane anchor of lipopolysaccharide. This modification masks negative charges and increases PA resistance to cationic antimicrobial peptides, including the antibiotic colistin, which is used to treat CF patients. We validated the RNAseq data using gas chromatography-mass spectrometry, which confirmed that PA added Ara4N residues to lipid A 24 hours after introduction to the SA biofilm. To test whether co-culture of PA with SA caused changes in colistin resistance, we assessed colistin sensitivity using CF isolates in a modified polymicrobial minimum inhibitory concentration assay with samples taken directly from the biofilm reactor. We found that several PA isolates became more sensitive to colistin, which could suggest that SA biofilms produce compounds that can synergize with colistin, triggering protective PA lipid A modifications. This hypothesis will be tested using *arn* operon mutant PA isolates in the future. These results represent a possible mechanism by which co-infection can lead to changes in antibiotic resistance, which could affect treatment strategies for CF patients.
P223- Developing a high-content screen to identify molecules that inhibit cyclic di-GMP-mediated antibiotic-resistance and biofilm formation.

Ying Hu, Professor Jeremy Webb, and Dr Shiqi An

University of Southampton, and National Biofilms Innovation Centre

The rapid emergence of resistant bacteria is occurring worldwide and posing a significant threat to global healthcare systems. Conventional antibiotics cannot efficiently eradicate these infections and therefore we urgently need new efficient anti-bacterial agents. Recent research attempts to identify new drug targets has focused on regulatory pathways. Regulatory systems that utilise intracellular cyclic di-GMP (c-di-GMP) as a second messenger are one such class of target. C-di-GMP is a signalling molecule found in almost all bacteria that acts to regulate an extensive range of processes including antibiotic resistance, biofilm formation and virulence.

We hypothesised that if some novel compounds can be found that inhibit Pseudomonas aeruginosa virulence during infection through modulate c-di-GMP signalling.

The reporter strain of Pseudomonas aeruginosa was labelled by bioluminescence and GFP. High-content screen models include bacterial cell-based screen and co-culture human bronchial epithelial cell-based screen were used in this experiment. After analysing the output data, the potential hits were further confirmed by dose response.

We have already designed and optimised two high-content screen models for screening natural compounds. And further dose-respond test show there are several natural compounds influence c-di-GFP level during bacterial cell-based, several natural compounds influence c-di-GFP level and inhibit Pseudomonas aeruginosa virulence during infection based on co-culture screen model. This study’ tool strains in combination of the co-culture biofilm model will allow us to identify potential small molecules that modulate Pseudomonas aeruginosa viability, biofilm formation, and host response during infection.
A silent pandemic, chronic, non-healing wounds e.g., diabetic foot ulcers, are a major cause of morbidity, with treatment and management representing significant economic and health burdens. The opportunistic pathogens *Staphylococcus (S.) aureus* and *Pseudomonas (P.) aeruginosa* are the most common species isolated from chronic wounds, contributing to prolific biofilm formation, and decreasing antimicrobial efficiency. Polydimethylsiloxane (PDMS), a biocompatible and, inexpensive to fabricate polymer, can undergo various modifications. The ability of the produced polymers to attract *S. aureus* and *P. aeruginosa*, either from the planktonic state, or while sessile in biofilms on *ex vivo* skin, was investigated using various combinations of PDMS; patterned (PT) or flat (FL) +/- triclosan 1% or 10%.

Patterned PDMS + 10% triclosan (PT 10%) attracted significantly more live *S. aureus* and *P. aeruginosa*, as determined via Colony Forming Units (CFU)/mL (*p<0.01), Scanning Electron Microscopy (SEM) (*p<0.01) and Confocal Scanning Laser Microscopy (CSLM) (*p<0.01). The released triclosan was not cytotoxic against either bacteria (*p<0.05) or primary cultures of human dermal fibroblasts (WST-1 *p<0.05). HPLC analysis highlights low level of phase release Fibroblast viability increased in the presence of PDMS, when subject to infection in co-culture via the Boyden chamber assay (*p<0.05). PT 10% demonstrated superior biofilm transfer from epidermis (*p<0.5), in comparison to all other polymers.

In summary, the unique topography of PDMS combined with triclosan attracted live bacteria. A co-infection model demonstrated the ability of the polymer to attract planktonic bacteria away from cultured dermal fibroblasts thereby increasing their viability. Successful establishment of biofilms on epidermal sheets from full thickness skin allowed the demonstration of the favourable polymer topography for biofilm removal from human skin. This promising data suggests potential for engineering a patterned polymer to physically transfer biofilms from wounds, and importantly lacks bactericidal properties which is vital in the quest to combat antimicrobial resistance.
Hey!

Did you know that your **EBF** accreditation is made of **seed paper**?

Pull the paper on a plate, cover it with water. 

Don’t let it dry out! Otherwise, the seeds will not germinate.

Once the first sprouts appear, cut and place the paper in a pot filled with soil.

Follow the instructions: useandplant.com

Let it grow (like your science) and see you all in the next Eurobiofilms!