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Eurobiofilms 2019
Poster Abstracts
Wed & Thur
No.’s 1 to 206
Mechanisms & Resistance

1: A glimpse into the survival of non-biofilm formers in a nutrient and moisture deficient environment - Amaeze N. J

2: Exposure to antibiotics Increases diversity and gene transfer in Salmonella typhimurium biofilms - Bartke K

3: Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis - Cavallo I

4: Clostridium perfringens biofilm: characterization and antibiotic tolerance - Dixon R.A

5: Development of a robust biofilm assay of S. pneumoniae to study adaptive evolution and the emergence of antibiotic resistance - Espinoza S

6: Combined effects of low incubation temperature, minimal growth medium and low hydrodynamics optimize Acinetobacter baumannii biofilm formation - Eze E.C

7: Assessment of the antimicrobial tolerance of archaeal biofilms - Fakhoury A

8: Bacterial suspended aggregates in high viscosity - Irie Y

9: Mycobacterium abscessus complex can be sensitized to antibiotics by breaking up bacterial aggregates and increasing oxygen availability - Kolpen M

10: Acquisition of plasmids containing a cephalosporin resistance gene reduces biofilm formation - Nesse L.L

11: Dynamics in biofilms with E. coli strains producing different matrix components - Nesse L. L

12: Predicting genetic determinants of antibiotic resistance in biofilm and planktonic Escherichia coli populations by adaptive laboratory evolution - Nesse L.L

13: Phenotypic and genotypic characterisation of biofilm-forming Salmonella enterica serovars isolated from pig and poultry production environments - Oastler C

14: In-vitro study of antibiotics affecting on new and establishing non-typeable Haemophilus influenzae biofilm - Obaid N.A
15: Selection of resistance in bacteria grown on antimicrobial surfaces in multidrug environments - Pietsch F

16: Increased plasmid uptake in a biofilm adapted, rugose phenotype was linked to the presence of flagella - Røder H.L

17: Cyclic-di-GMP synthesis and subsequent biofilm formation enhances dibenzothiophene biodegradation in *Rhodococcus erythropolis* - Cristina Solano C

18: Conditions under which glutathione disrupts the biofilms and improves antibiotic efficacy of both ESKAPE and non-ESKAPE species - Theerthankar D

19: Pathobionts of the gastrointestinal microbiota express BAP-like proteins with amyloid features to build biofilms - Valle J

20: Identification of adhesion-force induced gene expression, its force sensitivity and height distribution in Streptococcus mutans biofilms (Oral) - Wang C

21: Signal transduction genes in marine biofilms and the influence of signal molecules on marine biofilms development - Wang R

22: The search for PQS transport proteins using PQS affinity probe generated through metabolic labelling - Woo B.

23: Biofilm-evolved *Klebsiella pneumoniae* exhibit changes in capsule, fimbriae and c-di-GMP turnover - Zaborskyte G

24: Investigating the effect of tobramycin dry powder inhaler on the eradication of *Pseudomonas aeruginosa* biofilms - Aljalamdeh R

25: Characterising the structure and composition of biofilms formed by *Pseudomonas aeruginosa* under different shear conditions - Allan W

26: Modulation of immune cell activation by *Pseudomonas aeruginosa* biofilms; potential role of C-type lectin receptors (ORAL) - Almuhanna Y

27: Visible light for *P. fluorescens* biofilms inactivation - Angarano V

28: Prevalence and genetic variation of the *Pseudomonas aeruginosa* elastase gene in clinical isolates from cystic fibrosis patients - Barochia B

29: Key regulators of nitric oxide-mediated regulation in *Pseudomonas aeruginosa* biofilms dispersal (ORAL) - Cai Y

**Pseudomonas**

24: Investigating the effect of tobramycin dry powder inhaler on the eradication of *Pseudomonas aeruginosa* biofilms - Aljalamdeh R

25: Characterising the structure and composition of biofilms formed by *Pseudomonas aeruginosa* under different shear conditions - Allan W

26: Modulation of immune cell activation by *Pseudomonas aeruginosa* biofilms; potential role of C-type lectin receptors (ORAL) - Almuhanna Y

27: Visible light for *P. fluorescens* biofilms inactivation - Angarano V

28: Prevalence and genetic variation of the *Pseudomonas aeruginosa* elastase gene in clinical isolates from cystic fibrosis patients - Barochia B

29: Key regulators of nitric oxide-mediated regulation in *Pseudomonas aeruginosa* biofilms dispersal (ORAL) - Cai Y
30: Properties of *Pseudomonas aeruginosa* biofilm cells dispersed with various approaches - *Coenye T*  
31: Understanding phenotypic diversity of *P. aeruginosa* in chronic diabetic ulcers - *da Silva A.C*  
32: *Pseudomonas aeruginosa* quorum-sensing signal molecules interfere with human mesenchymal stem cells on bioactive coated 2D/3D titanium implants - *Damiati L*  
33: RNA-seq characterization of *Pseudomonas aeruginosa* grown in alginate bead model and comparison to in vivo infections (ORAL) - *Fritz B*  
34: Analysis of biofilm phenotypes of 350 clinical *Stenotrophomonas maltophilia* reveals high levels of phenotypic and structural heterogeneity - *Gudzu M*  
35: In vitro human peripheral blood mononuclear cell immune response to *Pseudomonas aeruginosa* biofilms - *Kaya E*  
36: Role of phenotypic switching in stability and persistence of *Pseudomonas aeruginosa* biofilms - *Mirani Z.A*  
37: Comparison of *ex vivo* porcine and human corneas as models for bacterial keratitis caused by *Pseudomonas aeruginosa* - *Okurowsk K*  
38: Evaluation of the antibiofilm activity of a Tetrasodium-EDTA complex-polymer against *Pseudomonas aeruginosa* in the drip flow bioreactor model - *Percival S.L*  
39: Development of a chronic *Pseudomonas* biofilm infection model in wax worms - *Robertson S.N*  
40: Effect of stress hormone epinephrine on *Pseudomonas aeruginosa* biofilm formation - *Rodrigues S*  
41: Simple pyranoside- and furanoside-functionalized glass surfaces: an attractive anti-bioadhesion strategy against *Pseudomonas aeruginosa* (ORAL) - *Scalabrini M*  
42: Changed antibody response following lung transplantation in cystic fibrosis patients with *Pseudomonas aeruginosa* biofilm infections - *Schwensen H*  
43: Drug resistance and biofilm production among *Pseudomonas aeruginosa* clinical isolates in a tertiary care hospital of Nepal - *Shrestha R*  
44: *Pseudomonas aeruginosa* exopolysaccharide Psl engages host C-type lectin receptors - *Singh S*
45: The use of nitric oxide donor pro-drugs to tackle *Pseudomonas aeruginosa* biofilms - *Soren O*  
46: Sensitising *Pseudomonas aeruginosa* biofilms to antibiotics and reducing virulence through novel target inhibition. - *Soukarieh F*  
47: Outer membrane vesicle formation by *Pseudomonas aeruginosa* biofilm cells - *Tashiro Y*  
48: Increased intracellular cyclic-di-AMP levels sensitisre Streptococcus gallolyticus subsp. gallolyticus to osmotic stress and reduce biofilm formation and adherence on intestinal cells - *Woo Keong Teh*  
49: Incorporating a cleaning step in the sanitation of drinking water systems of broilers reduces biofilms and inhibits the regrowth of multidrug-resistant *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* - *Vackier T*  
50: Identification of genes involved in *Pseudomonas aeruginosa* biofilm resistance to antibiotics - *Valentin J*  
51: *In vitro* synergistic activity of fosfomycin, ciprofloxacin and gentamicin combinations against *Pseudomonas aeruginosa* biofilms - *Wang L*

**Wounds & Skin**

52: Atopic dermatitis and healthy skin microbiota - *Bay L*  
53: Multispecies biofilm infected wounds murine model - *Cárdenas C*  
54: Interleukin 1-α and VEGF support the growth and persistence of biofilm-growing *Cutibacterium acnes* in individuals with acne - *Cavallo I*  
55: Relation between antibiotic susceptibility and biofilm formation capacity in strains isolated from infected orthopaedic devices - *Coraça-Huber D.C*  
56: Eradication of wound-relevant pre-formed biofilms following release of combination antibiotics from absorbable beads *in-vitro* - *Delury C*  
57. Micro/Nanostructured PBSA Membranes With Antibiofilm Properties As Chronic Wound Dressings - *Naila Bou Haidar*  
58: Cadexomer iodine delivers rapid & sustained broad spectrum antimicrobial activity and substantial biofilm disruption and kill in a clinically relevant wound model - *Forrest E.C*  
59: A biomimetic model of the chronic wound infection microenvironment - *Kadam S*  
60: A biomimetic, simulant wound fluid to investigate chronic wound biofilm pathogenesis and response to therapy - *Kaushik K.S*
61: Impact of the bone microenvironment on *Staphylococcus aureus* adhesion (ORAL) - Lamret F

62: A novel flow system to model chronic wound biofilms and test antimicrobial dressings - Maddocks S

63: Development of ‘smart’ wound dressings for biofilm sensing and control - Magee E

64: *Propionibacterium acnes* biofilm forming capacity: are phylotypes involved? - Pécastaings S

65: A fluorescent artificial wound eschar model for biofilm and debridement study - Percival S.L

66: In a laboratory model of diabetic foot infection, vancomycin and gentamicin loaded calcium sulfate beads were more effective than systemically achievable concentrations of antibiotics in reducing polymicrobial biofilms grown from clinical isolates - Price B

67: Application of furanone compounds for the modulation of biofilm formation in common wound pathogens - Proctor C

68: Internalization of *Cutibacterium acnes* in bone cells and its consequence on bacterial virulent behaviour - Reffuveille F

69: A surprising role of bacterial odor in human skin health - Sapir Ron-Doitch

70: Interactions between *Propionibacterium acnes* biofilm and different human cell types are strain dependent - Spittaels K-J

71: Risk factors for chronic biofilm related infection - Stewart P.S

72: One size does not fit all; the gap between standardized *in vitro* biofilm-infected wound models and *in vivo* clinical settings - Thaarup I.C

73: Phenotypic profile of biofilm and extended spectrum beta lactamases bacteria from patients with diabetic foot ulcers in Zaria-Nigeria - Usman Y
Multispecies & Interactions

74: Development and validation of an in vitro endodontic mixed biofilms model - Abusrewil S

75: Deciphering the matrix code: effect of interspecies interactions in multispecies-biofilm matrix (ORAL) - Amador C

76. Host-fungal interactions in the aetiopathogenesis of Orofacial granulomatosis - Anderson O.F

77: The effect of cannabigerol on quorum sensing and biofilm formation of Vibrio harveyi - Aqawi M

78: Enterococcus faecalis inhibits Klebsiella pneumoniae growth in polymicrobial biofilms - Ballén V

79: Investigating the relationship between bacterial vesicles and biofilm formation in E. coli - Brotherton, D. K

80: Development of a multi-species marine-based biofilm model for testing novel anti-microbial agents - Butcher M.C

82: Pre-clinical and clinical anti-biofilm efficacy of nitradian: a novel non-antibiotic brushing solution (periotabs) for teeth and gums to help reduce perio-diseases and dental-implant infections - De Wever B

83: Cold atmospheric pressure plasma significantly reduces polymicrobial cariogenic biofilm - Figueira L.W

84: Listeria monocytogenes and Salmonella Typhimurium dual-species biofilm: development and inactivation with Cold Atmospheric Plasma - Govaert M

85: Exploring antibiotic persistence and polymicrobial communities of biofilms from an ex-vivo perspective - Hassan M.M

86: Microbial communities of central venous catheters - Hola V

87: Investigating the microbiological response to periodontal therapy - Johnston W

88: Antibiotic susceptibility patterns of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis strains isolated from subgingival biofilm samples in Switzerland in the last 37 years - Karygianni L

89: The use of raman technologies for oral bacteria - Kriem L

90: A potential role for Fusobacterium nucleatum c-di-nucleotides production in (multispecies) biofilm formation - Kuehne S.A
91: The role of quorum sensing in the development of *Microcystis aeruginosa* blooms: gene expression - *Lamas-Samanamud G*

92: Polymicrobial denture-associated biofilms: in vitro interactions and infection modelling - *Morse D.J*

93: PRESENCE OF AHL-TYPE QUORUM SENSING MOLECULES IN ORAL SAMPLES - *Andrea Muras*

94: Quorum sensing-inducing subpopulation specific eDNA production in *Streptococcus mutans* - *Nagasawa R*

95: Microfluidic experiments of bacterial biofilms associated with biliary stents: the role of fluid flow on biofilm formation by clinical isolates - *Cardenas C*

96: The impact of smoking on subgingival biofilms and host antimicrobial peptides in patients with chronic periodontitis - *Soldati K.R*

97: Prebiotic potential of native plaque DNase enzymes to control oral biofilms - *Rostami N*

98: Assessing cleaning and bacterial contamination on endoscopy unit surfaces - *de Sousa Santos L.C*

99: Polymicrobial distribution of bacteria controlled using core-shell microcapsules - *Takahashi K*

100: Cross-domain signaling: bacterial response to archaeal quorum sensing - *Thompson T.P*

101: A novel rapid prototyping tool for suspended biofilm growth media - *Tsagkari E*

102: Developing commercially relevant complex biofilm models: limitations for standardisation - *Young T*
Novel Agents

103: Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis - Artini M

104: ANTIBIOFILMOGRAM®: Improve antibiotic therapy with a complementary method - Badel-Berchoux S

105: Antimicrobial activity of biocide-releasing PDMS substrates - Barbieri L

106: New antimicrobial peptide disrupts mono- and dual-species biofilms of Pseudomonas aeruginosa and Staphylococcus aureus - Bessa L.J

107: Biofilm-related antimicrobial cross-resistance: lesson learned from an old hydroxyquinolines - Bidossi A

108: Novel oleanolic and maslinic acids derivatives as a promising treatment against bacterial biofilm in nosocomial infections: an in vitro and in vivo study - Blanco-Cabra N

109: Evaluation of novel XF-Drugs - Board-Davies E.L

110: A strong inhibitory effect of heather honey, propolis and medicinal plant extracts on biofilm formation by pathogenic bacteria - Billah Z

111: Antimicrobial sensitization through quorum quenching - Falà A.K

112: In-vitro effect of antibiotic loaded calcium sulfate beads on bacterial growth from infected diabetic foot ulcer tissue - Julie Fletcher

113: Bioactivity and phenolic characterization of different medicinal and aromatic plants - Gomes F

114: The potential use of probiotics to control biofilm formation in urinary catheters - Gomes L.C

115: Biofilm inhibiting activity of selenium compounds - Kincses A

116: Traditional essential oils as new potential antimicrobials and biofilm formation inhibitors - Lazar V

117: Elasnin, a bacteriostatic agent that has potent antibiofilm activities against Gram-positive bacteria - Long L

118: Gelatin microparticles as carriers for the delivery of antimicrobial peptides - Mann K
119: Synthesis and evaluation of novel quaternary ammonium salts and development of bacterial biofilms by MBEC assay for future examination of these compounds - Markova A.  
120: Investigating the synergy between antimicrobials and cold atmospheric plasma - Clarke J-A.  
121: The effect of disinfectants on quinolone resistant E.coli (QREC) in broiler production - Osland A.M.  
122: The effect of antimicrobials on Pseudomonas aeruginosa PAO1 biofilm formation - Ocampo C.  
123: A new weapon against biofilm: a lipopeptide from Antarctica (ORAL) - Parrilli E.  
124: Efficacy of Tetrasodium-EDTA alone and in a complex with metal ions against mono and mixed species biofilms - Percival S.L.  
125: The in vitro cytotoxicity study of novel T-EDTA complexes - Percival S.L.  
126: Generation and screening of polyphenol rhamnosides with antibiofilm properties - Peuker T.  
127: Deciphering the novel cellulose degradation mechanism of the ruminal bacterium Fibrobacter succinogenes S85 - Raut M.P.  
128: Beneficial biofilms as smart bioactive interfaces to maintain a balanced oral microbial community - Ren Q.  
129: Bacteriophages for eradication of clinically relevant biofilms - Rice C.  
130: Improving the plasma activated liquids efficacy for the inactivation of L. monocytogenes and S. Typhimurium biofilms - SMET C.  
131: Novel polycationic photosensitizers for antibacterial photodynamic therapy - Tiganova I.G.  
132: Bacteriophage: potentials for enhancing anti-biofilm therapy (ORAL) - Townsend E.  
133: The effect of fosmidomycin prodrugs against Acinetobacter baumannii biofilms - van Charante F.  
134: Phage-encoded miniDNases as a novel source of enzyme-based antibiofilm strategies - Marie Van der Gucht.  
135: Nanocarriers with conjugated antimicrobials to eradicate pathogenic biofilms evaluated in vitro and in vivo - van der Mei H.C.
136: Metal formulations have the potential for use as antimicrobials in controlling healthcare associated infections - Whitehead K.A

137: Charge-reversible carbon dots for biofilm treatment - Wu Y

**Staphylococcus**

138: The human skin bacteria Staphylococcus epidermidis fermentation end product ameliorates UVB-induced ROS generation through production of free electron transfer - Balasubramaniam A

139: Furvina synthetic derivatives inhibit quorum sensing and biofilms of *Staphylococcus aureus* - Borges A

140: *Cutibacterium acnes* clinical isolates are able to modify the attachment, formation and structure of *Staphylococcus aureus* biofilms - Brown H.L

141: PepR, a viral-derived peptide, is efficacious against *Staphylococcus aureus* biofilms - Sandra N. Pinto

142: Facing the in vitro challenge in *Pseudomonas aeruginosa* and Staphylococcus aureus coexistence - del Mar Cendra M

143: Modelling *Staphylococcus aureus* biofilm on infected chronic wounds - Yanyan Cheng

144: Does mazEF have a role in *S. epidermidis*’ biofilm dormancy? - Gaio V

145: Virulence factor expression dominates the *S. aureus* transcriptomic signature of human infection (ORAL) - Ibberson C.B

146: Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in biofilm-related infections: insights through network reconstruction and creation of a new online database - Jorge P

147: Dabigatran has anti-biofilm properties and enhance antibiotic efficacy of experimental *Staphylococcus aureus* endocarditis (ORAL) - Lerche C.J

148: Demonstrating the efficacy of cold atmospheric gas plasma against biofilm of *Staphylococcus aureus* (ATCC 6358) - Onuoha O

149: A systematic comparison of factors affecting the antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* - Regan H.C

150: New anti-biofilm PDMS-based coating reducing biofilm formation of *Staphylococcus epidermidis* - Ricciardelli A
151: Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis - Marco Artini

152: Repurposing metal chelators to combat staphylococci biofilms - Richter K

153: Decontamination effect of the novel water vapor plasma generator prototype on staphylococcal biofilm - Růžička F

154: Antibacterial efficacy of cold atmospheric plasma on methicillin-resistant Staphylococcus aureus biofilm - Salgado B

155: Characterization of Staphylococcus aureus biofilms formed on modified paper-based arrays: understanding biofilm dynamics and impact of surface properties - San-Martin-Galindo P

156: Nanostructured surfaces prevent the formation of Pseudomonas aeruginosa and Staphylococcus aureus biofilms - Tolordava E

Candida

157: Candida albicans biofilm heterogeneity modifies persistence following sodium hypochlorite and EDTA treatment - Alshanta O-A

158: Saving the prey: Pseudomonas aeruginosa quorum sensing augments Candida albicans antifungal resistance - Bandara H.M.H.N

159: Evaluation of the antifungal activity of chitosan against Candida auris using an in vivo infection model - Brown J

160: Employment of polyclonal antibody anti-CR3-RP Ab in the treatment of biofilm of Candida albicans and Candida auris resistant to common antifungals - Dekkerová J

161: Candida albicans enhances initial biofilm growth of Cutibacterium acnes under aerobic condition - Imbert C

162: Spirulina sustainable lipid extracts and their vectorization to combat C. albicans biofilms - Imbert C

163: Transcriptional profiling of biofilm formation by the emerging fungal pathogen Candida auris - Kean R

165: Identification of *Candida* biofilm-related genes and expression of inflammatory biomarkers in RVVC - **Mckloud E** 182
166: Impairment of *Candida albicans* adhesion on dielectric surfaces (SiO$_2$) containing or not AgNPs by absorbed proteins (ORAL) - **Roques C** 183
167: In vitro polymicrobial bacteria-fungal biofilm model in the context of prosthetic joint infections - **Ruiz-Sorribas A** 184
168: *Candida auris* exhibits resilient biofilm characteristics *in vitro*: implications for environmental persistence - **Bryn Short** 185
169: Preliminary study into the effects of tobacco smoke on *Candida albicans* - **Williams M** 186
170: Control of *Candida auris* infections by using selected hospital surface disinfectant und new biocides - **Zatorska B** 187

**Surfaces & Methods** 188
171: Nucleating biofilms using polymers for use in biotechnology - **Adoni P** 188
172: Biocidal performance of a metal oxide coating on surface treated polyethylene - **Alemi F** 189
173: Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates - **Allkja J** 190
174: Modulating an antimicrobial release approach by dopamine chemistry to fight infections associated to orthopedic implants - **Alves D** 191
175: Flow dynamics and material surface properties influence ureolytic biofilm development and encrustation - **Blood N** 192
176: Detection of bacterial porphyrin fluorescence from *in vitro* biofilm models (ORAL) - **Smith A. C** 193
177: Efficiency of copper alloys touch surfaces against biofilm formation - **Colin M** 194
178: Optimising phosphate treatment in UK drinking water systems to prevent plumbosolvency: evaluation of its impact on biofilm development - **del Olmo G** 195
179: Discovery of a polymer resistant to biofilm, swarming and biomineralization for the prevention of catheter-associated urinary tract infections - **Dubern J-F** 196
<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>Author(s)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>Novel antimicrobial silicones for prevention of catheter-associated urinary tract infections (CAUTIs)</td>
<td>Duggan K</td>
<td>197</td>
</tr>
<tr>
<td>181</td>
<td>Multi-mode microscopy to elucidate early stages in biofilm formation</td>
<td>Farthing N.E</td>
<td>198</td>
</tr>
<tr>
<td>182</td>
<td>Characterization of microcosm biofilm regrowth on titanium surfaces after various decontamination treatments</td>
<td>Jiang Y</td>
<td>199</td>
</tr>
<tr>
<td>183</td>
<td>Bioactive glass granules inhibit mature bacterial biofilms on the surfaces of cochlear implants</td>
<td>Kirchhoff L</td>
<td>200</td>
</tr>
<tr>
<td>184</td>
<td>Scalable cell factories in membrane-based bioreactors</td>
<td>Leonov P</td>
<td>201</td>
</tr>
<tr>
<td>185</td>
<td>Biofilm inhibition of nitric oxide releasing titanium surfaces</td>
<td>Li M</td>
<td>202</td>
</tr>
<tr>
<td>186</td>
<td>Determinants of metabolic activity and biofilm aggregate sizes and distribution in a new <em>in vivo</em>-like biofilm model</td>
<td>Lichtenberg M</td>
<td>203</td>
</tr>
<tr>
<td>187</td>
<td>Characterization of microbial diversity in Czech mineral springs</td>
<td>Maťátková O</td>
<td>204</td>
</tr>
<tr>
<td>188</td>
<td>Assessing the Impact of chemically engineered surface modifications with respect to attachment, survival and the development of microbes at the cellular level</td>
<td>Mulhall R</td>
<td>205</td>
</tr>
<tr>
<td>189</td>
<td>Using <em>in vitro</em> models of the farm environment to assess the biofilm-forming abilities of pig and poultry production associated <em>Salmonella</em> enterica serovars</td>
<td>Oastler C</td>
<td>206</td>
</tr>
<tr>
<td>190</td>
<td>Structure and metabolism of engineered enhanced current-producing biofilms</td>
<td>Otero F.J</td>
<td>207</td>
</tr>
<tr>
<td>191</td>
<td>Micro-scale topographies instruct bacterial attachment to surfaces</td>
<td>Romero M</td>
<td>208</td>
</tr>
<tr>
<td>192</td>
<td>Influence of phosphate dosing to prevent plumbosolvency on biofilm formation and risk of mobilisation in an experimental chlorinated drinking water distribution systems</td>
<td>Rosales E</td>
<td>209</td>
</tr>
<tr>
<td>193</td>
<td>Propidium iodide staining underestimates viability of adherent bacterial cells</td>
<td>Rosenberg M</td>
<td>210</td>
</tr>
<tr>
<td>194</td>
<td>Can anammox surface (S-) layer protein nucleate biofilm formation?</td>
<td>Seviour T</td>
<td>211</td>
</tr>
<tr>
<td>195</td>
<td>Bacterial adhesion and early biofilm formation on soft surfaces: towards a better understanding of bacteria mechanosensing abilities and physico-chemical interactions at play</td>
<td>Straub H</td>
<td>212</td>
</tr>
<tr>
<td>196</td>
<td>Bacterial film formation at oil-water interfaces</td>
<td>Subbiahdoss G</td>
<td>213</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------</td>
<td>--------------------------</td>
<td></td>
</tr>
<tr>
<td>197</td>
<td>Construction of the analysis method for monitoring the physiological</td>
<td>Takabe K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>properties of individual cells in biofilm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>198</td>
<td>Study the adhesion and biofilm formation of diatoms on hydrophilic</td>
<td>Florian T</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and hydrophobic surfaces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>199</td>
<td>To form a biofilm or not- that is the question for <em>Salmonella</em> under</td>
<td>Lene K. Vestby</td>
<td></td>
</tr>
<tr>
<td></td>
<td>exposure to furanon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>Assessing the role of pharyngeal cell surface glycans in Group A</td>
<td>Vyas H.K.N</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus</em> biofilm formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>Biofilm hotspots in the food processing environment (ORAL)</td>
<td>Wagner E.M</td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>Covalent lectin inhibition and application in bacterial biofilm imaging</td>
<td>Wagner S</td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>Validation of the EPA approved single tube method using a wide range</td>
<td>Westgate S</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of biocides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>204</td>
<td>Bacteria on technical surfaces under the influence of shear forces</td>
<td>Wieland B</td>
<td></td>
</tr>
<tr>
<td>205</td>
<td>Effect of Faraday waves on the bacterial attachment and biofilm</td>
<td>Xia H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>formation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>206</td>
<td>Active layer fluctuations drive a critical pinning transition in biofilm</td>
<td>Young E</td>
<td></td>
</tr>
<tr>
<td></td>
<td>surface roughness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Wednesday 4th September

Mechanisms & Resistance

1: A glimpse into the survival of non-biofilm formers in a nutrient and moisture deficient environment - Amaeze N. J

Amaeze N. J¹, Akinbobola A¹, Ramage G², Williams C¹ and Mackay W²
¹University of West of Scotland & ²University of Glasgow

Introduction: The persistence of microorganisms in the healthcare environment and their survival of stress is often associated with the ability of microorganisms to adhere to surfaces and form biofilms. Bacterial adherence is the first step in the formation of biofilms without which there may be no biofilm.

Aim: In this study, the survival of non-biofilm formers in a semidry and nutrient deficient environment was investigated using an adherence mutant and a wild type strain.

Methodology: SrtA mutant and Staphylococcus aureus NCTC8178 at inoculum size of 1x 10⁸/ml were grown in 5% tryptic soy broth at 35° C following a serial sequence of hydration and long dehydration periods for 12days in 24 well microtiter plates. The survival of the mutant and the wild type were determined using cell recovery by colony forming unit on nutrient agar plates and cellular matrix composition by confocal laser scanning microscopy (CLSM) using fluorescent dyes.

Results: Cell recovery at 12days for srtA mutant and S. aureus NCTC8178 were log₁₀ 8.19±0.18 and 8.25±0.18 respectively. CLSM showed a matrix of cells dominated by intact green fluorescing cells of S. aureus NCTC8178 and a matrix of compromised red fluorescing cells of srtA mutant. S. aureus NCTC8178 cells were organised in microcolonies rich in protein. SrtA mutant cells had a few aggregates of cells surrounded by zones of green extracellular DNA (eDNA).

Conclusion: Cell recovery by CFU count showed that there was no significant influence of adhesion proteins on the attachment of biofilm and non-biofilm formers to surfaces. Physiologically srtA mutants were impacted heavily by dehydration and poor nutrition stress in comparison with the wild type strain. The overproduction of eDNA by srtA mutant may have helped in its attachment. Understanding microbial persistence in the environments will serve as a milestone for the prevention of healthcare associated infections.
2: Exposure to antibiotics Increases diversity and gene transfer in *Salmonella typhimurium* biofilms - **Bartke K**

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**Introduction:** In nature, bacteria mostly live in sessile communities called biofilms, characterized by a complex extracellular matrix consisting of lipids, proteins, and extracellular DNA. Bacteria in biofilms are more refractory to antibiotics than bacteria in planktonic cultures. Biofilms could favour the spread of mobile genetic elements and are therefore interesting as potential hotspots for horizontal genetic transfer (HGT) of antibiotic resistance genes.

**Hypothesis and aims:** To investigate the effect of exposure to antibiotics on biofilm population structure, and HGT within a biofilm. We hypothesised that antibiotic exposure would damage a mature biofilm, making it susceptible to invasion by planktonic bacteria and increasing the spread of resistance genes.

**Methodology:** *S. typhimurium* biofilm was allowed to mature on a peg system by undisturbed growth at 26°C for 72h. Biofilm was then incubated with a second *S. typhimurium* strain carrying a conjugative plasmid for a further 72h. During this second exposure, antibiotic from sub-MIC to above MIC, were present. Bacteria were removed from pegs and plated on selective media to quantify strains and transconjugants.

**Results:** We found that exposure of a mature biofilm to antibiotic influenced the ability of planktonic bacteria to invade the biofilm, and transfer a conjugative plasmid within the biofilm. These effects on biofilm population structure and HGT occurred even at sub-MIC antibiotic concentrations.

**Conclusion:** Infections involving bacterial biofilms are often chronic or associated with treatment failure, requiring multiple courses of antibiotic treatment. Here, we show that treatment of biofilms with antibiotics can lead to an increase in genetic diversity within the biofilm and increase the spread of antibiotic resistance genes.
3: Microbial biofilm correlates with an increased antibiotic tolerance and poor therapeutic outcome in infective endocarditis - Cavallo I


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Introduction: Infective endocarditis (IE) is associated with high rates of mortality. Prolonged treatments with high-dose intravenous antibiotics often fail to eradicate the infection, frequently leading to high-risk surgical intervention. By providing a mechanism of antibiotic tolerance, which escapes conventional antibiotic susceptibility profiling, microbial biofilm represents a key diagnostic and therapeutic challenge for clinicians.

Hypothesis and aims: This study aims at assessing a rapid biofilm identification assay and a targeted antimicrobial susceptibility profile of biofilm-growing bacteria in patients with IE, which were unresponsive to antibiotic therapy.

Methodology: Patients with surgically-treated IE were enrolled in the study. The level of biofilm production was assessed by the clinical Biofilm Ring Test® (cBRT) and confocal microscopy. Microbial susceptibility profiles to conventional drugs was determined by the microdilution broth method for both planktonic and biofilm cells.

Results: Staphylococcus aureus was the most common isolate (50%), followed by Enterococcus faecalis (25%) and Streptococcus gallolyticus (25%). All microbial isolates were found capable to readily adhere and produce large and structured biofilms. As expected, antibiotic treatment either administered on the basis of antibiogram or chosen empirically among those considered first line antibiotics for IE, including ceftriaxone, daptomycin, tigecycline and vancomycin, were not effective at eradicating biofilm-growing bacteria. Conversely, antimicrobial susceptibility profile of biofilm-growing bacteria indicated that teicoplanin, oxacillin and fusidic acid were most effective against S. aureus biofilm, while ampicillin was the most active against S. gallolyticus and E. faecalis biofilm, respectively.

Conclusion: Biofilm-producing bacteria, from surgically treated IE, display a high tolerance to antibiotics, which is undetected by conventional antibiograms. The rapid identification and antimicrobial tolerance profiling of biofilm-growing bacteria in IE can provide key information for both antimicrobial therapy and prevention strategies.
**4: Clostridum perfringens biofilm: characterization and antibiotic tolerance - Dixon R.A**

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**Introduction:** *Clostridium perfringens* is a Gram-positive, anaerobic bacterium associated with the etiology of human and animal diseases including necrotizing enterocolitis (NEC) in very premature infants and necrotic enteritis (NE) in poultry worldwide which is responsible for enormous economic losses. Although pathogenic bacteria form biofilms in order to survive and resist antimicrobial treatment by attaching to each other and to a substrate by an extracellular polymeric substance (EPS), their presence with anaerobic organisms is under-researched.

**Hypothesis and aims:** The aim of this study was to evaluate the biofilm-forming potential of *C. perfringens* isolated from broiler chicken, free-range poultry environments and preterm human neonates.

**Methodology:** The susceptibility of *C. perfringens* either grown as biofilms was assessed using traditional crystal violet staining assays in microtiter plates or planktonically using the broth microdilution method to conventional antibiotics respectively.

**Results:** All fifty four (54) *C. perfringens* isolates tested in this study were shown to form biofilms. 7 (13%) were strong biofilm producers, 31 (57%) were moderate biofilm producers and 16 (30%) were weak biofilm producers according to well established criteria. There was no significant difference in the strength of biofilms formed from clinical (NEC in neonates or NE in chicken) or commensal isolates from poultry environment and healthy chicken. As anticipated, the formation of biofilms protected *C. perfringens* from the action of antibiotics since they showed less susceptibility to antibiotics than when growing as single cells. The percentage resistance of tested isolates to antibiotics as either single cells or biofilms was 53% and 82% for penicillin, 53% and 100% for bacitracin, 88% and 100% for gentamicin and 76% and 91% for tetracycline respectively.

**Conclusion:** This study showed that *C. perfringens* growing in biofilms significantly reduces effective antibiotic susceptibility. To prevent and control biofilm-related contamination and infections, there is an urgent need for alternative therapeutic strategies.
Development of a robust biofilm assay of *S. pneumoniae* to study adaptive evolution and the emergence of antibiotic resistance - Espinoza S

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**Introduction:** Bacteria grow in at least two different states: 1) planktonically as free-swimming single cells; and 2) as biofilms in complex organized bacterial communities embedded in an extracellular matrix of polymeric substances. Bacterial biofilms are known to diminish the effectiveness of antibiotics, posing a challenge for successful clearance in a clinical setting.

**Hypothesis and aims:** bacterial adaptive evolution has been shown to differ between bacteria growing planktonically or in a biofilm. 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, maintenance and recalcitrance have been studied in many species but little is known for the opportunistic pathogen *Streptococcus pneumoniae*.

**Methodology:** Here we develop a method to create a reliable, consistent and long-term biofilm assay, which allows for examining *S. pneumoniae*’s biofilm population dynamics. 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). This allows for adaptive evolution experiments to be carried out for the first time with *S. pneumoniae* in biofilms, enabling the monitoring of the emergence of antibiotic resistance.

**Results:** Using our biofilm assay, we have successfully adapted multiple parallel *S. pneumoniae* populations to three different antibiotics over a period of 40 days. Our results show *S. pneumoniae* biofilms are using a different strategy of adaptation than planktonic growth.

**Conclusion:** We aim to uncover how *S. pneumoniae* undergoes adaptive diversification in biofilms in contrast to planktonic growth. As such, we intend to establish a more realistic model to study bacterial adaptation towards antibiotic resistance during an infection.
6: Combined effects of low incubation temperature, minimal growth medium and low hydrodynamics optimize Acinetobacter baumannii biofilm formation - Eze E.C

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Introduction: Biofilm formation is an important virulence factor expressed by microorganisms. It shields and protects microbial cells from host immune responses, antibiotics and other anti-infectives.

Hypothesis and aims: It is hypothesized that microbial cells demonstrate enhanced biofilm formation in nutrient limiting environment. The aim of this study was to investigate if limiting environmental conditions act synergistically to promote biofilm formation in multidrug resistance Acinetobacter baumannii.

Methodology: Biofilm was cultivated using quantitative microtiter plate method. The combined effects of temperature, medium and shear force were determined by measuring adherence (OD$_{570}$ nm) following incubation at 26 °C, 30 °C and 37 °C for 24 hr. when biofilm was cultivated with minimal nutrient medium (EAOB) and nutrient-rich medium (TSB) without or with agitation at 50 rpm. Antibiotic susceptibility test of selected antimicrobials were tested with Kirby-Bauer disc method. P < 0.05 was considered statistically significant for all the tests.

Results: A noticeable variation in adherence was observed among the isolates cultured with both media. Biofilm forming capacity of the isolates range from 0.09 to 0.33. Majority of the isolates had their relative biofilm-forming capacity significantly higher than the positive control, Acinetobacter baumannii ATCC 19606. The biofilm biomass during growth in nutrient-rich medium (TSB) without shaking was significantly different ($p < 0.05$) among the three temperatures tested compared with when cultured in EAOB without shaking. A positive correlation was observed between biofilm formation and resistance to imipenem ($r = 0.2889; \ p = 0.05$). There is a statistically significant difference among the median of the three source groups ($p < 0.05$) compared with the median between the source groups.

Conclusion: This observation extended further the view that A. baumannii biofilm formation is enhanced when nutrient-poor medium was used at room temperature (26 °C) with or without agitation compared to growth at 37 °C.
7: Assessment of the antimicrobial tolerance of archaeal biofilms - Fakhoury A

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Introduction: Archaea, first proposed as a fundamentally distinct domain of life by Woese & Fox (1977), are unicellular prokaryotes which share many common features with bacteria, however, genetic and metabolic analysis has revealed that archaea are in fact more closely related to eukaryotes. Most archaea colonise extreme environments however, archaea are known to be metabolically diverse, coexist and interact with bacteria and eukaryotes in complex environments. Recently, our group have isolated extremely halophilic archaea from a thalassohaline Triassic halite deposit, Kilroot salt mine, in Northern Ireland. We have demonstrated widespread quorum sensing molecule production, biofilm formation and antimicrobial production among extremely halophilic archaea. In addition, we observed surprisingly high levels of inherent antimicrobial resistance among these halophilic archaea.

Hypothesis and aims: These findings indicate that archaea may resemble bacteria in respect of antimicrobial tolerance and resistance development. This work aims to understand whether archaeal biofilms exhibit elevated tolerance to antimicrobial agents (antibiotics and biocides) compared to their planktonic counterparts?

Methodology: Initially, archaea were screened for antimicrobial sensitivity using a Kirby-Bauer disk method and their ability to form biofilms assessed in a range of growth media. Subsequently, MIC and Minimum Biofilm Eradication Concentrations (MBEC) for Haloferax volcanii DS2 and a range of halophilic archaeal isolates from Kilroot salt mine were collected. Biofilm formation was confirmed by both SEM and confocal microscopy.

Results: Archaeal isolates exhibited variable sensitivity to a wide range of antibiotic and biocidal compounds. Analysis of whole genome sequence data for these archaeal isolates also reveals the presence of putative antimicrobial resistance genes. We observe significantly elevated tolerance to a range of antimicrobial compounds for example H. volcanii DS2, when grown as biofilms exhibited biofilm tolerance to rifampicin > 8 fold higher than MIC and to both benzalkonium chloride and 1-dodecyl-3-methylimidazolium chloride was 128 fold higher than MIC. Tolerance to cetylpyridinium chloride was greater than 500 fold MIC.

Conclusion: Archaeal biofilms exhibit significantly elevated tolerance to a range of antimicrobial compounds. Ongoing studies are examining the effect of longer-term antimicrobial exposure of archaea in both planktonic and biofilm modes of growth, on tolerance and resistance development.


**8: Bacterial suspended aggregates in high viscosity - Irie Y**

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**Introduction:** Cystic fibrosis patients produce large amounts of sputum in their respiratory tract that obstruct their airways. Bacterial pathogens colonise these thick sputa, and cause chronic respiratory infections. Upon colonisation, the bacteria form suspended aggregates. Long considered to be a type of biofilm growth, while these aggregates have shared features with surface-associated biofilms such as elevated tolerance against antibiotics, attachment factors such as extracellular biofilm polysaccharides are not required to develop aggregates and therefore may represent a distinct and uncharacterised growth format of bacteria.

**Hypothesis and aims:** Bacterial suspended aggregates reduce interspecies competition and promote multispecies microbiota in viscous environments.

**Methodology:** Inoculation of bacteria in low density into high viscosity media (0.8% w/v agar) leads to formation of suspended bacterial aggregates. Co-inoculation of more than one strain or species in 1:1 ratio will result in mixed aggregates. We evaluate the resulting ratio and compare against low viscosity conditions where the bacteria do not aggregate.

**Results:** Based on microscopy results, individual aggregates appear to be outgrowths derived by clonal expansion from a single bacterium.

**Conclusion:** The bacteria in the aggregates formulate spatially segregated micro-communities. This prevents competition between one bacterial aggregate to another and may contribute to multi-species co-existence in viscous environments such as on mucosal surfaces that otherwise may involve outcompeting species in spatially mixed environments.
Mycobacterium abscessus complex can be sensitized to antibiotics by breaking up bacterial aggregates and increasing oxygen availability - Kolpen M

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Introduction: Pulmonary infection with the multidrug-resistant Mycobacterium abscessus complex (MABSC) is difficult to treat in individuals with cystic fibrosis (CF). MABSC grows as biofilm aggregates in the CF lung, which is known to have anaerobic niches. How aggregation and anoxic conditions affect antibiotic tolerance is not well understood.

Hypothesis and aims: We sought to determine whether disaggregation and oxygen availability sensitize MABSC isolates to recommended antibiotics.

Methodology: We tested susceptibility of the following antibiotics amikacin, azithromycin, cefoxitin, ciprofloxacin, clarithromycin, imipenem, kanamycin, linezolid, moxifloxacin, rifampicin, tigecycline and sulfamethizole + trimethoprim for 33 isolates from 22 CF patients with MABSC infection and a reference strain. Isolates were grown in Müeller-Hinton broth with and without the disaggregating detergent Tween®80 (5%). Time-kill curves at day 1 and 3 were generated for oxic and anoxic amikacin treatment in four-fold dilutions from 2 to 512 mg L⁻¹. In addition, confocal laser scanning microscopy and micro-respirometry were used to visualize biofilm growth patterns.

Results: Disruption of MABSC aggregates increased susceptibility to amikacin, tigecycline, kanamycin, azithromycin, imipenem, cefoxitin and clarithromycin (P<0.05, n=29-31). In addition, oxygenation enhanced bacterial killing by amikacin in disaggregated MABSC isolates (P<0.05) by 1-6 log using 2-512 mg L⁻¹ of amikacin.

Conclusion: This study helps explain why current drug susceptibility testing correlates poorly to treatment outcomes. Aerobic culturing of planktonic isolates in vitro does not resemble the hypoxic conditions in CF lungs. Biofilm disruption and increasing O₂ availability during antibiotic therapy may be new therapeutic strategies for chronic MABSC infection.
10: Acquisition of plasmids containing a cephalosporin resistance gene reduces biofilm formation - Nesse L.L

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Introduction: Cephalosporin resistant E. coli (CRE) and quinolone resistant E. coli (QREC) has been isolated from the broiler production chain through NORM-VET monitoring programme for antimicrobial resistance. The CR-gene, bla<sub>CMY-2</sub>, in these strains is plasmid bound, whereas QR were mostly due to mutations in chromosomal genes. Little has been known on the biofilm forming abilities of the CRE and QREC strains, and the effect of the CRE resistance plasmids on biofilm formation.

Hypothesis and aims: The aim was to compare the biofilm forming abilities of CRE and QREC, and study the effect of the bla<sub>CMY-2</sub> containing plasmid on biofilm formation by QREC strains.

Methodology: Biofilm formation of 91 CRE and 110 QREC was studied using the Crystal violet microtiter plate assay at 37 °C. Two plasmids, one IncK and one IncI, containing bla<sub>CMY-2</sub> were separately conjugated into nine QREC strains, resulting in 13 transconjugants. Growth was measured in microtiter plates, swimming rate on motility agar, and biofilm size on CongoRed agar, all assays at 37 °C.

Results: Mean biofilm production in microtiter plates by the QREC strains were twice that of the CRE strains (mean OD<sub>595</sub> 1.505 ± 0.964 vs 0.711± 0.706, respectively). Mean biofilm production by QREC transconjugants was 71.3 % (CI 57.5, 85.0) of their respective wild types. Mean swimming rate was also reduced (82.2 %, CI 72.6, 91.8), whereas mean growth rate and mean biofilm size on CongoRed agar was at the same level as the wild type.

Conclusion: QREC were in general better biofilm formers than CRE. Conjugation with plasmids containing bla<sub>CMY-2</sub> reduced biofilm formation in QREC in microtiter plates, but not on CongoRed plates. Furthermore, conjugation reduced swimming rate, but not growth rate. The results may indicate that the plasmids reduced biofilm formation through interfering with adhesion, rather than affecting the general vitality of the transconjugants.
11: Dynamics in biofilms with *E. coli* strains producing different matrix components - *Nesse L. L*

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**Introduction:** On CongoRed agar, *E. coli* display different biofilm morphotypes indicating the composition of the matrix. The morphotype RDAR indicates both cellulose and curli fimbriae in the matrix, BDAR only curli, and PDAR only cellulose. RDAR is believed to be most beneficial for biofilm persistence.

**Hypothesis and aims:** The aim was to study the dynamics of BDAR-PDAR dual biofilms, and see whether they together would produce biofilm with RDAR morphology.

**Methodology:** Two BDAR and two PDAR *E. coli* strains were used to make single and dual biofilms. For dual biofilms, the inoculum ratios were 30:70, 50:50 and 70:30. Biofilms were formed on glass slides in LB w/o NaCl, 20 °C, 2 days, and cfu in the biofilm and in the broth were enumerated. Using the same inoculates, biofilms were also produced on CongoRed agar plates incubated in 20 °C and visually inspected for 10 days.

**Results:** On glass slides, PDAR strains alone were much better biofilm formers than BDAR strains alone, and PDAR also dominated in the dual biofilms. This was in contrast to the planktonic phase where BDAR strains outnumbered PDAR, even though both displayed similar growth rates when grown alone. On CongoRed plates, all pairs produced biofilm with RDAR morphology the first three days. Thereafter they separated into producing biofilm in a spatial manner, where the ratio of the strains reflected the growth ratio observed in the planktonic phase of the glass slide assay.

**Conclusion:** When attachment was needed, the ratio in dual biofilms was depended on the biofilm forming abilities of each of the strains. When attachment was not needed, the ratio was dependent on the relative planktonic growth of the strains. Grown together, *E. coli* strains each lacking one of the matrix components produced a biofilm matrix with both components. However, after few days they started growing separate biofilms.
Introduction: Growing antimicrobial resistance (AMR) is an increasing cause of morbidity and mortality, and a significant global threat. Therefore it is crucial to study the mechanisms of antibiotic resistance as well as its emergence and dissemination. One way to analyze the acquisition of de novo mutations conferring antibiotic resistance is adaptive laboratory evolution. Prior studies have mainly been performed on planktonic cultures. However, increased mutation rates in the biofilm environment have been reported. Consequently, biofilms may be hot spots for development of antibiotic resistant genotypes.

Hypothesis and aims: We hypothesize that the biofilm environment might be supportive of resistance development, and provides a specific niche for mutational events.

Methodology: In this study we use biofilm as hot spot of antibiotic resistance and for studying evolutionary paths to antibiotic resistance. Four different *Escherichia coli* strains isolated from poultry are being used. All are susceptible to both ciprofloxacin (MIC ≤0.015 mg/L) and tetracycline (MIC ≤2 mg/L). We expose biofilm and planktonic subpopulations of these strains to gradients of increasing concentrations of the antibiotics ciporfloxacin and tetracycline in 37 °C over a four weeks period, and monitor the evolution of antibiotic resistance and other mutation events.

Results: Results from comparisons of the geno- and phenotypes of *E. coli* after exposure to increasing concentrations of antibiotics in the planktonic and biofilm state will be presented.

Conclusion: The present work will help to understand the dynamics of resistance development in biofilms and planktonic cultures.
13: Phenotypic and genotypic characterisation of biofilm-forming *Salmonella* enterica serovars isolated from pig and poultry production environments - Oastler C

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**Introduction:** *Salmonella* was the second most commonly reported zoonosis in the EU in 2017 (EFSA, 2018). *Salmonella* has been isolated from pig and poultry production environments and this poses a risk of infection to pigs and poultry, and ultimately humans through the food chain. Multiple factors contribute to *Salmonella* survival in the farm environment, including biofilm-formation. The protective mechanism of biofilm-formation may facilitate the persistence of *Salmonella* in the environment, and allow it to survive cleaning and disinfection.

**Hypothesis and aims:** It is hypothesised that variation in *Salmonella* biofilm-forming ability is due to differences in genotype, and phenotypic expression. Work aimed to assess biofilm-formation, genotype and phenotype of isolates recovered from commercial pig and poultry environments.

**Methodology:** Biofilm-formation was evaluated for up to 72 hours, at realistic farm temperatures of 20 or 25°C, using a crystal violet microtiter plate assay. Motility was assessed using motility test medium. Morphotype and colour of colonies on colonization factor antigen agar plates supplemented with Congo Red identified cellulose production and fimbriae expression. For genotypic characterisation, the isolates were sequenced and the pan-genome explored to find genes associated with biofilm-formation.

**Results:** Serovars associated with pig production, or regulated in the UK poultry industry, were selected for further study: *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Infantis. Also included were persistent *Salmonella* 13:23:i:- isolates from a UK hatchery. Variations in biofilm-forming ability and phenotypic characteristics were observed across the isolate panel. The majority of isolates showed moderate and strong biofilm formation. Although all isolates were motile, the degree of motility differed with incubation temperature. Morphotypes, such as rdar and RpoS mutants, showed differences in fimbriae and cellulose expression.

**Conclusion:** Investigation of genes associated with biofilm-formation and their relationship to phenotypic characteristics is on-going, and will further our understanding of the factors associated with biofilm-formation in the farm environment.
14: In-vitro study of antibiotics affecting on new and establishing non-typeable Haemophilus influenzae biofilm - Obaid N.A

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Introduction: A range of upper and lower respiratory tract infections caused by Nontypeable Haemophilus influenzae (NTHi) are frequently treated with antibiotics such as amoxicillin and azithromycin. These infections may persist and antibiotic therapy may fail to eradicate the infection. The persistence of the infection may be related to biofilm formation which may not be easily managed by conventional antibiotic treatment. The laboratory measurement of antibiotic susceptibility is based on the minimum inhibitory concentrations (MICs) for particular NTHi clinical isolates in the planktonic state. The treatment with antibiotics sometimes could affect the biofilm and paradoxically may enhance the production of sessile cells.

Hypothesis and aims: This study aims to examine the effect of four classes of antibiotics at two concentrations, on the formation of NTHi biofilm.

Methodology: Two isolates were used; (NTHi A1) with high biofilm formation and (RdKW20) with low biofilm formation to assess the effect of sub-MIC and MIC of four antibiotics on biofilms; β-lactams (amoxicillin;AMX and cefotaxime;CTX), macrolide (azithromycin;AZM) and fluoroquinolone (ciprofloxacin;CIP). These antibiotics were applied on two stages of biofilm; adding antibiotics at 0 h “the initiation of new biofilm” and adding antibiotics after 24 h formed biofilm “on existing biofilms” using microtitre plate assay method (MTP assay).

Results: The results showed that AMX at the MIC concentration, impacted on existing biofilm of NTHi A1 and caused formation of more biofilm material. With sub-MIC of AMX and MIC of the other antibiotics, there were no effects on new biofilm formation for NTHi A1. No changes on biofilm formation were shown by RdKW20 with addition of antibiotics compared to untreated biofilm except the increase in biofilm with sub-MIC of AMX.

Conclusion: This in-vitro method provided a direct examination method on the impact of antibiotics for biofilm formation of an NTHi clinical isolate which indicate on the behavior of this clinical isolate after exposure to antibiotic. The standard isolate RdKW20 (weak biofilm formation) showed a slight increase in biofilm after exposure to AMX.
15: Selection of resistance in bacteria grown on antimicrobial surfaces in multidrug environments - Pietsch F

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Introduction: Biofilms are regarded as a common cause of chronic infections on medical devices. Preventive and therapeutic strategies against biofilm infections commonly involve applications of multiple antimicrobial substances: antimicrobial coatings on the implanted biomaterials in combination with systemically administered antibiotics. While this practice of combination therapy harbours the risk of developing cross-resistance, it might also provide the possibility to implement specific antimicrobial-antibiotic combinations (AACs) that can slow down the selection of antibiotic resistant strains.

Hypothesis and aims: Specific AACs can exert combinatorial effects on the growth of susceptible and antibiotic-resistant Pseudomonas aeruginosa that either suppress or increase their individual effects. Our aim is to identify AACs with antagonistic or synergistic effects on pseudomonal biofilms and to understand their impact on selection of resistant strains. Specifically, we want to identify AACs that select for and against antibiotic resistance during biofilm formation.

Methodology: We screened for AACs that cause antagonistic or synergistic effects on planktonic P. aeruginosa. To study the effect of antimicrobial-antibiotic exposure on resistance selection in bacterial biofilms, we will grow resistant and sensitive strains on PDMS surfaces with and without antimicrobial coatings and expose them to antibiotics.

Results: Several combinations with synergistic or antagonistic interaction on the growth rate of P. aeruginosa were detected. We observed a strong antagonism when combining the antimicrobial substance chlorhexidine with the carbapenem drug meropenem. A meropenem-resistant mutant showed a selection advantage in low concentrations of chlorhexidine combined with a sub-inhibitory concentration of meropenem over the wild-type. No antagonistic effect was observed for the same combination when E. coli was exposed to chlorhexidine and meropenem, suggesting a non-chemical basis for the observed effect on P. aeruginosa.

Conclusion: Gaining a better understanding about resistance selection during biofilm formation on biomedical surfaces will enable us to mitigate against biofilm-associated antimicrobial resistance.
Introduction: Life in biofilm selects for adapted variants, expressing a distinct biofilm phenotype. Gene expression is altered in such variants, resulting in overproduction of matrix components, rugose colony morphotypes, reduced swimming capabilities, elevated c-di-GMP levels and other phenotypic changes. However, the ability of these variants to engage in horizontal gene transfer (HGT), e.g. by receiving plasmids, remains unexplored. Xanthomonas retroflexus undergoes phenotypic variation generating two morphologically different variants, termed smooth and rugose. The rugose variant has mutations in genes encoding a diacylglycerol cyclase predicted to synthesize cyclic-di-GMP leading to the rugose phenotype.

Hypothesis and aims: The aim of the present study was to assess the capability for plasmid uptake by a rugose biofilm variant of X. retroflexus and identify underpinning mechanisms of the observed differences.

Methodology: Plasmid uptake was identified by quantification of fluorescing cells by flow cytometry. The results showed that the rugose X. retroflexus variant exhibited an increased frequency of plasmid uptake compared to the smooth variant. To test if this was linked to cell physiology or changes in matrix composition, we compared the proteomes of the two strains. The smooth variant displayed increased abundance in proteins associated with motility compared to the rugose variant. Based on this, fliM (encoding part of the flagella) was knocked out in X. retroflexus to create a non-flagellated version. The ΔfliM mutant exhibited increased plasmid uptake compared to the smooth variant at levels comparable to those of the rugose variant.

Results: To further examine the impact of flagella on HGT, we tested the plasmid uptake for various flagella mutants of P. putida. Complete loss of flagella promoted plasmid uptake, compared to the presence of nonfunctional flagella for P. putida.

Conclusion: These results show that loss of flagella can lead to enhanced plasmid uptake and that nonfunctioning flagella can decrease the ability to receive plasmids, thus emphasizing the importance of cell physiology on plasmid uptake.
Introduction: Biofilms are structured communities of microorganisms that grow adhered to inert or living surfaces due to a self-produced matrix in which they are encased. Inside the biofilm, bacteria exhibit a superior tolerance to physicochemical insults and harsh reaction conditions. These properties are of great interest in industrial and environmental biotechnology, such as in the field of biocatalysis of toxic compounds.

Hypothesis and aims: We hypothesized that genetically engineered bacteria that gain the ability to form a biofilm might show improved metabolic capabilities. As a proof of concept, we have used Rhodococcus erythropolis IGTS8 that harbors the dszABCD genes, encoding the functional units of the 4S pathway, through which dibenzothiophene (DBT) is desulfurized into 2-hydroxybiphenyl (2HBP). We aimed at analyzing whether engineered R. erythropolis biofilm cells might provide an improved biotechnological strategy for the removal of the recalcitrant sulfur of aromatic heterocycles present in fuels.

Methodology: Three different strategies were used to promote biofilm formation in R. erythropolis: i) exogenous addition of the Bap (Biofilm Associated Protein) protein; ii) heterologous expression of the icaADBC operon responsible for PIA/PNAG exopolysaccharide synthesis and iii) heterologous expression of a diguanylate cyclase (DGC), and thus, overproduction of c-di-GMP. To avoid bottlenecks in the conversion of DBT into 2HBP, plasmid cloning and genetic engineering of dsz cassettes were performed. Bioconversion efficiency was analyzed using resting cell assays followed by HPLC quantification of DBT and 2HBP.

Results: Heterologous expression of a DGC in R. erythropolis drives c-di-GMP synthesis and leads to a highly aggregative phenotype which results in a significant increase in the bioconversion of DBT into 2HBP.

Conclusion: Biofilm formation by R. erythropolis can be driven by the overproduction of the second messenger c-di-GMP. Resulting biofilm cells expressing an engineered dsz cassette can be considered an attractive and efficient strategy for enhancing DBT biodesulfurization.
18: Conditions under which glutathione disrupts the biofilms and improves antibiotic efficacy of both ESKAPE and non-ESKAPE species - Theerthankar D

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Introduction: Bacterial resistance to antibiotics has significantly increased in recent decades, raising concerns in hospital and community settings. Novel, innovative strategies are needed to combat resistance and to diminish the likelihood of recurrent transmission. In this study, we investigated whether glutathione (GSH) can act as a biofilm disruptor, and enhance antibiotic effectiveness against various bacterial pathogens.

Hypothesis and aims: To develop a novel therapeutic strategy using GSH to disrupt biofilm formation and enhance antibiotic efficiency in killing bacteria.

Methodology: To investigate the effect of GSH, antibiotics and enzymes on disruption and killing of ESKAPE and non-ESKAPE biofilms we used techniques includes biofilm viability (resazurin assay), biofilm biomass quantification (crystal violet assay), confocal microscopy complemented with ImageJ software, Mechanism of GSH mediated biofilm disruption was determined using circular dichroism, Qubit fluorometer techniques. The role of GSH in facilitating human fibroblast cells (HFF-1) growth, confluence and viability were assessed using various biochemical assay and microscopy imaging techniques.

Results: GSH treatment exhibited a >50% decrease in biofilm viability in all bacterial species used in this study and considerable change in the biofilm architecture as evidenced using confocal imaging technique. The mechanism of GSH-mediated biofilm disruption is due to a concentration-dependent increase in GSH acidity that triggers destabilization and cleavage of the biofilm matrix components. Enzymatic (DNase I, amylase and Proteinase K) treatment of biofilm revealed that extracellular DNA (eDNA) and polysaccharide are essential for biofilm stability. Combination of GSH, antibiotic and DNase-I showed the greatest reduction in biofilm viability. Additionally, GSH alone and in combination with antibiotic was shown to foster HFF-1 growth and confluence while inhibiting bacterial adhesion and colonization.

Conclusion: GSH acidity plays a key role in biofilm disruption of many antibiotic resistance bacterial isolates through cleaving of biopolymers present in biofilm matrix and consequently enhances antibiotic efficacy.
19: Pathobionts of the gastrointestinal microbiota express BAP-like proteins with amyloid features to build biofilms - Valle J

Matilla L and Valle J

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Introduction: Recent insights into bacterial biofilms have induced a paradigm shift towards the recognition of functional amyloids as common building block structures of the biofilms. Amyloids are attractive extracellular building material because they generally resist to harsh denaturing conditions and proteases and their polymerization occurs in the absence of energy. Our group has demonstrated that the staphylococcal Bap protein, a member of the BAP family of proteins, is processed and fragments containing the N-terminus of the protein become aggregation-prone and self-assemble into amyloid-like structures.

Hypothesis and aims: Based on our results obtained with the staphylococcal Bap protein, we propose to study the amyloidogenic properties of BAP-like proteins express by pathobionts of the gastrointestinal microbiota.

Methodology and Results: We used the curli-dependent amyloid generator C-DAG-system to test the amyloid-forming propensity of BAP-like proteins. We detected the presence of extracellular amyloid aggregates by analyzing the capacity of the strains to bind Congo Red dye and by detecting the presence of fibrillar structures by electron microscopy. We confirmed the amyloid properties by using biophysical in vitro assays, microscopic approaches and specific dye-binding analyses of purified recombinant amyloids regions.

Conclusion: In this regard, a previously unnoticed interplay between the host immune system and the microbiota-derived amyloids occurs that under specific conditions might cause pathologies related to protein misfolding.
20: Identification of adhesion-force induced gene expression, its force sensitivity and height distribution in Streptococcus mutans biofilms (Oral) - Wang C

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Introduction: Bacterial adhesion to a surface is the initial step of biofilm formation, accompanied by nanoscopic cell wall deformation and stimulated surface-adaptation of initially adhering bacteria through alterations in the expression of a lot of genes. Adhesion force sensed by initial colonizers differs on different surfaces and has a limited functional distance up to 0.5 µm into a biofilm. Yet it is unknown how far adhesion-force sensitive genetic programming extends into a mature biofilm.

Hypothesis and aims: luxS quorum sensing system which coordinates communication in Streptococcus mutans biofilm was hypothesized to impact the extension of adhesion-force sensitive genetic programming into a mature biofilm. Therefore, this study aims to investigate how adhesion-force induced gene expression spread in a mature biofilm and to what extent quorum-sensing controls it in later biofilm inhabitants, residing further away from the substratum surface.

Methodology: S. mutans UA 159 (wild type) and S. mutans UA 159 ΔluxS (quorum-sensing deficient mutant) were used in this study. Adhesion forces between bacterial strains and four different solid surfaces were tested by using atomic force microscope. Biofilm thickness and structure on all surfaces were analyzed using optical coherence tomography (OCT) after 5 h and 24 h of growth. Biofilms were then collected and sliced. Gene expressions in whole biofilms and biofilm slices were determined using RT-qPCR.

Results: The gene expression of brpA, comDE and gbpB in 5 h old biofilms were up-regulated with increasing adhesion forces sensed by the bacteria. In 24 h old biofilms, adhesion-force induced gene expression and emergent extracellular polymeric substances production was stronger for the parent strain than for the quorum-sensing deficient mutant, but only up to a height of around 30-40 µm above the substratum surfaces.

Conclusion: Initial colonizers of a substratum surface sense adhesion forces directly, which triggers the gene expression and quorum-sensing system. Bacteria in a biofilm 40 µm away from the surface still show adhesion-force induced gene expression.
21: Signal transduction genes in marine biofilms and the influence of signal molecules on marine biofilms development - Wang R

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Introduction: Microbes utilize complicated signal transduction systems to respond to environmental stimuli. Due to complex multi-species interactions, microbial community in natural biofilms enhances the intricate conditions. However, the signal transduction in marine biofilms has hardly been comprehensively explored.

Hypothesis and aims: The type and abundance of signal transduction genes in natural marine biofilms significantly differ from those in seawater microbial communities; Particular signal molecules play important roles in shaping the microbial community of biofilms.

Methodology:

- Biofilms and seawater sampling across oceans
- DNA extraction and metagenomic analyses of natural biofilm and seawater samples
- Signal molecule treatment experiment and metagenomic analyses
- Bacterial isolation, Pseudomonas quinolone signal (PQS) treatment experiment, and transcriptomic analyses

Results: Metagenomic profile of signal transduction genes in marine environment: classification of signal transduction genes revealed distinct patterns between the biofilms (n = 101) and the seawater samples (n = 91) which were collected worldwide, indicating the specificity of signal transduction system in marine biofilm communities; Most signal transduction genes were enriched in marine biofilms, especially the genes related to quorum sensing; and the abundance variation of signal transduction genes was less in biofilm bacterial communities; Different signal molecules had individual impacts on the composition and functional genes of marine biofilms evidenced through the signal molecules treatment experiment, and the biofilms treated by PQS demonstrated the least similarity compared to the control; PQS had unambiguous influence on gene expression during the biofilm development of a bacterium isolated from a marine biofilm.

Conclusion: Certain signal transduction systems, such as the PQS, take distinguishing part in regulating microbe-microbe interactions in marine biofilms and contribute to the differentiation between surface-associated and free-living microbial communities, highlighting the important roles of small signal molecules with low concentration in the marine ecosystem.
The search for PQS transport proteins using PQS affinity probe generated through metabolic labelling - Woo B.

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Introduction: Pseudomonas Quinolone Signal (PQS) is a quorum sensing signal of Pseudomonas aeruginosa which plays a vital role in virulence regulation and the development of antibiotic resistant biofilms. While it is known that PQS can induce the formation of outer membrane vesicles (OMVs) as a vehicle for trafficking in the bacterial communities, very little is known about the bidirectional transport of PQS within cells, between the bacterial inner and outer membranes.

Hypothesis and aims: Due to the hydrophobic nature of PQS, we hypothesized that its trans-envelope transport would require specific protein machineries. The aim of our study is to understand the uptake and export process of PQS through the use of PQS affinity probe.

Methodology: Affinity-based protein profiling, metabolic labeling.

Results and Conclusion: A probable ABC transporter and two RND efflux systems are involved in the uptake and export process of PQS respectively. Altogether, these experiments have provided greater understanding of the transport pathway of PQS and revealed potential targets for the development of novel anti-virulence agents.
23: Biofilm-evolved *Klebsiella pneumoniae* exhibit changes in capsule, fimbriae and c-di-GMP turnover - Zaborskyte G

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**Introduction:** *Klebsiella pneumoniae* frequently causes medical device-related infections due to biofilm formation, but the factors affecting it remain understudied in this species.

**Hypothesis and aims:** Our aim was to analyse the mutations selected during experimental evolution in a biofilm model and compare them with the changes observed during *K. pneumoniae* hospital outbreak.

**Methodology:** Surface-attached biofilms were grown on plastic pegs (in-house developed modular version of the Calgary device with insertable pegs). Ten lineages of three clinical strains (IA565, CAS55 and an ESBL-outbreak isolate) were subjected to five cycles of biofilm growth, one cycle lasting for 48 h and involving attachment, maturation, and dispersal. Evolved mutants were whole-genome sequenced and selected mutants were characterised with respect to biofilm formation (CFU/peg and crystal violet staining), fitness in planktonic cultures and resistance to human serum.

**Results:** Most mutants carried single mutations associated with type 3 fimbriae (*mrkD*), c-di-GMP turnover (EAL-domain proteins, e.g. *yhjH*) and capsular polysaccharides (*wzc/etk* tyrosine kinase). IA565 strain also repeatedly had a point mutation in a putative bacteriocin/T6SS effector gene. Evolved mutants had up to 30x higher CFU/peg, but planktonic growth rate did not change. *Wzc/etk* mutants had the most extreme biofilm phenotype (long strings of biomass stretching from the pegs) and colonies completely stuck on agar. Unexpectedly, *wzc/etk* mutants were highly sensitive to human serum in planktonic state. Mutations in *mrkD, etk* and *yhjH* at different positions were also present in isolates from a clonal *K. pneumoniae* outbreak at Uppsala University Hospital.

**Conclusion:** We identified a range of novel mutations leading to increased biofilm formation in *K. pneumoniae* using an experimental evolution approach. Mutations in the most frequent targets (type 3 fimbriae, c-di-GMP turnover and capsular polysaccharide production) were also observed during the clinical outbreak. This suggests possible clinical relevance with regards to the virulence or persistence of infection and will be studied further.
Wednesday 4th September

Pseudomonas

24: Investigating the effect of tobramycin dry powder inhaler on the eradication of Pseudomonas aeruginosa biofilms - Aljalamdeh R

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Introduction: Biofilms are sessile communities of microorganisms embedded within a self-generated extracellular polymeric matrix, which consists of polysaccharides, DNA, and/or proteins. Such biofilms are found for instance in adults with cystic fibrosis, with pulmonary infections with the Gram-negative bacterium Pseudomonas aeruginosa being particularly common and responsible for a high mortality rate among CF patients. This infection in CF patients is commonly managed with antibiotic dry powder inhalers, one of which is the aminoglycoside tobramycin. The activity of tobramycin has been well characterized in vitro.

Hypothesis and aims: Hypothesis: We hypothesize that different particle sizes of tobramycin dry powder inhaler can influence the anti-biofilm activity against Pseudomonas aeruginosa biofilm infections. Aim: Establishing a better in vitro model to test activity of dry powder inhaler antibiotic because the current models that have been used are not very representative for lung infections. Therefore, a better model would provide a significant advantage as this could be used, for instance, to improve the formulation of dry powder inhalers.

Methodology: In this project, we utilized the Next Generation Impactor (NGI), which is a pharmaceutical instrument used to separate particles into size fractions. We used the NGI to separate tobramycin particles into different sizes and tested the influence of these particles on eradication of P. aeruginosa biofilms, which were grown as colony biofilms that closely mimics conditions in the lung where biofilms are grown on a substrate-air interface.

Results: Preliminary evidence indicated smaller tobramycin particles are better in eradication of P. aeruginosa biofilms as compared to larger particles.

Conclusion: Our results may represent a step towards improving the formulation of tobramycin dry powder inhalers to be effective in eradicating P. aeruginosa biofilms.
25: Characterising the structure and composition of biofilms formed by *Pseudomonas aeruginosa* under different shear conditions - Allan W

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**Introduction**: Biofilms provide physical, mechanical and chemical protection of microbes from their external environment; removal requires harsh chemicals and abrasive cleaning. This protective mechanism has a broad impact upon a wide range of fast-moving consumer goods, and biofilm contamination during manufacture can impact on productivity, product recalls and significant economic cost to industry for cleaning, sanitization and scrappage. Biofilms formed by *Pseudomonas aeruginosa* (Ps. a.), a major contaminant of industrial processes, have yet to be studied in-depth with respect to physical and biological changes that occur in response to different fluid shear conditions.

**Hypothesis and aims**: The central aim of this work was to understand and elucidate the biological response of *Ps. a.* biofilms when grown under different fluid flow conditions: focusing on structure and polysaccharide production (Psl, Pel), to characterise the mechanisms by which *Ps. a.* produces phenotypic responses to fluid shear.

**Methodology**: The CDC Bioreactor (CBR) was used to investigate the effect of shear stress on biofilm formation by *Ps. a.* strains PA01 and PA14, under low shear and high shear conditions. The bioreactor was run for 24 hours in batch, and for a further 72 hours as a continuous culture. At 24, 48 and 72 hour timepoints, polycarbonate coupons were removed from the device, and analysed by confocal laser scanning microscopy (CSLM) and biochemical assays.

**Results**: The effect of shear forces on production of EPS components and their impact on biofilm morphology will be described. In particular, the productions of the exopolysaccharides Psl and Pel, which are differentially produced by strains PA01 and PA14, will be compared for different shear conditions.

**Conclusion**: Hydrodynamic conditions impose shear stress on *Ps. a.* biofilms, in turn affecting the relative proportions of structural components and thereby biofilm architecture.
26: Modulation of immune cell activation by *Pseudomonas aeruginosa* biofilms; potential role of C-type lectin receptors (ORAL) - Almuhanna Y

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**Introduction:** Colonization and persistent infection by *Pseudomonas aeruginosa* (PA) are important causes of mortality and morbidity in individuals with underlying conditions. The ability of PA to form drug-resistant biofilms is considered essential to establish chronic infection. PA biofilms are enclosed in an extracellular polymeric substance (EPS) that protects bacteria from harsh environments. The polysaccharides Psl and Pel are EPS components that promote biofilm formation. No information is available on immune receptors specifically involved in PA biofilm recognition.

**Hypothesis and aims:** We hypothesise that EPS recognition by innate immune cells could modulate immune activation through the engagement of C-type lectin receptors. The aim of this study is to explore the ability of lectin receptors expressed by human dendritic cells (huDCs), Mannose Receptor (CD206, MR), DC-SIGN (CD209) and Dectin-2 (CLEC6A) to directly recognise PA biofilms and potentially modulate the immune response.

**Methodology:** Here, we used binding assays and confocal microscopy to study binding of lectin receptors to PA biofilms. To investigate if biofilm EPS composition influences immune activation, huDCs expressing DC-SIGN, MR and Dectin-2, were incubated with fixed PA biofilms and cytokine production was quantified at 4 and 18 hours.

**Results:** We detected binding of DC-SIGN, MR and Dectin-2 to PA biofilms. Binding was calcium-dependent, could be inhibited by relevant sugars, and was observed in biofilms formed by PAO1 and clinical isolates. DC-SIGN, MR and Dectin-2 ligands within biofilms showed differential distribution. HuDCs were incubated with wells containing Psl+/Pel+, Psl+/Pel− and Psl−/Pel+ biofilms alongside wells containing bacteria non capable of biofilm formation Psl−/Pel−. Preliminary results indicate selective induction of IL-1β (4 and 18 h) and IL-23 (only 4 h) by biofilm-containing wells, with biofilms containing more Psl tending to induce less IL-23.

**Conclusion:** Our results indicate that biofilms can directly engage receptors expressed by human immune cells and modulate cellular activation.
Introduction: Bacteria are ubiquitous and they may cause clinical infections (e.g., in catheters), corrosion, loss of efficiency in different processes (e.g., water process). Nowadays, many strategies are used to control microbial growth and development (e.g., antibiotics, UV light, etc.). Nevertheless, when bacteria are in the vicinity of an interface they start to grow as a biofilm, producing the extracellular polymeric substances, becoming more resistant and less susceptible to biocides and disinfectants. An alternative solution could be the utilization of visible light to inactivate microbial biofilms, in particular violet light at 400 nm.

Hypothesis and aims: The interaction of photons with defined energy causes the cell death. Light produces reactive oxygen species dangerous for the cells. The goal of this work is to investigate the killing effect of a specific wavelength. The use of light emitting diodes represents a future perspective for the realization of devices based on lower consumptions that do not use chemistry.

Methodology: Biofilms of *P. fluorescens* are grown on a polystyrene surface. They are exposed to the light treatment with a high tech device with 400 nm of wavelength. Inactivation of microorganisms is carried out and the Geeraerd *et al.* (2000) model is used to fit the data. Different irradiances are tested. The inactivation parameters are estimated using Geeraerd *et al.* (2000) model.

Results: The wavelength 400 nm in the VIS region is able to inactivate *P. fluorescens* when it is grown as biofilm. The maximum specific inactivation rate seems not to be affected by the irradiance, while the residual population depends on the irradiance emitted by the LEDs.

Conclusion: *P. fluorescens* biofilms can be successfully inactivated by means of 400 nm light.
28: Prevalence and genetic variation of the *Pseudomonas aeruginosa* elastase gene in clinical isolates from cystic fibrosis patients - *Barochia B*

*Cai Y*1,2,3, **Barochia B**1,2,3, Allan R1,2,3,4, Spyriniski N6, Castandet J, Connett G3,4,5, Faust S2,3,4,5, Everett M6 and Webb J1,2,3,4

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**Introduction:** *Pseudomonas aeruginosa* as an opportunistic pathogen of the cystic fibrosis (CF) lung and is capable of producing a number of virulence factors which assist in biofilm formation and early transient infection of the host. LasB is an important quorum-sensing dependent non-specific metalloprotease which is a potential target for anti-virulence therapeutics that inhibit pathogenesis in CF. LasB is capable of cleaving multiple protein targets within the respiratory tract including immune factors and CFTR. The frequency of detection alongside the mutations of this protein have thus far been previously uncharacterized, therefore providing the scope for this study.

**Hypothesis and aims:** We hypothesised that LasB will be highly conserved among CF isolates of Pa, but that adaptations to the biofilm lifestyle within the lung may lead to genetic variation which could impact virulence. This study aimed to evaluate the prevalence of LasB in isolates from CF patients alongside analyse the frequency of mutations within this gene.

**Methodology:** 136 expectorate samples were collected from 49 individual CF patients of which two had a familial relationship. 48 patients were undergoing exacerbation and 78% of the patients were chronically infected with Pa. The genome was extracted from the isolates. PCR amplification of the LasB gene and Sanger sequencing was used.

**Results:** The study isolated 106 Pa strains of which 77.4% (82) had the LasB gene directly detectable by PCR. Sequencing of the gene identified 3 distinct LasB gene variants which could possibly influence protein activity.

**Conclusion:** The study identified 3 lasB gene variations that are observed consistently across a CF Pa population from different patients, indicating a survival advantage of these mutations to Pa within the CF lung. Such genetic variation could potentially impact protein activity and will be the subject of further study. Furthermore, variation in the LasB protein may impact in the design of a therapeutic interventions against this target.
29: Key regulators of nitric oxide-mediated regulation in *Pseudomonas aeruginosa* biofilms dispersal (ORAL) - Cai Y

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**Introduction:** Previous studies have shown that low dose nitric oxide (NO) can trigger *Pseudomonas aeruginosa* biofilm dispersal by modulating the levels of the intracellular secondary messenger cyclic dimeric guanosine monophosphate (c-di-GMP). Diguanylate cyclase (GGDEF motif) and phosphodiesterase (EAL/HD-GYP motif) activities are responsible for the synthesis and hydrolysis of c-di-GMP, respectively. Various sensor domains have been found to link environmental cues to GGDEF and EAL/HD-GYP activities modulation, of which PAS and MHYT domains are of interest due to their potentials to bind NO.

**Hypothesis and aims:** In *P. aeruginosa* PAO1, a total of 12 proteins containing either PAS-DGC+/PDE were thought to be responsible for the NO-induced biofilm dispersal and were selected as our targets for investigation of their relationships between NO responses and biofilm phenotypes.

**Methodology:** A range of NO donors were first tested for their efficacies on dispersing *P. aeruginosa* biofilms using microtiter plate assay. Gene deletion was then applied to 12 protein candidates followed by phenotypic assays including microscopic biofilm structural and dispersal analysis, intracellular c-di-GMP level measurement, agar-based motility assays and extracellular polymeric substance measurement.

**Results:** Results suggested a correlation between NO-induced swarming promotion and biofilm dispersal under the regulation of 3 potential PDEs, such as previously reported RbdA (PA0861). Deficiency in both twitching and swimming motility under the regulation of another 2 potential PDEs, such as previously reported FimX (PA4959), altered the 3D structures of the biofilms and led to enhanced biofilm dispersal.

**Conclusion:** *P. aeruginosa* PAO1 depends on 3 key PAS-GGDEF-EAL structure proteins to coregulate flagellar motility and biofilm dispersal upon NO challenge. Pili deficiency modulates mature biofilm structure and facilitates biofilm dispersal, which may provide potential new targets for therapeutic drug design.
30: Properties of *Pseudomonas aeruginosa* biofilm cells dispersed with various approaches - Coenye T

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**Introduction:** Biofilm dispersal is regarded as a possible strategy to combat biofilm-related infections. Various methods to disperse a biofilm have been described, but it is currently unknown how the method of dispersion influences the phenotype of the dispersed cells.

**Hypothesis and aims:** Our goal was to compare properties of *P. aeruginosa* biofilms dispersed with various approaches.

**Methodology:** *P. aeruginosa* PAO1 biofilms were grown in flow-cells at room-temperature for 4 days. Biofilms were dispersed by adding 500 µM sodium nitroprusside (SNP), a 10-fold increase of the C-source (Glutamate), stop and restart flow after 1 hour (stop/start-flow) or by VNB’s. VNB disruption is induced by adding gold nanoparticles to the biofilm prior to submitting the biofilm to a green (561 nm) pulsing laser. This creates nano-sized water-vapor bubbles which disrupts the biofilm. The dispersed cells were collected and c-di-GMP was extracted and quantified via LC-MS/MS. We determined the gene expression profiles via Illumina-based RNA sequencing. Virulence in *G. mellonella* was assessed and susceptibility to tobramycin (10 µg/mL), colistin (16 µg/mL) and ciprofloxacin (5 µg/mL) was determined via time-kill assays. As control, we used spontaneously-dispersed cells.

**Results:** A lower c-di-GMP concentration was observed in VNB and stop/start-flow dispersed cells compared to spontaneous and SNP dispersed cells. 251 genes were only differentially expressed in VNB dispersed cells, including genes involved in virulence (e.g. hcnBC, toxA). However, no significant difference was observed in virulence in *G. mellonella*. RNA-sequencing showed significant expression of RND exporters (e.g. mexCD), this resulted in stop/start-flow and VNB dispersed cells to be more susceptible to colistin after 1 hour and 5 hours of treatment respectively.

**Conclusion:** Lower c-di-GMP concentrations and the differential expression of the genes involved in virulence and antibiotic susceptibility did not lead to phenotypical differences in virulence, but does result in differences in susceptibility to colistin.
Introduction and aims: Chronic wounds (CW) are a common complication of diabetic ulcers (DUs), which are a major burden to health care systems worldwide and can result in lower limb amputation due to the intractability of the infection. In DUs there is a high probability of the infecting bacteria evolving considerable phenotypic and genetic diversity, as has previously been shown for chronic cystic fibrosis lung infections. However, it is not known whether this is also the case for DUs, and whether diversity impacts on virulence and antibiotic resistance.

Methodology: Bacterial populations were isolated from different samples from patients with DUs, and phenotypic diversity was investigated in Pseudomonas aeruginosa populations through the analysis of phenotypes traditionally associated with pathogenicity, and through whole genome sequencing.

Results and conclusion: Phenotypic variation in P. aeruginosa isolates taken from different patients was observed, but little variation within the same CW. Antibiotic resistance was found to increase during the course of infection, and it became apparent that P. aeruginosa colonisation in DUs is via a single strain per ulcer, and potentially per patient. In one patient, two distinct P. aeruginosa phenotypic profiles were found, so a detailed genomic analysis between isolates was carried out, including a full characterisation of the single nucleotide polymorphisms and a comparison of their transcriptomes using RNAseq. The results obtained suggest that the loss of flagellum may have facilitated evasion of the innate immune system, such that the blood isolates were able to go undetected and so spread systemically causing the rapid decline in the patient’s health.
32: *Pseudomonas aeruginosa* quorum-sensing signal molecules interfere with human mesenchymal stem cells on bioactive coated 2D/3D titanium implants - Damiat L


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Introduction: Titanium is a common material used for the orthopaedic and dental implants. However, it is recognized that it has suboptimal hard-tissue differentiation and antibacterial properties. *Pseudomonas aeruginosa* is associated with orthopaedic infections and releases a wide range of toxins and tissue-degrading enzymes. The production of virulence factors is controlled by interbacterial communication in a process known as quorum sensing (QS). In improving the osteogenic properties of the material, we have developed an ultra-thin polyethylacrylate (PEA) coatings applied to 2D and 3D Ti via plasma polymerization. PEA causes spontaneous unraveling of fibronectin (FN) upon contact which aids the absorbance of with ultra-low doses of growth factors, such as BMP2. 3D Ti scaffolds were produced using the selective laser melting technique (SLM), very efficient for scaffolds with different sizes and shapes, with good dimensional accuracy.

Hypothesis and aims: Understanding the MSCs response to the QS factors to aid the improvement of the osteoinductive properties of our PEA/FN/BMP2 coated Ti scaffolds.

Methodology: Alkaline hydrothermal treatment was applied to produce an antimicrobial high-aspect ratio nanotopographies on the Ti scaffolds. PEA/FN/BMP2 coatings were applied and scaffold physical and chemical characteristics were studied pre- and post-coating. *P. aeruginosa* were cultured on the substrates and the number of viable microbial cells was determined by quantitation of ATP. MSCs were cultured on the test substrates in the presence of QS virulence factors (C12-HSL and C4-HSL) and cell viability was analysed by flow cytometry using annexin V, JC-1 and the cell cycle analysis. MSC bone mineralisation in response to the test substrates was examined using Raman spectroscopy, calcein blue and Alizarin red staining.

Results: ATP release suggested a reduction in *P. aeruginosa* adherence on the Ti coated surfaces. Also, a significant reduction in MSC viability was observed in the presence of long carbon chain QS factors (C12-HSL) but not in the shorter one (C4-HSL). Ti surfaces with PEA/FN/BMP2 coating showed an improvement in MSC growth, adhesion and bone mineralisation compared with uncoated substrates. Moreover, the 3D Ti lattices with 900 µm diameter struts had better MSCs adhesion and growth.

Conclusion: Here we demonstrate the potential to fabricate 3D Ti implants with topographies that reduce microbial viability and polymer coatings that enhance osteogenesis of MSCs in-vitro.
33: RNA-seq characterization of *Pseudomonas aeruginosa* grown in alginate bead model and comparison to in vivo infections (ORAL) - Fritz B

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**Introduction:** Bacteria grown in many laboratory systems exhibit a fundamentally different physiology compared to bacteria found in chronic infections. This suggests that conclusions drawn from *in vitro* laboratory systems may not translate well to actual chronic infections. We have developed an alginate bead model which appears to more closely resemble the physiology of *Pseudomonas aeruginosa* during chronic infections. RNA-seq was performed to further validate the applicability of the model, understand how the entire transcriptome changes in the model under varying environmental conditions, and compare the model against true chronic infections.

**Hypothesis and aims:** The aim of this project was to establish overall similarities and contrasts between bacterial physiology in the alginate bead model and in chronic infection to allow further development of the model. We hypothesize that older, anoxic beads will more relevantly, but not completely, resemble bacterial physiology during chronic infections.

**Methodology:** *P. aeruginosa* PAO1 was inoculated into alginate beads grown in shaken cultures under three conditions (O₂, O₂+10mM NO₃⁻, and Anoxic +10mM NO₃⁻) for 24 or 48hr. RNA was purified from these beads and sequenced by Illumina sequencing. Differential gene expression analysis was performed to identify differentially expressed genes among different conditions. Additional comparisons were also performed between the beads and several types of chronic infection tissues.

**Results:** Analysis is still ongoing and we expect that important processes such as quorum sensing, nutrient acquisition and antibiotic tolerance will represent important contrasts between the beads and in vivo infections.

**Discussion:** We hope that implementation of these considerations into the bead model will lead to a more representative model and also allow other model systems to be adapted to chronic infection research.
Introduction: The multidrug resistant opportunistic pathogen *Stenotrophomonas maltophilia* is a biofilm forming and gram-negative bacterium. It contribute to disease progression in cystic fibrosis patients and is found in wounds and on catheter surfaces.

Aims: The aim is to identify the processes and genes involved in the biofilm formation of *S. maltophilia*. Therefore, we sequenced the genomes of over 350 clinical and 40 environmental isolates and investigated their biofilm profile. The genome data together with the biofilm analysis and other phenotypic and metabolic data will generate the largest data set of *S. maltophilia* and its biofilm formation on a genus and pangenome-wide level.

Methodology: Biofilm assays were done in microtiter plates, flow cells or µ-slides (Ibidi, Wisconsin, US). Genomes were sequenced using NGS technologies and phylogenetic trees constructed as previously published (Steinmann, Front Microbiol. 2018; 9:806).

Results: The analysis of 300 clinical and environmental *S. maltophilia* isolates revealed a strong variation in biofilm forming ability. 2 % of all isolates formed very strong biofilms, while 12 % formed strong, 77 % formed moderate and 9 % formed weak biofilms. Clinical and environmental isolates do not differ in their biofilm forming abilities. Analyses of biofilm 3D-structures of 40 isolates identified high heterogeneity in the biofilm matrix and appearance independent of the strains and their phylogenetic position within the genus. Furthermore, a high variation in resistance of 35 isolates towards the antibiotic colistin was detected.

Conclusion: We speculate that under *in vivo* conditions *S. maltophilia* also displays varying biofilm architectures on a strain-specific level. However, no correlations between biofilm forming abilities, 3D-structure and resistance to the antibiotic colistin were observed. Transcriptome data for selected isolates are underway to estimate the biofilm formation on a global level to analyze if the high variation in biofilm architecture correlate with *S. maltophilia* strain specific expression patterns.
**Introduction:** Human host immune response to bacterial biofilms represents an important but poorly investigated research topic. Although several studies investigated neutrophil or monocyte response to biofilms, only few of these focused on human peripheral blood mononuclear cells (PBMCs). Low viability of human cells upon incubation with biofilms makes difficult to study immune cells-biofilm interactions.

**Hypothesis and aims:** Aim of the present study was to establish an in vitro immune cell-biofilm interaction model that could sustain the viability of host cells for at least 24h and investigate PBMC immune response to *Pseudomonas aeruginosa* (PA) biofilms.

**Methodology:** Preformed biofilms were obtained in microtiter plates by incubating PA for 8-24h in RPMI medium with 10% human plasma. Human PBMCs were added onto biofilms or planktonic bacteria. Following 24h-incubation: i) supernatants from the wells were used to analyze cytokine production by PBMCs, and incubated with preformed biofilms to evaluate any effect on their growth; ii) viability and activation of PBMCs was assessed; iii) biofilm-forming bacteria were determined by CFU count.

**Results:** Although the viability of PBMCs incubated with biofilms decreased, the cell death was <15% even at 24h. PBMCs were significantly more activated in the presence of preformed biofilms compared to PBMCs incubated alone, with an activation of almost 50% of natural killer (NK) cells. Differences in activation and cytokine production were observed between PBMCs incubated with biofilms or planktonic bacteria. Interestingly, incubation of preformed biofilms with supernatants of PBMC-biofilm co-cultures caused a significant increase (2-10 times) in biofilm-forming bacteria.

**Conclusions:** These results demonstrated that: i) it is possible to study PA biofilms in vitro, in conditions optimal to human immune cells; ii) PA biofilm formation is enhanced in the presence of PBMCs and/or PBMC components, suggesting a possible bacterial defensive strategy against immune response; iii) biofilms activate and induce cytokine production of PBMC subsets more than planktonic bacteria.
Introduction: *P. aeruginosa* is one of the versatile bacteria that evenly survive in clinical and community environs. Production of exopolysaccharides is a natural tendency of *P. aeruginosa* that enable these pathogens to adhere on animate and inanimate objects and protect them from the toxic effect of antibacterial agents.

Hypothesis and aims: This study was designed to explore the role of different phenotypes of *P. aeruginosa* in the development, stability and persistence of biofilm.

Methodology: A total of seventeen (17) waterborne biofilm producing strains of *P. aeruginosa* were studied. These isolates were identified on the basis of typical phenotypic characters, i.e. growth on cetrimide agar and by amplification of 16S rDNA. Tube method was used for development of biofilms on glass slides and growth and exopolysaccharides production was measured after 18h, 24h, 36h, 48h, 72h and 96h of incubation. The Crystal violet assay was used for quantification of biofilms. Population and phenotypic variance were studied by the drop plate method. The hydrophobicity of strains was evaluated by the bacterial adhesion to apolar solvent test.

Results: Study showed that the subject isolates of *P. aeruginosa* adopted a biofilm life style after 36h of incubation at 35°C. After 24h the adhesion started, but it was reversible and easily dispersed by simple washing. However, after 36h the irreversible adhesion, difficult to disperse, was noticed. The biofilm consortia harbor three different phenotypes: i. wild types, showed typical *P. aeruginosa* characters on Cetrimide agar; ii. Slow growers, showed poor pigmentation and take >36h for colony development, and iii. Small colony variants (SCVs) are metabolically inactive very slow growing and producing pinpointed non pigmented colonies. Interestingly, increase of incubation time of biofilm consortia results in strong adhesion and dominance of SCVs. Comparative analysis showed that these phenotypes i.e. SCVs were highly hydrophobic and persistent in biofilm consortia due to the production of excessive amounts of exopolysaccharides.

Conclusion: This study showed that phenotypic heterogeneity is a characteristic feature of *P. aeruginosa* biofilms and all of these phenotypes have a major role in stability and persistence of biofilm consortia.
Comparison of ex vivo porcine and human corneas as models for bacterial keratitis caused by Pseudomonas aeruginosa - Okurowsk K

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Introduction: Bacterial keratitis is a typical biofilm infection. In Asia and Africa 1.5 - 2 million cases of infectious keratitis, caused by various species of bacteria and fungi, are recorded annually. LV Prasad Eye Institute in India alone recorded 1200 patients with infected cornea per year 30% of whom lose their vision due to lack of appropriate timely interventions. Pseudomonas aeruginosa is a typical pathogen isolated from patients with keratitis. Infections caused by P. aeruginosa are often antibiotic-resistant, involve biofilm formation and therefore difficult to treat.

Hypothesis and aims: The aim of this study was to determine the time taken by P. aeruginosa to establish a biofilm on ex vivo cornea models.

Methodology: We compared porcine and human ex vivo model of cornea infected with two strains of P. aeruginosa (PAO1 and PA14). Excised corneoscleral rims from porcine and human eyes were maintained in organ culture medium and infected using 10^6 cells at 37°C. Bacteria were introduced by making a wound using a scalpel and the progression of infection was followed by viable plate counts at frequent intervals up to 48 hours from inoculation. Transition of P. aeruginosa PA01 from an acute planktonic infection to a chronic biofilm-based infection was followed using a green fluorescent protein as a molecular marker. Fluorescent images were recorded using confocal microscopy at frequent intervals up to 48 hours from inoculation.

Results: Results of viable plate count analysis indicated that both strains of P. aeruginosa were able to establish an infection on the human and porcine cornea. Concurrent degradation of the corneal tissue was also observed. Significant biofilm formation by P. aeruginosa PA01 was seen from 18 hours of infection.

Conclusion: Infection progressed similarly in human and porcine cornea indicating that porcine cornea can be used as a reliable model for predicting the efficacy of novel early intervention strategies for treating Pseudomonas keratitis in humans.
38: Evaluation of the antibiofilm activity of a Tetrasodium-EDTA complex-polymer against *Pseudomonas aeruginosa* in the drip flow bioreactor model - Percival S.L

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**Introduction:** Biofilms are formed when bacterial cells adhere to a surface and each other and secrete extracellular polymeric substances (EPS), encasing themselves in a matrix. There is increasing evidence showing the presence of biofilms on surfaces, such as wounds, can lead to infection, inflammation and delayed wound healing.

**Hypothesis and aims:** The aim of this study was to evaluate a Tetrasodium-EDTA complex-polymer against a *Pseudomonas aeruginosa* biofilm formed on various platforms using the drip flow bioreactor model.

**Methodology:** Two models were developed using different surfaces and immature (24 H) and mature (48 H) biofilms of *Pseudomonas aeruginosa*. The first model was set up in accordance with ASTM E2647 13. This involved growing a biofilm for 48 H on a glass coupon. The second model was set up using adsorbent pads and membrane filters. Tetrasodium-EDTA complex-polymer were applied for 24 H before neutralising and either scraping or sonicating the samples. Biofilm density was determined by performing serial dilutions and plating onto agar.

**Results:** Tetrasodium-EDTA complex-polymer demonstrated antibiofilm activity against an immature and mature biofilm of *P. aeruginosa* in the drip flow bioreactor model, showing a significant log reduction in biofilm cell density.

**Conclusion:** Tetrasodium-EDTA complex-polymer demonstrated efficacy against *P. aeruginosa* biofilms and should be investigated further for treatment of acute and chronic wounds.
39: Development of a chronic *Pseudomonas* biofilm infection model in wax worms - *Robertson S.N*

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**Introduction:** The wax worm (*Galleria mellonella*) has gained acceptance as an alternative model for studying microbial infections and testing new antimicrobials. Notably, when used for *Pseudomonas aeruginosa* PAO1 infections, mortality is often rapid, indicating an acute infection. Chronic infections are often associated with biofilm formation, antimicrobial tolerance and long-term persistence. The *P. aeruginosa* Gac/Rsm system has been shown to be key in the switch from chronic to acute infection.

**Hypothesis and aims:** We hypothesised that knockouts of the Csr/Rsm system should display reduced mortality in the wax worm model resulting in a chronic biofilm-mediated infection. We aimed to test this to determine the suitability of *G. mellonella* as a chronic in vivo biofilm infection model using *rsmA* and *rsmN* mutants combined with visualization of the infection progression.

**Methodology:** PAO1 Rsm knockouts were used to infect *G. mellonella*. CFU counts were performed to determine if survival was due to clearance of infection and/or establish if proliferation occurred in vivo. Visualisation of infection was undertaken using lux expressing PAO1.

**Results:** Significantly increased survival was observed with Δ*rsmA* (p < 0.0001), Δ*rsmN* (p < 0.0346) & Δ*rsmAN* (p < 0.0001). CFU counts of Rsm knockout infected wax worms confirmed that clearance of the infection did not occur in the majority of wax worms, suggesting long term persistence of infection (> 72 h). Visualisation of PAO1 infection was carried out through bioluminescence detection.

**Conclusion:** We show that *P. aeruginosa* Rsm knockouts appear to produce a chronic-like infection in *G. mellonella*. We are currently validating this model through biofilm visualization and the testing of known antimicrobial and anti-virulence compounds.
**INTRODUCTION:** Hosts signals such as hormones, neurotransmitters or immune system molecules have been shown to modulate the bacterial physiology. Among them, catecholamines hormones epinephrine/norepinephrine, released by stress, physical effort or used therapeutically as inotrope were shown to affect bacterial behaviors of various Gram-negative bacteria. *Pseudomonas aeruginosa* is an opportunistic pathogen, often involved in nosocomial infections and responsible of chronic infections in immunocompromised patients, which is likely to be in presence of these hormones in human body.

**Hypothesis and aims:** Biofilm formation of *P. aeruginosa* is closely related to its virulence and is often implicated in chronic infections. We therefore decided to evaluate the effect of various concentrations of epinephrine on *P. aeruginosa* biofilm formation.

**Methodology:** Effect of epinephrine at different concentrations was examined on *P. aeruginosa* PAO1 biofilm formation under hydrodynamic conditions in a flow-cell system. Bacteria were allowed to attach to the glass side for 2h in presence of epinephrine, and a flow of LB medium (containing epinephrine) was then applied for 24h. Biofilm biovolume and architecture were monitored using Confocal Laser Scanning Microscopy.

**Results:** Biofilm formation of *P. aeruginosa* was found to be increased when continuously exposed to epinephrine and this effect depended on the hormone concentration used. A two-fold higher biovolume of the biofilm was seen when the bacteria were exposed to 10 µM of epinephrine. Moreover, microscopic image analysis also showed that epinephrine modified *P. aeruginosa* adhesion on abiotic surface by inducing cells aggregation.

**Conclusion:** In this work, *P. aeruginosa* seems to sense epinephrine stress hormone and respond by increasing its biofilm formation capacity. This result suggests that increase of epinephrine concentration in case of acute stress could promote biofilm formation of *P. aeruginosa*. A better understanding of these mechanisms and the identification of *P. aeruginosa* adrenergic putative sensor may be an interesting path to develop new antibacterial strategies against this clinical pathogen.
**Introduction:** All immersed materials are vulnerable to the bacterial colonization and their development in biofilm. The resulting health, economic and environmental issues involve developing materials which avoid the bacterial attachment with non-toxic properties. One possible approach is the use of amphiphilic copolymers, especially PDMS-PEG ones. However, PEG units are subject to oxidation phenomena. Therefore, the use of carbohydrates-coated surfaces appears as one of the best non-biocidal and non-toxic strategy. Indeed, sugars afford an extensively possibility of structural modulations with their numerous functional groups. Although pyranosides are largely described, the biological impacts of furanosides are still to be elucidated. Their absence in mammal species and their known bacteriostatic activities provide an interest as potential anti-bioadhesion agent.

**Methodology:** In this work, we examined the anti-bioadhesion activities of glass surfaces presenting simple monosaccharides such as D-glucose, D-galactose and D-mannose in both, pyranose and furanose configurations.

**Results:** The adhesion studies with *Pseudomonas aeruginosa* showed promising anti-bioadhesion activities from glycosidic surfaces which appeared to depend of the configuration. Molecular interactions and thermodynamic studies confirmed that the adhesion engaged long-range interactions, rather than the other specific interactions. Finally, preliminary assays on biofilm development exhibited promising anti-biofilm results from glycoside-functionalized surfaces.

**Conclusion:** To conclude, these encouraging results add more evidences that a few atomic layers of monosaccharides may minimize the bacterial adhesion and the biofilm maturation.
42: Changed antibody response following lung transplantation in cystic fibrosis patients with *Pseudomonas aeruginosa* biofilm infections - Schwensen H

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**Introduction:** Specific *Pseudomonas aeruginosa* (PA) precipitating immunoglobulin G antibodies in serum are correlated with PA biofilm infection and are used as diagnostic and prognostic markers in cystic fibrosis (CF).

**Hypothesis and aims:** The aim of this study was to examine the change of antibody response against PA biofilm infections following bilateral sequential lung transplantation (LTx) in CF.

**Methodology:** PA antibodies and airway bacteriology were retrospectively evaluated in 20 CF patients with chronic PA infection, who underwent LTx between 2001 and 2016 at Rigshospitalet, Copenhagen. The number of precipitating anti-PA antibodies were examined one year before LTx and up to five years after LTx. Monthly airway culture results were examined in the five-year period.

**Results:** Nine patients were re-infected with PA within the first month after LTx and remained infected in the entire five-year observation period. Seven patients became infected later in the observation period. All patients experienced a drop in PA antibodies following LTx. Median antibody drop from one year pre-LTx to one year post-LTx was significant (p<0.0001). No patients reached the pre-LTx precipitin level in the following five years, and the median pre-LTx level was higher than the median highest post-LTx level (p=0.002). Total IgG had a similar significant drop after LTx (p=0.0007). Interestingly, PA antibody titres post-LTx were similar to the antibody titres at the beginning of PA biofilm infection years earlier.

**Conclusion:** Following LTx a significant and continuous reduction in antibodies against PA biofilm infection was observed, which was consistent with the post-LTx course of total IgG. PA antibody titres post-LTx were similar to titres at the beginning of PA biofilm infection. Post-transplantation immunosuppressants have a documented impact, however, other factors may be involved in the antibody reduction.
43: Drug resistance and biofilm production among *Pseudomonas aeruginosa* clinical isolates in a tertiary care hospital of Nepal - *Shrestha R*

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**Introduction:** Clinical isolates of *Pseudomonas aeruginosa* often exhibit multidrug resistance due to their inherent ability to form biofilms. Drug resistance in *Pseudomonas aeruginosa* is a major clinical problem, especially in the management of patients with nosocomial infections and those admitted to ICUs with indwelling medical devices.

**Hypothesis and aims:** To evaluate the biofilm forming abilities of the clinical isolates of *Pseudomonas aeruginosa* and to correlate biofilm formation with antibiotic resistance.

**Methodology:** A total of 90 consecutive isolates of *P. aeruginosa* obtained from various specimens collected from patients visiting the Manipal Teaching Hospital, Pokhara, Nepal between January 2018 - October 2018 were studied. Isolates were identified by standard microbiological methods. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method. All the isolates were tested for their biofilm forming abilities by employing the tissue culture plate assay.

**Results:** Of the 90 *Pseudomonas aeruginosa* isolates maximum i.e 42 (46.6%) were from patients in the age group of > 50 years. Majority (30; 33.3%) of the isolates were obtained from sputum samples. However, percentage isolation from other specimens like urine, endotracheal tube (ETT), pus, eye specimens and blood were 18.9%, 16.7%, 16.7%, 7.8% and 6.7% respectively. All the isolates were sensitive to polymixin B and colistin, 91.1% of the organisms were sensitive to imipenem, and more than 80% to aminoglycosides (80% to gentamicin, 83.3% to amikacin). A total of 29 (32.2%) organisms were biofilm producers. Maximum numbers of biofilm producing strains were obtained from ETT (8 of 15; 53.3%), pus (8 of 15; 53.3%) and blood (2 of 6; 33.3%) i.e from all invasive sites. None of the isolates from noninvasive specimens such as conjunctival swabs were biofilm positive. Significantly higher numbers of biofilm producers (23 of 29; 79.3%) were found to be multidrug resistant as compared to non-biofilm (6 of 61; 9.8%) producers (p=0.000).

**Conclusion:** *Pseudomonas aeruginosa* colonization leading to biofilm formation in deep seated tissues and on indwelling devices is a therapeutic challenge as majority of the isolates would be recalcitrant to commonly used antipseudomonal drugs. Effective monitoring of drug resistance patterns in all *Pseudomonas* clinical isolates should be a prerequisite for successful patient management.
**44: Pseudomonas aeruginosa** exopolysaccharide Psl engages host C-type lectin receptors - Singh S

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**Introduction:** Chronic *Pseudomonas aeruginosa* (PA) infection is characterised by the presence of drug and host-resistant biofilms, and is especially devastating in conditions such as cystic fibrosis and burns. PA biofilm formation is reliant on the exopolysaccharides (EPS) Psl and Pel. Additionally, there is evidence that PA EPS modifies host immunity. However, the host receptors involved in PA EPS recognition are unknown.

**Hypothesis and aims:** Based its mannose-rich structure, we hypothesised that Psl would engage host C-type lectin receptors (CLRs) on immune cells, thereby modulating immunity to PA biofilms. The aims were; 1) to prepare and characterise purified Psl from PA biofilms; 2) to determine whether mannose receptor (MR), DC-SIGN, and Dectin-2 bind to Psl; 3) to study the effect of Psl on human immune cells.

**Methodology:** Psl from PAO1ΔwspFΔpel was isolated and characterised. Binding of purified Psl to CLRs was determined by ELISA, bio-layer interferometry, confocal microscopy, and inhibition of sugar uptake by eukaryotic cells expressing specific CLRs. Human dendritic cells (huDCs) were treated with Psl, following which: 1) CLR location was visualised, and 2) the cytokine profile was quantified at 4 and 24 h.

**Results:** Psl preps were found to be predominantly carbohydrate with 1-3% protein. The carbohydrate component consisted of 87% mannose, 15% glucose, and ~3% each rhamnose and galactose. Lipopolysaccharide (LPS) presence in the preps could not be ruled out. Psl bound MR, DC-SIGN, and Dectin-2 in a dose-dependent, calcium-dependent manner. Pre-incubation with Psl reduced the uptake of MR and DC-SIGN ligands in cell lines expressing DC-SIGN/MR and huDCs. The cytokine profile of huDCs treated with Psl was similar to that of LPS-treated cells. However, there was an indication that Psl could modulate huDC responses to infectious stimuli.

**Conclusion:** Psl binds specifically to MR, DC-SIGN, and Dectin-2. The biological consequences of this binding are being investigated.
45: The use of nitric oxide donor pro-drugs to tackle *Pseudomonas aeruginosa* biofilms - Soren O


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**Introduction:** For cystic fibrosis (CF) patients, *Pseudomonas aeruginosa* poses an immense therapeutic burden. Lung inflammation, bronchiectasis and respiratory function decline are heavily associated with both recurrent and chronic pulmonary infections. Conventional antibiotics are seldom able to eradicate the infection as it is heavily associated with the biofilm mode of growth. Previous research by our group has shown low dose nitric oxide (NO) can disperse *P. aeruginosa* biofilms and opens the door to novel therapeutic regimes.

**Hypothesis and aims:** In this study, NO-releasing prodrug compounds, termed cephalosporin-3'-diazeniumdiolates are investigated as a potential novel treatment strategy. Cleavage of the compounds is dependent on bacterial specific enzyme β-lactamase, hence NO is only released at the site of infection and minimizes the risk of systemic effects. Previous work has shown these compounds to disperse biofilms formed by laboratory isolate PAO1; here we investigated the efficacy of these agents against clinical CF isolates of *P. aeruginosa*.

**Methodology and Results:** The release of NO from the compounds was analyzed using a chemiluminescence system, with results confirming the presence of NO following addition of penicillinase. Biofilms formed by PAO1 and multiple clinical CF isolates demonstrated a dose-dependent reduction in total biomass following treatment with lead compound DEA-CP (DEA-NONOate cephalosporin prodrug), as quantified by crystal violet staining. Using confocal laser scanning microscopy, we were able to demonstrate the ability of DEA-CP to improve to efficacy of antibiotics against biofilms of a clinical *P. aeruginosa* isolate. Furthermore, newer generation cephalosporin-3'-diazeniumdiolates were developed and we were able to demonstrate evidence of these compounds having both anti-biofilm and anti-microbial effects.

**Conclusion:** Overall, these data suggest that these novel NO-releasing products offer a potential new treatment for *P. aeruginosa* infection in CF, able to subvert tolerance mechanisms associated with the biofilm phenotype and render cells more susceptible to conventional antibiotics.
Introduction: *Pseudomonas aeruginosa* represents one of the top priority pathogens according to the WHO due to its high level of antimicrobial resistance. This organism controls the production of virulence traits at the bacterial population level through quorum sensing (QS). *P. aeruginosa* has several QS systems, one of which, the Pseudomonas Quinolone System (*pqs*), uses 2-alkyl-quinolones (AQ) as signal molecules. The major AQ signal molecule in *P. aeruginosa* is 2-heptyl-3-hydroxy-4(1H)-quinolone (PQS) which controls the expression of the *pqsABCDE* operon involved in the biosynthesis of AQs. This operon is under the transcriptional control of the LysR-type transcriptional regulator PqsR. The *pqs* system plays a key role in controlling the production of virulence factors and biofilm formation representing a key drug target for virulence attenuation.

Hypothesis and aims: To identify molecules that are able to inhibit the interaction of PQS with PqsR leading attenuation the virulence of this organism, sensitising biofilms to the action of antibiotics.

Methodology: *In silico* screening was employed to identify ligands that inhibit the interaction of PQS with PqsR. This was then followed by validation using a bioreporter assay. The identified hits underwent a structure activity relationship to enhance their affinity for PqsR and improve their physiochemical properties. The antagonists were also subjected to a series of phenotypic analyses using *P. aeruginosa* cultures including pyocyanin, AQ signal quantitation and biofilm inhibition assays.

Results: We have identified a highly potent PqsR inhibitor (SEN089) which has significantly reduced pyocyanin expression and production of AQ signals. Moreover, SEN089 sensitised biofilm to tobramycin treatment with almost complete eradication within 6 h.

Conclusion: SEN089 represents promising novel anti-virulence compound against *P. aeruginosa* infections as it sensitises biofilm to antibiotic treatment.
47: Outer membrane vesicle formation by *Pseudomonas aeruginosa* biofilm cells - Tashiro Y

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**Introduction:** Many bacteria secrete outer membrane vesicles (OMVs) from bacterial surface to extracellular milieu. OMVs have important roles in several biological functions, including efflux of cellular substances, delivery of genetic materials and virulence. While OMV secretion increases at the biofilm condition, the mechanism on high MV secretion under the biofilm condition has not fully understood.

**Hypothesis and aims:** We hypothesized that the mechanism of OMV biogenesis at planktonic and biofilm conditions are different. The aim of this study is to understand the mechanism on OMV biogenesis at biofilm condition in *Pseudomonas aeruginosa*.

**Methodology:** OMVs are extracted from the supernatant of shaking culture (planktonic condition) and static culture (biofilm condition) of *P. aeruginosa* PAO1 by ultracentrifuge. OMVs were quantified using lipophilic dye FM4-64. The composition of phospholipid was analyzed by thin liquid chromatography (TLC) and fatty acid composition is analyzed by gas chromatography. Anionic phospholipid is detected by the 10-N-nonyl acridine orange (NAO).

**Results:** OMV formation at biofilm condition was significantly more than that at planktonic condition. OMV formation was not enhanced in extracellular polymeric substance synthesis mutants (*pel* or *psl*). The TLC analysis indicated that the ratio of cardiolipin increases at biofilm. The ratio of unsaturated fatty acids also increased at biofilm condition. Cardiolipin was distributed in planktonic cells but localized heterogeneously at biofilm cells; it was localized at cell poles and OMV-forming sites.

**Conclusion:** We found that OMVs are secreted at cardiolipin-rich sites in biofilm cells. These results provide a new insight into the importance of phospholipid distribution on OMV secretion in biofilm cells.
Increased intracellular cyclic-di-AMP levels sensitise Streptococcus gallolyticus subsp. gallolyticus to osmotic stress and reduce biofilm formation and adherence on intestinal cells - Wooi Keong Teh

Wooi Keong Teh, Shaynoor Dramsi, Tim Tolker-Nielsen, Liang Yang, Michael Givskov

Introduction:

Streptococcus gallolyticus subsp. gallolyticus (shortened to S. gallolyticus) is an emerging pathogen responsible for septicemia and infective endocarditis in the elderly, and has a strong association with the occurrence of colorectal cancer in endocarditis patients. Previous studies have identified a few S. gallolyticus virulence factors such as Pil1 and Pil3 pilus, however, not much is known about the signal(s) governing the induction of virulence.

Hypothesis and aims:

We hypothesised that the second messenger c-di-AMP may be important for the pathogenicity of S. gallolyticus through its regulation on virulence factors expression and biofilm formation. We therefore aimed to investigate the regulatory roles of c-di-AMP in S. gallolyticus.

Methodology:

We first constructed a S. gallolyticus c-di-AMP phosphodiesterase deletion mutant. We subsequently characterised the mutant and investigated the mutant’s ability in surface attachment and biofilm formation. We also performed a genomic-wide transcriptomic analysis to better understand the regulatory roles of c-di-AMP.

Results:

We found that compared to the parental strain, S. gallolyticus c-di-AMP phosphodiesterase deletion mutant displayed a 1.5-fold higher intracellular c-di-AMP levels, is more sensitive to osmotic stress and is morphologically smaller. We also found that the mutant formed less biofilm, attached less efficiently and formed less cell aggregates on human intestinal cells. Our genome-wide transcriptomic analysis indicated that c-di-AMP regulates many biological processes in S. gallolyticus, including the expression of various ABC transporters and disease-associated genes encoding bacteriocin and Pil3 pilus.

Conclusion:

We showed that c-di-AMP plays pleiotropic roles in S. gallolyticus, controlling the tolerance to osmotic stress, cell size, biofilm formation on abiotic surfaces, adherence and cell aggregation on human intestinal cells, expression of Pil3 pilus and production of bacteriocin. These data indicates that c-di-AMP may constitute a key regulatory molecule for S. gallolyticus host colonisation and pathogenesis.
49: Incorporating a cleaning step in the sanitation of drinking water systems of broilers reduces biofilms and inhibits the regrowth of multidrug-resistant *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* - Vackier T

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**Introduction:** A previous study, it was shown that despite regular disinfection, the drinking water systems of broiler houses showed high presence of *S. maltophilia* and *P. aeruginosa*. These bacteria are opportunistic pathogens and are frequently associated with respiratory tract infections and their intrinsic resistance to many drugs make treatment of these infections difficult. Although most of these infections are nosocomial, cases of community-acquired *P. aeruginosa* pneumonia are reported and the prevalence of community-acquired *S. maltophilia* infections is increasing. However, the transmission routes of these bacteria are poorly understood.

**Hypothesis and aims:** The hypothesis of this study was that the current sanitation of the drinking water systems of broilers insufficiently removed biofilms of multidrug resistant *S. maltophilia* and *P. aeruginosa* from which they can spread to wider communities. The aim of the study was to optimize the sanitation protocol to further reduce biofilms of these microorganisms in the drinking water system.

**Methodology:** The disc diffusion method was used to evaluate the antibiotic resistance, according to CLSI guidelines. Biofilms were grown in a biofilm reactor model, mimicking real-life conditions, under continuous flow. Sanitation was optimized by combining a cleaning step prior to disinfection as opposed to the current practices which consist of only a disinfection step.

**Results:** For *P. aeruginosa* the majority of the isolates (63%) were extensively drug-resistant and only 11% didn't show multidrug-resistance. For *S. maltophilia*, the isolates were predominantly (75%) multidrug-resistant with only 8% not exhibiting multidrug-resistance. A sanitation protocol consisting of a combination of a cleaning and disinfection step further reduced the microbial load and inhibited the ability of the biofilm to regrow compared to a sanitation protocol consisting of only a disinfection step.

**Conclusion:** The results suggest the incorporation of a cleaning step in the sanitation protocol of the drinking water system of broiler to reduce biofilms of multidrug-resistant bacteria.
50: Identification of genes involved in *Pseudomonas aeruginosa* biofilm resistance to antibiotics - Valentin J

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Introduction: Bacteria living in biofilms tolerate much higher antibiotic concentrations compared to planktonic bacteria and survive long enough to evolve antimicrobial resistance (AMR). Biofilms can cause persistent, hard-to-treat infections and exhibit intrinsic properties that promote the development and transmission of AMR. *Pseudomonas aeruginosa* is responsible for many biofilm-related infections and listed as top priority for research and development of new antibiotics by the World Health Organization.

Hypothesis and aims: We speculate that there are biofilm specific genes responsible for antibiotic resistant bacteria in a biofilm. Thus, our aim was to identify the genes involved in resistance of *P. aeruginosa* in a biofilm. By doing so, new bacterial targets could be highlighted and be used to develop antibacterial agents or compounds improving the efficiency of current antibiotics.

Methodology: A *P. aeruginosa* mutant library was screened to assess the resistance of biofilms toward different antibiotics. Biofilms formed by each mutant were exposed to different concentrations of antibiotics, e.g. colistin, followed by re-growth in the absence of the antibiotic.

Results: Measuring the ability of biofilms to overcome antibiotic treatment revealed the importance of several genes. The mutant PA3552 missing ArnB, a known resistance to colistin, was used as positive control. Among others, we identified the response regulator CbrB, involved in nutrient uptake, as important for biofilm formation and subsequent biofilm resistance. We also found that a gene annotated as probable transcriptional regulator participated in colistin resistance and its absence led to the eradication of the biofilm just like PA3552. Several other unknown genes involved in resistance have been identified and are currently under investigation.

Conclusion: Screening a *P. aeruginosa* mutant library allowed us to identify key genes involved in biofilm resistance. Characterization of promising genes is in progress to understand the underlying mechanism of resistance.
In vitro synergistic activity of fosfomycin, ciprofloxacin and gentamicin combinations against Pseudomonas aeruginosa biofilms - Wang L

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Introduction: Ciprofloxacin is recommended as anti-biofilm therapy for P. aeruginosa periprosthetic joint infection. With ciprofloxacin monotherapy, resistance in gram-negative bacteria was observed.

Hypothesis and aims: We evaluated in vitro synergistic activity of fosfomycin, ciprofloxacin and gentamicin combinations against biofilms formed by P. aeruginosa strains.

Methodology: P. aeruginosa ATCC 27853 and 7 clinical isolates were used. MIC values were determined by Etest. Biofilms were formed on porous sintered glass beads for 24h and exposed to antibiotics for further 24h. Viability of bacteria on the glass beads after antibiotic treatment was detected by cfu counting of the sonicated beads. The minimum biofilm eradication concentration (MBEC) was defined as the lowest concentration of antibiotic required to kill biofilm cells (no colonies on plate counts; <20cfu/mL). Synergistic activity against biofilm was evaluated by calculation of the fractional inhibitory concentration index (FICI). According to it, synergism was defined as MBEC reduction by 2-fold compared to the lowest MBEC of single substance.

Results: Most strains were susceptible to tested antibiotics, except Pa6 (resistant to gentamicin) and Pa7 (resistant to ciprofloxacin). The biofilm susceptibility to each antibiotic varied widely among clinical isolates. Among 8 tested isolates, synergism was observed in 4 isolates (50%) with fosfomycin/ciprofloxacin, in 5 isolates (62.5%) with fosfomycin/ ciprofloxacin and 6 isolates (75%) with ciprofloxacin/gentamicin.

Conclusion: The gentamicin/ciprofloxacin combination showed the highest activity against P. aeruginosa biofilms, flowed by fosfomycin/gentamicin and fosfomycin/ ciprofloxacin combination.
**Wednesday 4th September**

**Wounds & Skin**

**52: Atopic dermatitis and healthy skin microbiota - Bay L**

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**Introduction:** Human skin microbiota protects against pathogens and maintain healthy skin, but if the microbial balance is disturbed, skin disorder and infections may occur. In Atopic Dermatitis (AD) skin, the impaired barrier function leads to alteration of the microbial composition, and during flares of eczema, an immense reduction is expected within the microbial diversity.

**Hypothesis and aims:** We hypothesize that the distribution and composition of the microbiota in AD skin is altered relative to healthy skin. Our aim was to investigate epidermal skin microbiota within different layers and habitats of AD skin.

**Methodology:** This study investigated the epidermal microbiota in 15 tape strip layers, at three skin habitats (hand back, elbow pit and chin) in 15 healthy volunteers and 15 AD patients suffering from flares of eczema at hand back and/or in elbow pit. The tape strips were cultivated and the bacteria were identified by matrix-assisted laser desorption-ionization – time of flight mass spectrometry (MALDI-TOF) and visualized by confocal laser scanning microscopy (CLSM).

**Results:** We demonstrated that in AD skin, the bacterial load was significantly (*) increased and the bacterial composition was altered compared to the composition in healthy skin. In particular Staphylococcus aureus was more frequently present in lesional than in non-lesional AD skin. Furthermore, the microbiota highly differed between habitats but not between layers, except in healthy sebaceous skin in which *Cutibacterium acnes* was significantly (*) more present in the mid layer of epidermis. Lastly, CLSM images confirmed our findings by visualizing the distribution and size of bacterial aggregates in different tape strip layers of epidermal skin.

**Conclusion:** We found an unexpected increase in bacterial genera within flares of eczema in lesional AD skin on the hand back and in the elbow pit. The dominance of *Staphylococcus* spp., including *S. aureus*, was particularly significant to the AD lesions.
Introduction: Chronic wounds cannot heal due to impairment of tissue regeneration process. This mainly by the persistent infection of multispecies biofilms, driving chronic inflammation, evasion of host's immune responses, induction of hypoxia, and tissue degradation. Most animal biofilm infection models are based on single-species biofilms. Moreover, how these biofilms affect each stage of the regeneration process remains unclear. To evaluate novel treatments, in vivo robust models resembling infection processes, species diversity and biofilm related clinical symptoms in chronic wounds patients, must be developed.

Hypothesis and aims: To establish a murine model of multispecies biofilm infected wounds, and characterize their effect on general health parameters and skin regeneration.

Methodology: Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis were inoculated at $10^2$ cells/ml each on artificial skin scaffolds, and incubated in M9 media for 24 h. Bacteria-containing scaffolds were implanted onto full skin bilateral wounds in female C57/BL6 mice. Animals were daily treated with ciprofloxacin (30mg/kg IP) and supervised for general health parameters. After 10 days mice were euthanized, and lymphoid organs, blood and scaffolds were collected for weight, CFU counting, bacterial presence by MALDI-TOF MS, and histologic analyses.

Results: Bacteria-containing scaffolds implanted on skin turned into biofilm-infected wounds. At early stages, mice suffered fever and 10-15% weight loss, recovering at latest days. Wounds showed infection with $>10^8$ CFU/g scaffold and presence of the three bacterial species. Blood samples at day 10 did not show viable bacteria by plating in blood agar media. Size and weight of lymphoid organs was increased by the biofilm infection, whereas tissue histology showed a diminished skin integrity.

Conclusion: Implantation of seeded scaffolds onto wounds resulted in multispecies biofilm-infections, with high bacterial loads and impaired skin regeneration. Health parameters were affected at early stages, but the inflammatory systemic response was sustained, validating this multispecies biofilm-infected wounds model.
54: Interleukin 1-α and VEGF support the growth and persistence of biofilm-growing *Cutibacterium acnes* in individuals with acne - *Cavallo I*

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Introduction: Acne vulgaris is a common inflammatory disorder of the sebaceous follicles, affecting more than 80% of young adolescents and often persisting into adulthood. *Cutibacterium acnes* plays a role in the pathogenesis of acne lesions, although the mechanism(s) are poorly understood.

Hypothesis and aims: The study investigated the role of *C. acnes*, and the impact of biofilm production in acne lesions. Besides, we assessed the effect of skin inflammatory molecules, in bacterial growth and persistence.

Methodology: Samples were collected from the healthy skin, microcomedon, comedon, papule and pustule, respectively, from 30 acne patients. Biofilm production was measured by the clinical BioFilm Ring Test in all *C. acnes* isolates. Tape adsorption tests were performed on the skin of acne patients and in 10 healthy controls, to measure the levels of skin inflammatory molecules. *In vitro* studies were performed to evaluate the response of *C. acnes* isolates to different concentrations of inflammatory molecules.

Results: *C. acnes* strains were found in all acne patients. Nevertheless, a significantly higher prevalence of *C. acnes* was found with both inflamed (papule and pustule) and noninflamed (comedon) acne lesions, as compared with microcomedon and healthy skin. All the strains analyzed were able to produce biofilm. The level of interleukin (IL)-α, and vascular endothelial growth factor (VEGF) were higher in the skin of acne patients as compared to control subjects. Additionally, both IL-1α and VEGF selectively promoted a concentration-dependent increase of *C. acnes* growth.

Conclusion: 1) The presence of *C. acnes* increases according to the progression of acne lesions; 2) the increased level of IL-1α and VEGF observed in the skin of acne patients may play a role at promoting the growth of *C. acnes*; 3) biofilm production by *C. acnes* may contribute at sustaining bacterial adhesion and chronic persistence in acne.
55: Relation between antibiotic susceptibility and biofilm formation capacity in strains isolated from infected orthopaedic devices - Coraça-Huber D.C

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Introduction: Several studies have been performed with the aim to understand the mechanisms involved in biofilm formation and its influences on the activity of antibiotic substances. In this study, the relation between antibiotic susceptibility and biofilm forming capacity in strains isolated from patients undergoing implant-related infections treatment was evaluated.

Methodology: Antibiotic susceptibility tests were carried out on strains isolated from patients with implant-related infections. Also, the ability of these isolates to form biofilms in vitro was evaluated by counting the colony forming units, by measuring the metabolic activity of biofilm cells and by analysing the morphology of the formed biofilms using scanning electron microscopy.

Results: In total 140 isolated strains were selected for this study. A significant difference on the capacity of biofilm formation was observed between the isolates (P<0.0001). The number of colony forming units and the metabolic activity of the bacteria forming biofilms varied between the isolates. The morphological analysis showed that not all strains formed biofilms with three-dimensional structures covered with slime-like substances. Some of the strains which showed multiple antibiotic resistances were not able to form biofilms in vitro. The opposite was also observed.

Conclusion: The tolerance to antibiotic substances is not directly related to the capacity of forming biofilms in vitro in bacteria isolated from infected orthopaedic devices. These findings can lead to further studies aiming to identify the link between antibiotic resistance mechanisms and activation of biofilm genes in strains infecting orthopaedic implants.
56: Eradication of wound-relevant pre-formed biofilms following release of combination antibiotics from absorbable beads *in-vitro* - Delury C

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Introduction: Successful treatment of periprosthetic joint infection requires surgical intervention alongside antimicrobial therapy targeting surface-adhering microorganisms. The objective of the study was to assess the ability of synthetic recrystallised calcium sulfate beads* (SRCS) or β-tricalcium phosphate/calcium sulfate bi-phasic beads** (TPCS) containing a mixture of vancomycin and gentamicin (VG) or vancomycin and tobramycin (VT) to effectively eradicate pre-formed biofilms *in-vitro*.

Methodology: Single species *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms were established on polycarbonate coupons within a CDC biofilm reactor. Biofilms were established in a batch model for 72 hours prior to processing. Biofilms were exposed to a challenge plate containing suspended SRCS beads or TPCS beads containing a mixture of VG or VT. Positive and negative controls were tested concurrently. All testing was performed in triplicate. The challenge plate was incubated for 24 hours at 37°C ± 2°C. Students T-Tests were performed on the raw data to determine the significant effect of the test items.

Results: Negative controls retained $6.78 \pm 0.23 \text{Log}_{10}\text{CFU/mL}^{-1}$ and $6.60 \pm 0.23 \text{Log}_{10}\text{CFU/mL}^{-1}$ from *P. aeruginosa* and *S. aureus* biofilms respectively. No viable organisms were recovered from biofilms exposed to the positive control or those exposed to SRCS or TPCS beads containing a mixture of VG/VT within detection limits. This equated to an average log reduction in *P. aeruginosa* of >5.78 Log_{10} CFU/mL^{-1} and an average log reduction in *S. aureus* of >5.60 Log_{10} CFU/mL^{-1} (p < 0.001) against SRCS beads and an average log reduction in *P. aeruginosa* of >5.94 Log_{10} CFU/mL^{-1} and an average log reduction in *S. aureus* of >4.08 Log_{10} CFU/mL^{-1} (p < 0.001) against TPCS beads.

Conclusion: Exposure of the biofilm to SRCS and TPCS beads containing a mixture of VG or VT resulted in eradication of pre-formed biofilms in the test method described. Further assessment is required to confirm clinical performance.
57. Micro/Nanostructured PBSA Membranes With Antibiofilm Properties As Chronic Wound Dressings - Naila Bou Haidar

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Bacterial biofilms present in roughly 80% of chronic wounds, and are a major obstacle to healing [1]. In such cases, traditional antibiotic therapies remain most often ineffective. Antibiotic tolerance or resistance of biofilm bacteria may increase a 1000-fold compared to that for planktonic bacteria [2]. Moreover, antibiotics do not specifically target the biofilm. Hence, the development of new therapeutic strategies targeting the biofilm, are essential and urgent [3]. In that respect, we propose, here, the development of a micro/nanostructured asymmetric poly(butylene-succinate-co-adipate) (PBSA) membrane (AM) for the controlled delivery of a protein (Discobolin B, noted DB) specifically aiming the biofilm matrix (Figure 1) [4].

The use of hydrophilic porogen agents, polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG), were shown to promote greater porosity along with pore interconnections. Furthermore, increased porosity did not weaken the mechanical strength of the formed membranes in dry and wet state. Using bovine serum albumin (BSA) as a model protein, we demonstrated that the protein loading and release from the PBSA membranes were influenced by membrane microstructure (e.g. porosity) and likely residual porogen. Furthermore, protein kinetics release curves showed a fast initial slope followed by a second slower release over 24 hours. Cross-sectional confocal laser scanning microscopy (CLSM) images revealed a heterogeneous distribution of fluorescein isothiocyanate model protein (FITC-BSA) throughout the entire membrane, in both the dense and porous layer. PBSA membranes loaded with DB, were highly efficient in inhibiting and dispersing pre-formed Staphylococcus epidermidis biofilms. The PBSA-PVP AM displayed the highest antibiofilm activity. Furthermore, in vitro cytotoxicity assays using two-dimensional monolayer cultures of human HaCaT cells and a fully differentiated normal human epidermis RHE model revealed that both unloaded and DB-loaded PBSA-PVP membranes exhibited good biocompatibility, and hence are likely adequate for wound dressing applications.

REFERENCES
**Introduction:** Infection has long been known as an important factor in delayed wound healing. More recently, an examination of clinical wound samples indicated biofilms were present in at least 60% of the chronic wounds. Furthermore, preclinical models of wound biofilm have provided evidence that biofilm contributes to delayed wound healing.

**Hypothesis and aims:** Activity of cadexomer iodine (CI) products† was assessed over their wear time *in vitro* against a broad spectrum panel of wound pathogens and against mature biofilms in a model incorporating clinically relevant features.

**Methodology:** CI† were tested against a broad-spectrum of microorganisms over 0.5-72 h treatment periods using a log reduction method (n=6). CI† were further challenged against mature (72 h) *S. aureus* and *P. aeruginosa* colony biofilms in a clinically relevant wound model incorporating 3.9% (w/v) bovine serum albumin and 0.5% (v/v) blood for 72 h (n=10).

**Results:** CI† achieved mean log reductions (compared to 0 h control) ≥4.00 colony forming units (CFU)/sample at 0.5 h and were maintained for the 72 h treatment period against all wound pathogens tested. CI† achieved mean log reductions (compared to gauze control) in excess of 9.00 CFU/sample against *S. aureus* & *P. aeruginosa* biofilms. Imaging techniques further indicated the capability of CI to physically disrupt the protective biofilm matrix.

**Conclusion:** Clinically CI has been shown to have a significant effect on the bioburden of chronic wounds and produces higher healing rates in venous leg ulcers compared to standard care. This combined with rapid & sustained broad spectrum antimicrobial activity and the substantial capacity to disrupt and kill mature biofilms in the presence of clinically relevant factors, further supports a role for CI in the effective treatment of chronic wounds.
Introduction: Chronic wounds are a major healthcare burden, associated with high morbidity and reduced quality of life. Microbial infections, often presenting as polymicrobial biofilms, are the single most common cause of impaired wound healing. An infected chronic wound is a complex microenvironment, with interactions between host cells (keratinocytes and fibroblasts), matrix components (collagen, elastin) and microbial elements. Current in vitro and microfluidic model systems of chronic wound infections include select host and matrix elements, as well as microbial components, but fail to recapitulate the complex 3D microarchitecture and microenvironment of the infected wound bed.

Hypothesis and aims: We are developing a biomimetic model of chronic wound infection which recapitulates the complex infected wound microenvironment, including the 3D microarchitecture of various key components, and their interactions with each other. Using a Transwell platform, host cellular elements such as fibroblasts and keratinocytes will be spatially co-cultured in a stratified manner, along with matrix components, to mimic the wound bed.

Methodology: To recapitulate the infected microenvironment, wound co-pathogens P. aeruginosa and S. aureus will be grown as multi-species biofilms on top of and in close association with host cell layers. This will reconstitute the 3D microarchitecture of the infected wound bed. To validate the pathophysiology of this microsystem, we will study key parameters in host cells such as cell viability and proliferation, production of growth factors, enzymes, and signaling elements. Biofilms will be evaluated for their density, distribution, susceptibility to antibiotics, and production of bacterial metabolites.

Conclusion: In this manner, this biomimetic model will not only faithfully recapitulate the microarchitecture and microenvironment of the chronic infected wound bed, but also provide selective and precise control on key contributing factors. This could be leveraged to study the composite effects of current and novel therapeutic approaches on various contributing factors in the chronic wound infection microenvironment.
60: A biomimetic, simulant wound fluid to investigate chronic wound biofilm pathogenesis and response to therapy - Kaushik K.S

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Introduction: Chronic, non-healing wounds are characterized by the presence of recalcitrant microbial biofilms, along with host cells, matrix elements, and immune signals, constituting a complex wound infection microenvironment. Dynamic and interactive communication across these factors, influences infection resolution and therapeutic outcome. The components mediating these effects are reflected in the surrounding wound fluid, composed of a range of nutritional substrates, signaling and inflammatory factors, and remodeling enzymes. Chronic wound fluid from patients has been shown to affect various components of the infection microenvironment, including host cell proliferation and migration, and degradation of matrix components. However, its effects on biofilm formation, physiology and response to therapeutics have not been explored. This is important given that wound biofilm formation is intricately associated with the surrounding milieu and the constituents of wound fluid are likely to affect bacterial processes.

Hypothesis and aims: We aim to develop a biomimetic, simulant in vitro wound fluid that mimics the composition of nutrients, growth factors, inflammatory mediators, and enzymes, to that found in typical chronic wound fluid. The simulant wound fluid will be leveraged to study the effects of biofilm formation, structure and physiology in this microenvironment, as well as test the effects of treatment approaches. We hypothesize that this simulant wound fluid will closely recapitulate the surrounding milieu of the wound bed, thereby enabling a better understanding of biofilm formation and effects of therapeutics under clinically relevant conditions.

Methodology: A simulant wound fluid will be developed with relevant components and their concentrations, based on previous literature. Using this in vitro wound fluid, a range of assays will be performed to assess bacterial biofilm growth, structure and function, such as biofilm formation, microscopic architecture of biofilms, susceptibility of biofilms to antibiotic treatments, microbial signaling and gene expression. For these assays, we will use common wound copathogens Pseudomonas aeruginosa and Staphylococcus aureus, which will also recapitulate the polymicrobial state of bacterial biofilms in chronic wounds.

Conclusion: While the wound microenvironment is known to affect the infection state, little is known of the impact of the chronic wound fluid on biofilm development, structure, and susceptibility. This simulant wound fluid will provide a biomimetic, clinically relevant milieu to better understand the dynamics of chronic wound infection biofilms. Understanding the impact of the native nutrient, inflammatory and signaling conditions, on biofilm physiology and susceptibility can provide insights into the clinical state, thereby leading to new therapeutic tools and targets.
**61: Impact of the bone microenvironment on *Staphylococcus aureus* adhesion (ORAL) - Lamret F**

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**Introduction:** Bone joint infections due to implanted materials are mostly due to *Staphylococcus aureus*. To fight this kind of infections, prevention appears to be the best strategy. Thus, biofilm initiation in bone context needs to be better deciphered and antibiofilm strategies to be improved.

**Hypothesis and aims:** Our hypothesis is that the very complex and interconnected bone microenvironment increases the bacterial adhesion and biofilm maturation. Our final aim is to develop an in vitro model that mimicked this specific microenvironment in order to screen different antimicrobial molecules.

**Methodology:** To identify the main factors that influence biofilm formation in bone microenvironment, we determined biofilm biomass and number of live adhered bacteria in static model, with microscopy analysis to support our results. Different factors of bone microenvironment were tested: starvation, low oxygen rate, excess of magnesium and presence of bone cell products. The supernatant of osteoblast cell line were collected after being cultured in classic or inflammatory environment (TNF-α stimulation) to mimic post-surgery environment.

**Results:** Our first results showed that MSSA or MRSA strains did not have the same behaviors under the tested conditions. However, for both type of strains, the excess of magnesium in a very poor media increased the biofilm formation of *Staphylococcus aureus* (a 3 to 4 fold-increase, p<0.05) and the amino acids starvation seemed to induce biofilm formation under anaerobic conditions (a 7 to 9 fold-increase, p<0.05). Moreover, the presence of bone cells supernatant leads to an increase of live adhered bacteria and biofilm formation (a fold-increase between 2 and 5, p<0.05).

**Conclusion:** The bone microenvironment is complex but our results show that the parameters that mimicked this specific environment influence the bacterial adhesion of several strains of *Staphylococcus aureus*. Further investigations will help to understand how the different factors influence biofilm formation through the matrix production or stress response.
62: A novel flow system to model chronic wound biofilms and test antimicrobial dressings - Maddocks S

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Introduction: Several models exist for the study of chronic wound infection, but few combine all of the necessary elements to allow high throughput, reproducible biofilm culture with the possibility of applying topical antimicrobial treatments. Furthermore, few take into account the appropriate means of providing nutrients combined with biofilm growth at the air-liquid interface. We have designed a novel device that is 3D printed, straightforward to operate, and can be used to investigate single and mixed species biofilms, as well as the efficacy of antimicrobial dressings.

Hypothesis and aims: To demonstrate that it is possible to reproducibly culture mixed-species biofilms within our biofilm device, to allow for the study of biofilm composition and efficacy of topical antimicrobial dressings.

Methodology: Staphylococcus aureus and Pseudomonas aeruginosa were used as representative chronic wound isolates, being frequently co-isolated from chronic infected wounds. They were co-cultured for 96 hours under flow rates equivalent to a heavily exuding wound and the population assessed by analysis of competitive relative index. Chlorhexidine dressings were applied topically to established biofilms within the flow device and efficacy determined by determining log reductions in recovered bacteria.

Results: The novel flow device produced robust, reproducible data indicating that it has the potential to be a useful tool for the study of experimental biofilms to model chronic wound infection. A distinct Gram-negative shift was observed under conditions of flow that is not observed for static biofilms comprised of S. aureus and P. aeruginosa. Topical antimicrobials showed reduced effectiveness under flow, which might indicate that current analyses that rely on static biofilm models are not appropriate to ascertain the efficacy such treatments.

Conclusion: The flow device constitutes a new tool for modelling chronic wound biofilms, and the element of flow could be an important factor in shaping the composition of a mixed-species biofilm.
63: Development of ‘smart’ wound dressings for biofilm sensing and control - Magee E

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Introduction: Chronic wounds affect approximately 2% of the worldwide population and incur healthcare costs in the billions. Key to their persistence is the formation of microbial biofilms, which are accounted for in nearly 80% of all non-healing wounds. A paradigm shift in wound management has resulted in the emergence of smart dressings, which offer real-time monitoring of the wound condition without physical intervention.

Hypothesis and aims: The smart dressing presented herein aims to detect a range of biofilm-derived volatiles, with a striking colour change that can be visualised with the naked eye, providing 24/7, non-invasive monitoring of biofilm development in the wound bed.

Methodology: CO₂ sensors, containing xylenol blue dye, were tested against a range of wound pathogens inoculated onto an ex vivo porcine wound model. Digital images of the indicator film were captured each hour and split into red, green and blue (RGB) colour channels to yield semi-quantitative data. FT-IR was employed to measure the volume of CO₂ generated into the wound headspace by infected explants, in tandem with digital imagery of sensor films and viable counts of inoculated pathogens.

Results: All sensors monitoring wound pathogens underwent a marked colour change of blue to yellow, whilst sensors monitoring uninoculated control skin remained blue (no colour change). In addition, colour change of the indicator film was linearly correlated to the amount of CO₂ generated into the wound headspace and the wound bioburden, adding a diagnostic aspect to the xylenol blue sensor film.

Conclusion: The marked colour change exhibited by the xylenol blue sensor film is easily visualised with the naked eye, and can be directly correlated to the wound bioburden. This early warning, point-of-care technology is a promising candidate in combatting biofilm development in wounds and improving patient outcome.
64: Propionibacterium acnes biofilm forming capacity: are phylotypes involved? - Pécastaings S

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Introduction: Acne vulgaris is a common skin problem that results from abnormal inflammation and causes various lesions (hyperpigmentation, papules, comedos etc.). It mainly affects the face, the torso and the upper back and arms. The proliferation of Cutibacterium (formerly known as Propionibacterium) acnes is one of the factors involved in acne. C. acnes is a skin commensal and a Gram positive bacillus that resides in pilo-sebaceous follicles. Nevertheless, the presence of C. acnes does not solely account for acne and healthy and acne prone persons can both exhibit the same level of colonization by C. acnes. It is a well-known fact that the pathogenicity of C. acnes lies at least partly in its capacity to form biofilms. Biofilm formation by C. acnes also significantly reduces its susceptibility to antibiotics (vancomycin, clindamycin, erythromycin, levofloxacin, ciprofloxacin).

Hypothesis and aims: Since different phylotypes of C. acnes have various involvements in acne vulgaris, our goal now was to investigate biofilm formation capacities of strains of C. acnes belonging to different phylotypes.

Methodology: In order to better understand biofilm formation by C. acnes, an in vitro biofilm model in 24-wells microplates was optimized.

Results: First results show that C. acnes is able to form biofilms of approximately 10⁶ CFU/well after 72 h in a medium with a reduced concentration of nutrients (BB), a density that is comparable to biofilms obtained in rich media like BHI (Brain Heart Infusion) or RCM (Reinforced Clostridial Medium). Interestingly, BB does not support planktonic growth, contrary to BHI or RCM. Strains belonging to specific phylotypes form biofilms that are less dense than others.

Conclusion: The first results indicate different capacities to form biofilms by different phylotypes, which would correlate with the various implications of phylotypes in acne vulgaris.
65: A fluorescent artificial wound eschar model for biofilm and debridement study - Percival S.L

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Introduction: Many studies have demonstrated that biofilms are localized in the eschar rather than directly on the wound surface. Eschar is difficult to remove, and its presence can lead to a delay in wound healing. One of the most important tasks in chronic wound healing is to remove eschar and also slough which has additionally been shown to contain biofilms. However, there are no standard in vitro methods to evaluate the ability of wound dressing gels to breakdown eschar (debridement) and slough (desloughing).

Hypothesis and aims: To design a cost-efficient in vitro artificial wound eschar (AWE) system and to apply the system to examine the debridement efficacy of wound dressing gels.

Methodology: fAWE composed of 60% FITC-collagen, 10% Rhodamine-elastin, 10% Coumarin-fibrin, and 20% fibrin was made freshly by clotting fibrinogen with thrombin. fAWE was placed into cell culture inserts and 100l wound dressing gel was placed on top. Then the cell culture inserts were put into a 6 well plate with 5ml Tri-buffer. Another 5ml Tri-buffer with or without collagenase was added into the cell culture inserts. The 6-well-plate system was moved into a shaking incubator at 37C and sampled at set time points from 0 hour to 72 hours. The breakdown of each protein in the fAWE substrate was measured by detecting each fluorescent dye in the wells.

Results: Wound dressing gels presented different efficacies on the ability to breakdown fAWE. Surfactant in the wound gels increased exudation of small fragments/debris from cell culture inserts to culture wells.

Conclusion: The results of this study imply that the fAWE system used here can be used to measure the breakdown-degradation-decomposition of collagen, elastin and fibrin, which are the main components of wound eschar.
66: In a laboratory model of diabetic foot infection, vancomycin and gentamicin loaded calcium sulfate beads were more effective than systemically achievable concentrations of antibiotics in reducing polymicrobial biofilms grown from clinical isolates - Price B

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Introduction: Diabetic foot ulcers are a common complication of diabetes and 60% of these show signs of infection upon presentation in the clinic. These infections are often recalcitrant to treatment and become chronic in 45% of cases. Failure of antibiotics to treat infections is multifactorial but penetration of antibiotics to the extremities is often poor in diabetes sufferers, particularly those with comorbidities such as peripheral arterial disease.

Hypothesis and aims: We aimed to grow polymicrobial biofilms of clinical isolates derived from diabetic foot ulcers in vitro and then expose these biofilms to antibiotics modeling topical or systemic administration. We hypothesise that topical release of antibiotics at the site of the biofilm growth will be more effective in reducing bioburden than antibiotics administered to patients at concentrations achievable systemically.

Methodology: We harvested bacteria from debrided diabetic foot tissue of six subjects and used the same isolates to inoculate a collagen wound model and grew a polymicrobial biofilm. We added either calcium sulfate beads (CSB) (Stimulan Rapid Cure, Biocomposites Ltd), vancomycin and gentamicin loaded CSB, the systemic antibiotics used to treat the patient from which the debrided tissue was collected or a vehicle only control. After 72 hours further incubation the model was sectioned and bacteria enumerated. We calculated log reductions after exposure of biofilms to “topical” or “systemic” antibiotics.

Results: In five of the six biofilms the bioburden was significantly reduced (P>0.05) after exposure to loaded CSB: log reductions ranged from 2.5 to 8.2 and were on average 5.7. The biofilm for the subject with no significant difference included yeast. We found no significant differences in bioburden after exposure of the biofilm to antibiotics at expected systemic levels for any of the biofilms.

Conclusion: Topical release of antibiotics in close proximity to the biofilm has the potential to reduce bioburden to a greater extent than antibiotics at systemic concentrations.
**67: Application of furanone compounds for the modulation of biofilm formation in common wound pathogens - Proctor C**

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**Introduction:** It has been shown that the majority of non-healing wounds contain a bacterial biofilm and these biofilms prolong the inflammation, impair tissue repair and significantly delay wound healing. This often causes normal wounds to progress and become chronic, presenting a new range of problems for both patients and healthcare professionals including increased risk of secondary infection and further deterioration of the wound.

**Hypothesis and aims:** This project aims to assess the efficacy of several natural compounds in modulating the formation of biofilms in the wound pathogen *Pseudomonas aeruginosa DSM50071* when used at sub-inhibitory concentrations. We tested the efficacy of the plant derived compounds 4-hydroxy-2,5-dimethyl-3(2H) furanone (HDMF), 2-methyltetrahydrofuran-3-one (MTHF) and 3-hydroxy-4,5-dimethylfuran-2(5H)-one (sotolon).

**Methodology:** Minimum inhibitory concentrations for each compound were assessed by broth microdilution and confirmed using a TTC assay and streak plate method. Biofilm formation was assessed using a standard colorimetric crystal violet microtitre dish assay. Release of drug payload from hydrogels was assessed using a dissolution assay.

**Results:** Candidate molecules showed an ability to reduce *P. aeruginosa* biofilm formation. Treatment with HDMF resulted in a 77%, 77% and 88% reduction in biofilm when tested at 24h, 48h and 72h, respectively. Similarly, treatment with sotolon resulted in an 86%, 84% and 60% reduction in biofilm when tested at the same time points. Treatment with MTHF resulted in no significant reduction in biofilm formation at 24h, a reduction of 25% at 48h and a significant increase in biofilm at 72h. Proof of concept experiments have shown that when candidate molecules are loaded into a polymer hydrogel, they are efficiently released from it with 47.6% and 44.5% release of the total drug payload in sotolon and MTHF loaded gels respectively.

**Conclusion:** This data shows that furanones tested have a clear antibiofilm activity and that these compounds can be delivered to directly to a site from our novel hydrogel.
68: Internalization of \textit{Cutibacterium acnes} in bone cells and its consequence on bacterial virulent behaviour - Reffuveille F

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\textbf{Introduction:} \textit{Cutibacterium acnes} is a commensal skin organism also recognized as a pathogen in foreign body infection specifically in Bone Joint Infections (BJIs).

\textbf{Hypothesis and aims:} We hypothesize that \textit{C. acnes} bacteria from skin acquire virulent behavior after cell internalization.

\textbf{Methodology:} In this study, we evaluated the bacterial internalization rate by bone cells and the biofilm formation through Crystal violet staining and fluorescent microscopy methods of different \textit{C. acnes} clinical strains isolated from BJIs and from skin.

\textbf{Results:} We observed that \textit{C. acnes} isolated from BJIs form initially 2 fold-more biofilm than the strains isolated from a normal skin in our two biofilm models (Crystal violet and fluorescent staining, \(p=0.04\) and \(p=0.02\), respectively, Mann-Whitney test). The internalization rate in osteoblast-like cells was similar for all \textit{C. acnes} strains. However, the strains isolated from the skin showed a significant increase in biofilm formation after the osteoblast-like cells internalization (\(x2.3\pm0.07\); \(p=0.008\), Mann-Whitney test). The hydrophobicity was measured to investigate a major modification of cell wall responsible of this finding. The skin strain hydrophobicity is significantly less important than for the BJIs strains (11.4\pm3.9\% vs 42.24\pm5.5\% respectively; \(p=0.003\), Mann-Whitney test). Nevertheless, no change was observed after internalization suggesting that the biofilm capacity increase is not only supported by a bacteria surface modification. Then, the genomic background of \textit{C. acnes} strains was inquired to investigate the difference of virulence level between all tested strains. All BJIs strains have IAI phylotype.

\textbf{Conclusion:} In conclusion, we studied for the first time the impact of bacterial internalization by osteoblast-like cells on the virulent behavior of \textit{C. acnes} biofilm, which could explain the hided pathogenicity of this commensal bacterium.
A surprising role of bacterial odor in human skin health - Sapir Ron-Doitch

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Introduction: S. aureus and S. epidermidis are some of the most abundant human skin-associated bacteria, emitting a wide variety of volatile organic compounds (VOCs). These bacteria are usually found as a biofilm or biofilm-type colony on the skin. The human skin is constantly exposed to exogenous oxidative stressors. Its most important protection mechanism is the Nrf2-keap1 protective pathway. This pathway is activated by electrophilic molecules. However, the mechanism by which skin maintains Nrf2 in a constant alert condition has not been elucidated.

Hypothesis and aims: We hypothesized that S. aureus and/or S. epidermidis-derived VOCs may activate the Nrf2-keap1 pathway, leading to enhanced skin protection.

Methodology: Bacterial localization on ex-vivo human skin was evaluated by SEM; bacterial VOCs were analyzed using a GC-MS device in a direct HS-SPME sample injection of bacterial cultures grown in test vials; Nrf2 activation was evaluated in human keratinocytes (HaCaT cells) using immune-staining methods; oxidative stress protection was assessed in HaCaT cells/ex-vivo skin exposed to UVB light by Caspase 3 apoptosis evaluation assay.

Results: S. aureus treated ex-vivo human skin was significantly more resistant to UV-induced apoptosis (> 50% protection compared to control). The VOC 3-furaldehyde (3-FA) was found in the headspace of both S. aureus and S. epidermidis cultures. Its pure standard was shown to induce the Nrf2-keap1 pathway in HaCaT cells in the low µM concentration range. Moreover, pre-treatment of cells and skin with 3-FA led to protection against UV-induced apoptosis, reducing it by ≈ 30%.

Conclusion: The skin-associated bacteria-derived volatile metabolite 3-FA was able to induce the Nrf2-keap1 protective pathway in human skin cells. Furthermore, pre-treatment with 3-FA was beneficial to skin and cells, leading to increased survival under UV-induced oxidative stress. This suggests a pivotal role of the skin's microbiome in protection against exogenous stressors.
70: Interactions between *Propionibacterium acnes* biofilm and different human cell types are strain dependent - *Spittaels K-J*

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**Introduction:** *Propionibacterium acnes* is one of the most prevalent members of the human skin flora. However, colonization and biofilm formation by this bacterium in the skin’s pilosebaceous units is an important contributing factor in the pathogenesis of acne. Recent studies have shown that *P. acnes* populations differ considerably between acne patients and healthy individuals, with some strains being more prevalent in normal skin while others appear to be associated with acne.

**Hypothesis and aims:** The goal of the present study is to determine whether differential association of *P. acnes* strains with acne or healthy individuals is due to differential interactions with relevant human skin cells (SZ95 sebocytes and HaCaT keratinocytes).

**Methodology:** Adhesion of various *P. acnes* strains (associated with healthy skin, acne or both) to SZ95 and HaCaT cells was quantified after 48h of infection by plating, and visualized by microscopy following a modified Gram staining. The release of the proinflammatory cytokines IL-1β, IL-8, and TNF-α of both skin cell lines following infection was quantified using ELISA assays.

**Results:** Overall, acne-associated strains of *P. acnes* showed higher adhesion to SZ95 sebocytes, compared to strains from healthy skin. This difference was not found for the HaCaT keratinocytes. Preliminary results show that all strains tested were able to induce IL-8 release in both the SZ95 and the HaCaT cell lines, but no significant differences were observed between the tested strains. Interestingly, acne-associated *P. acnes* strains induced higher levels of IL-1β and TNF-α in SZ95 cells than *P. acnes* strains associated with healthy skin. A similar trend for TNF-α was found in the HaCaT cells, although not statistically significant.

**Conclusion:** Our results demonstrate that *P. acnes* strains typically associated with acneic or healthy skin interact differently with human skin cells, although a wider panel of *P. acnes* strains needs to be investigated to confirm these differences.
**71: Risk factors for chronic biofilm related infection - Stewart P.S**

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**Introduction:** The use of implanted medical devices is associated with a small but clinically important risk of foreign body infection. A key question is: Why do some patients develop chronic infection associated with an implanted device but most do not?

**Hypothesis and aims:** Risk factors matter and provide clues about the etiology of device-related infections.

**Methodology:** The literature on risk factors for five categories of device related chronic infections was surveyed: cardiovascular implantable electronic devices (CIEDs), hernia meshes, prosthetic hip and knee joints, prosthetic shoulder joints, and breast implants. Drawing on 48 published reports, two quantitative measures of the importance of a particular risk factor were examined: 1) the percentage of the studies analyzing that risk factor that determined a statistically significant effect, and 2) either an odds ratio (OR) or hazard ratio (HR). The first measure indicates the consistency of the effect and the second measure indicates the magnitude of the effect.

**Results:** Risk factors can differ from one device to the next. The overall ranking of risk factors from most important to least important (based on the percentage of studies reporting a statistically significant effect) was: 1) immunomodulation/steroid therapy, 2) renal disease/hemodialysis, 3) diabetes, 4) smoking, 5) high BMI. The overall ranking of risk factors according to the strength of the effect, from stronger effect to lower effect (based on odds or hazard ratio), was: 1) immunomodulation/steroid therapy, 2), smoking 3) diabetes, 4), high BMI, 5) renal disease/hemodialysis.

**Conclusion:** This analysis of risk factors supports the concept of a vulnerable subpopulation of individuals – those with systemic co-morbidities or interventional therapies that compromise innate immunity - who are the ones at risk. The limitations of in vitro and animal models of chronic device-related infections are discussed in this context as are implications for research and clinical practice.
Introduction: Progressively, biofilms are becoming more recognized as the reason why chronic wounds fail to heal. As a result of this, a whole new market for therapeutic products specifically targeting biofilms in wounds has emerged. In order to develop new antibiofilm-specific wound dressings, a need has arisen for appropriate *in vitro* wound models which simulate the complex environment found in biofilm-infected wounds. Nonetheless, as there is much we still do not know about the variety of factors contributing to the high tolerance found in such biofilms, mimicking these conditions *in vitro* is still a work in progress. In this rapidly evolving field several different models have been, and still are, continuously being created attempting to provide a solid and relevant platform on which new antibiofilm therapeutics can be tested.

Hypothesis and aims: We hypothesize that the current standardized biofilm methods are not correlated with the present knowledge of chronic biofilm infections and our aim was to elucidate the discrepancies which exist between the currently used standardized biofilm models and the translation of these into *in vivo* clinical settings.

Methodology: Clinical observations along with literature research and comparisons to current standardized methods.

Results: There is still no harmonized consensus regarding the use of standard models for testing antibiofilm efficacy of new treatments. To date, only a handful of standardized protocols deals with the elimination of nosocomial relevant biofilms. Yet, the applicability of these models in a clinical situation is often lacking. The gap between the currently recognized *in vitro* standard models and the *in vivo* medical setting is simply too great, and as a result of this, antibiofilm-specific wound dressings often fail to produce satisfying results when applied *in vivo*.

Conclusion: There is a need to recognize that the standardized methods are not suitable for all circumstances; one size does not fit all.
**73: Phenotypic profile of biofilm and extended spectrum beta lactamases bacteria from patients with diabetic foot ulcers in Zaria-Nigeria - Usman Y**

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**Introduction:** Diabetic Foot ulcers are the most common, costly and devastating complications of diabetes mellitus that is associated with severe morbidity. Biofilms are considered to be a major biological agent that encourages antibiotic resistance and inevitably present multiple obstacles to the clinicians in treating wounds like diabetic foot ulcers.

**Hypothesis and aims:** This study sought to determine the prevalence of bacterial colonization, biofilm formation and Extended Spectrum Beta Lactamases (ESBL) production from patients with diabetic foot ulcers in Zaria

**Methodology:** A hospital based cross-sectional studies conducted at Ahmadu Bello University Teaching Hospital and Hajiya Gambo Sawaba General Hospital, Zaria-Nigeria. A total of ninety seven consecutive non-duplicate patients with diabetic foot ulcer were enrolled and had their wound biopsy collected using a sterile technique. A standard microbiological technique was used in isolation and identification of the bacteria. Biofilm formation and ESBL detection were done using microtitre plate method and Double Disc Synergy Test respectively.

**Results:** Out of the 97 samples analyzed, 49(50.5%) had positive culture results. Of this, *Staphylococcus aureus* was most occurring pathogen, 11(22.4%), then *Escherichia coli*, 9(18.4%), *Proteus mirabilis*, 7(14.3%), *Pseudomonas aeruginosa*, 6(12.2%), *Acinetobacter baumannii*, 6(12.2%), *Enterococcus faecalis*, 4 (8.2%), while *Citrobacter freundii* and *Morganella morgani*, 3(6.1%) were the least. Out of the 49 isolates, 45(91.8%) were biofilm producers, of this, 24(48.9%) were strong biofilm producers, 14 (28.6%) were moderate biofilm producers, 7(14.3%) were weak biofilm producer and were not biofilm producing bacteria. The prevalence of ESBL producing bacteria was 24.5%. Ten (83.3%) of the ESBL producing bacteria were biofilm producers. Of this, 6 (50%), 3(25), 1(83.3%) ESBL producers were strong, moderate and weak biofilm producers, respectively.

**Conclusion:** Due to the high prevalence of biofilm producing bacteria observed in this study, a paradigm shift in the management of patients with diabetic foot ulcers is commended.
**Wednesday 4th September**

**Multispecies & Interactions**

**74: Development and validation of an *in vitro* endodontic mixed biofilms model - Abusrewil S**

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**Introduction:** Endodontic disease, an infection of the root canal space is a significant cause of dental morbidity worldwide. One of the main aims of root canal treatment is elimination of microorganisms from the root canal system. Endodontic infections are of biofilm aetiology and are associated with key oral bacterial pathogens. *In vitro* studies of strategies for management of endodontic infections have been hampered by the lack of validated model systems.

**Hypothesis and aims:** To develop and validate an *in vitro* model of a mixed bacterial/fungal endodontic biofilm.

**Methodology:** *C. albicans, S. gordonii, P. gingivalis* and *F. nucleatum* were identified as candidate biofilm members from the literature. Culture conditions were optimised. Microbial cell density, media, and incubation conditions for single and mixed species biofilms were explored. The growth characteristics of biofilms in three different incubation conditions in 1:1 RPMI/TSB media were compared. Additionally, biofilms grown in a CO₂ incubator in 1:1 RPMI/TSB media with or without 10% FBS were assessed. Biofilms grown in a CO₂ incubator in 1:1 RPMI/TSB or RPMI/THB media were also compared. Viability was measured by XTT assay and biomass was quantified by crystal violet assay. Biofilm compositional analysis was performed using PCR. Morphological and architectural features of the resulting biofilms were characterised using scanning electron microscopy.

**Results:** Mixed species biofilm composed of *Candida albicans, Streptococcus gordonii, Porphyromonas gingivalis* and *Fusobacterium nucleatum* are viable with representation of all species detectable during a culture period of 48 h. Specific culture conditions were identified that allowed optimal growth of the endodontic mixed species biofilm model. A 1:1 mixture of RPMI/THB medium with 10% FBS and a CO₂ incubation condition was identified as supporting optimal biofilm formation of the four single species and mixed species biofilm.

**Conclusion:** The current study describes the development of a validated mixed species endodontic biofilm model. This provides the platform for evaluation of interactions within endodontic biofilms and between the biofilm and clinically relevant substrates. This model system will allow an enhanced understanding of current antimicrobial strategies employed in root canal treatments and allow exploration of novel means of biofilm disruption/inhibition within the root canal system.
Introduction: The biofilm matrix contributes to establishment of microbial cells on very diverse surfaces and provides protection against the different hostile conditions the cells encounter in clinical, environmental and industrial settings such as desiccation, predation, virus or antimicrobials. Despite its documented impact in those settings, characterization of its composition and attribution to specific producing organisms yet remains scarcely understood in multispecies biofilms.

Hypothesis and aims: In this work, we aim at circumventing such a problem studying two different microbial consortia isolated from natural environments: a 4-species soil consortium and a 3-species wastewater community.

Methodology: We performed an in silico search of matrix determinant homologues in Xanthomonas retroflexus—the most abundant strain in the soil consortium—and found an orthologue of the Fap amyloid cluster, described in Pseudomonas as a biofilm-scaffold contributing element. We deleted the fap cluster, replaced it in the 4-species model and compared biofilm structure and adhesion capability to the parental community.

Results: A Fap-deficient consortium—unlike the parental one—resulted in irregular substrate coverage and less sturdy biofilm structure in Ibidi-flow cells, even though adhesion did not change significantly. Furthermore, the fap mutant colonized the substrate poorly in single-species biofilm and displayed a rather filamentous structure, contrasting the wild-type strain displaying full substrate coverage. Moreover, we are currently applying a fluorescent-lectin screening on single-, dual- and multispecies biofilms to characterize the matrix glycoconjugates in both consortia, but also molecular strategies to assess species distribution and localized expression of matrix-encoding genes within biofilms.

Conclusion: Our data suggest the Fap amyloid as a structural component of the soil biofilm matrix, since its lack impacts the biofilm synergistic properties in the 4-species consortium. Chemical characterization in single-, dual- and multispecies biofilms will allow us to identify other matrix components and associate them to specific bacterial species in multispecies biofilms.
76. Host-fungal interactions in the aetiopathogenesis of Orofacial granulomatosis - Anderson O.F

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Introduction: Orofacial granulomatosis (OFG) is a chronic inflammatory disease of the oral cavity; the incidences of which are higher in the West of Scotland than anywhere else in the world. Little is known about the aetiopathogenesis of OFG. However, it has been suggested that fungal pathogens may play a role by driving a sustained dysregulated inflammatory response in the oral cavity; although this hypothesis remains controversial.

Hypothesis and aims: We hypothesised that oral candidal carriage would be associated with OFG and fungal pathogens play a role in disease immunopathogenesis. We specifically aimed to determine the presence and abundance of canidial species in the oral cavity of patients with OFG and investigate their association with salivary inflammatory biomarker profiles.

Methodology: Twenty-nine patients with OFG attending the Oral Surgery department of Glasgow Dental School and Hospital (GDSH) were recruited. In addition, 19 healthy volunteers were recruited from a pool of staff at GDSH. Patients and volunteers provided a saliva sample for inflammatory biomarker analysis using the Proximity Elongation Assay (PEA) (Olink, Uppsala, Sweden). In addition, an oral rinse sample was collected, and presence and abundance of candida species determined using CHROMagar™ plates (E&O Laboratories, UK)

Results: No significant differences in candida carriage in terms of abundance and species distribution was observed between patients with OFG and healthy volunteers. Patients with OFG had a distinct salivary inflammatory biomarker profile as determined using PEA technology.

Conclusion: Patients with OFG have a distinct salivary biomarker profile which could reveal clues towards the immunopathogenesis of the disease. However, the evidence to date suggests that candida species are not associated with the disease.
77: The effect of cannabigerol on quorum sensing and biofilm formation of *Vibrio harveyi* - Aqawi M

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**Introduction:** Cannabigerol (CBG) is a non-psychoactive cannabinoid present in cannabis in trace amounts. By now the activity of CBG is well known in eukaryotes. In contrast, little is known about its effect in prokaryotes.

**Hypothesis and aims:** Quorum sensing (QS) is cell to-cell communication between bacteria that involve a signaling network. *Vibrio harveyi* is a characterized model organism in QS. Here we investigate the potential role of CBG as an anti-quorum sensing and anti-biofilm agent against *V. harveyi*.

**Methodology:** The effect of CBG was tested on *V. harveyi*. For the planktonic growth, changes in bioluminescence and growth were recorded. The biofilm biomass was quantified by qPCR and scanning Electron Microscope (SEM) was used to visualize the biofilm. To investigate CBGs’ mode of action, gene expression and bioluminescence assay against mutant strains (genetic defects in the QS-cascade genes) of *V. harveyi* were performed. For the motility assay, motility halos were measured. PI influx as a measure of membrane permeability was checked by FACS.

**Results:** CBG was able to reduce the QS-regulated bioluminescence of *V. harveyi* (W.T.) and mutant strains without affecting the bacterial growth at concentration of 50, 20 and 2 µg/ml. Moreover, CBG downregulated QS-regulating genes including Lux M, Lux N, Lux P, Lux S and Lux Q in both biofilm and planktonic conditions. Furthermore, QS-mediated biofilm formation of *V. harveyi* was distorted in the presence of CBG at concentrations of 50 and 20 µg/ml. SEM confirmed the former results. Swimming motility of *V. harveyi* lessened significantly in a dose-dependent manner. Importantly, the ethanol in which the CBG was dissolved in, had no effect. At concentrations above the MIC, CBG appeared to disrupt the cell membrane permeability.

**Conclusion:** Evidence is provided for interference of CBG with the bacterial signal-transduction system, providing a new innovative way to tackle bacterial biofilm.
**78: Enterococcus faecalis inhibits Klebsiella pneumoniae growth in polymicrobial biofilms - Ballén V**

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**Introduction:** Indwelling devices related urinary tract infections are one of the most common biofilm infections of the urinary system, and polymicrobial colonization commonly occurs with long-term catheterization. In this way, bacteria in multispecies biofilms cooperate, compete or have neutral interactions according to the involved species. The aims of this work were to study the interspecies interactions in polymicrobial biofilms formed by *K. pneumoniae* and *E. faecalis*, two of the most common uropathogens.

**Methods:** In this study, mono and polymicrobial biofilm were evaluated by the crystal violet assay, reduction percentage in biofilm calculation, enumeration of colony-forming units and competitive index. Then, we tested four stages of lyophilized cell free supernatants (CFS) of *E. faecalis*: planktonic, exponential growth, biofilm and biofilm with adjusted pH against *K. pneumoniae* strains.

**Results:** The results showed total biomass volume decreased in polymicrobial biofilm and the reduction percentage was statistically significant. Enumeration of colony-forming units revealed a reduction statistically significant just with *K. pneumoniae* and the competitive index negative values indicated a competitive advantage of *E. faecalis* over *K. pneumoniae*. This reduction in colony-forming units was not observed when both bacteria grew in planktonic culture. Then, we demonstrated that biofilm CFS with pH between 4.0 and 4.3 had inhibitory effect over different biofilms of *K. pneumoniae*. This effect was no present when the pH of biofilm CFS was adjusted to 6.5, or when the planktonic and the exponential growth CFS was tested, suggesting that *E. faecalis* decreases pH in polymicrobial biofilm and inhibits the proper growth of *K. pneumoniae*.

**Conclusions:** *K. pneumoniae* and *E. faecalis* interact in a competitive manner. Both microorganism grew better in monomicrobial biofilms than in polymicrobial biofilms, and *E. faecalis* is able to modify the pH, according to their metabolic needs, which originate microenvironments favorable for the own growth but compromising the appropriate growth of *K. pneumoniae*. 
**Introduction:** Bacterial vesicles are 10 - 300nm spherical membrane structures constitutently released from all species studied so far. Such membrane vesicles are implicated in a wide range of roles including secretion of toxins, trafficking of virulence factors, inter-species signalling, and packaging of DNA for horizontal gene transfer. Despite great strides in the field, many details as to their categorisation, biogenesis, and their function within the human host, remain obscure. Since bacterial vesicles are consistently found within biofilms on electron micrographs, authors have long speculated a structural role. More recently, vesiculation has been suggested to aid both export of eDNA into the biofilm and attachment by altering cell surface hydrophobicity. As the two emerging disciplines of extracellular vesicles and biofilms cross paths, closer attention to the role vesicles play in the biofilm milieu is due. Using the model microorganism *Escherichia coli*, this research aims to characterise this functional relationship and establish methods for studying bacterial vesicles as they relate to biofilm.

**Hypotheses:**

- Co-incubation of *E. coli* with vesicle preparation strengthens biofilm
- Inhibiting vesiculation in turn reduces biofilm

**Methodology:** Vesicles were isolated from *E. coli* strains B and K12 using differential centrifugation, ultracentrifugation, ultrafiltration, and density gradient centrifugation. Vesicles were characterised using Nanoparticle Tracking Analysis and Bradford assay. Bacterial adhesion and growth were measured by crystal violet static biofilm assay using 96-well and 24-well microtiter plates in the presence of varying concentrations of vesicles over 24 hours.

**Results:** Preliminary results indicate biofilms appear thicker and more developed in samples supplemented with high concentrations of vesicles. This appears true also when incubating with vesicles from different strains. The trend occurs in a dose-dependent manner.

**Conclusions:** Further work will test the ability of chloramidine to reduce vesiculation via irreversible binding to Protein Arginine Deiminase. Eventually it is hoped to test additional species to ascertain whether this observation is species-specific.
80: Development of a multi-species marine-based biofilm model for testing novel anti-microbial agents - Butcher M.C

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Introduction: The near-constant replenishment of bacteria and fungi present in sea water can readily make an ideal biofilm environment out of any exposed surface. If allowed to continue unregulated, microbial accumulation can easily lead to the adherence of larger, more robust organisms which can severely alter the intended purpose of the original surface. The shipping industry alone suffers an estimated $60 billion dollars a year in increased fuel and antifouling maintenance costs.

Hypothesis and aims: Here we developed an in-vitro multi-species biofilm model, made up of known proponents of biofilm formation and common water-based bacteria, to emulate the effects of environmental biofouling. This, in turn, has provided a platform to test novel anti-microbial treatments and their ability to undermine the fouling process.

Methodology: A 7-species biofilm model was developed through co-culture of Shewanella algae, Cobetia marina, Pseudoalteromonas atlantica, Vibrio alginolyticus, Vibrio anguillarum, Cellulophaga lytica and Aspergillus brasiliensis using a staggered growth model. Each organism’s standard rate of growth was determined through growth curve analysis with the slowest growing organisms being introduced sequentially. Compositional analysis of the biofilm was assessed by quantitative PCR conformity was assessed through scanning electron microscopy. Assessment of anti-microbial interaction with the biofilm was achieved through alamarBlue cell viability assay, Crystal Violet Biomass assay and Live/dead RT-PCR.

Results: We observed the formation of a robust multi-species biofilm, with multiple target species represented within the biofilm being confirmed through Q-PCR. In anti-microbial treated biofilms, we noted a conformational and compositional difference between untreated marine biofilms and those treated with anti-fouling agents.

Conclusion: Our results have shown that we have produced a replicable, multi-species biofilm model suitable for in-vitro analysis relating to microbiota and anti-microbial testing in marine environments.
81: A biomimetic self-assembling hydrogel for the delivery of antimicrobials in the control of periodontal pathogens - Davies R.P.W

Davies R.P.W, Do T, Devine D and Kirkham J

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Introduction: Periodontal disease (PD) is a chronic, progressive inflammatory disease that is the 6th most common disease in the world and the most common cause of tooth loss in older adults. PD starts with inflammation of the gingival tissues in response to bacterial biofilms within the periodontal pocket.

Hypothesis and aims: We have developed a self-assembling peptide (SAP) platform as a potential therapeutic device for delivery of antimicrobial moieties (AMM). We hypothesise that SAP-AMM combinations will control PD-associated microbial load whilst simultaneously providing a biomimetic scaffold to regenerate periodontal tissues. We aimed to: 1) incorporate AMM without disrupting the SAP's ability to self-assemble; 2) quantify AMM release from the scaffold and 3) determine whether SAP-AMM combinations can control microbial load in vitro using an orally relevant biofilm.

Methodology: The self-assembling properties of SAP ‘P_{11-4}’ in combination with either doxycycline hyclate, metronidazole or cetylpyridinium chloride were investigated macroscopically and by TEM. AMM release from SAP scaffold gels was quantified by UV monitoring over time. A model five-species biofilm (Streptococcus salivarius, Actinomyces naeslundii, Fusobacterium nucleatum, Prevotella intermedia, Porphyromonas gingivalis) was cultured anaerobically for 10 days on the Calgary biofilm device. SAP-AMM combinations were added and incubated for 5 days after which the biofilm was harvested and viable bacteria enumerated.

Results: The SAP hydrogel incorporated each AMM homogenously without loss of assembling properties. Controlled AMM release was demonstrated over a period of 5 days. SAP peptide alone did not inhibit microbial growth, however, biofilms treated with SAP-AMM combinations resulted in complete bacterial inhibition at the concentrations used here.

Conclusion: Our data suggest that SAP P_{11-4} is an effective scaffold for the delivery of these antimicrobials. The combination of SAP and AMM controlled microbial load for model periodontal biofilms and may therefore offer a novel therapeutic opportunity for treating PD in the future.
**82: Pre-clinical and clinical anti-biofilm efficacy of nitradine: a novel non-antibiotic brushing solution (periotabs) for teeth and gums to help reduce perio-diseases and dental-implant infections - De Wever B**

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**Introduction:** Oral biofilms are an important casual element in gum and teeth related pathologies including gingivitis, periodontitis, peri-mucositis and peri-implantitis. Current standard treatment is based on the use of local chlorohexidine, often combined with systemic antibiotics. However efficacy is low and important side effects are observed.

**Hypothesis and aims:** Here we have tested a new treatment regime based on the use of a NitrAdine-based brushing solution (PerioTabs) for teeth and gums. NitrAdine is a nonantibiotic chemical formulation that has shown to have a very strong anti-biofilm efficacy against biofilms formed by a variety of bacteria, fungi and viruses. The aim of this study is to demonstrate pre-clinical and clinical efficacy of PerioTabs in perio and dental implant related diseases.

**Methodology:** 2 clinical studies in 40 gingivitis, periodontitis, peri-mucositis and periimplantitis patients were conducted. After routine SRP, patients were instructed to brush teeth and gums daily for 2 minutes with a freshly prepared PerioTabs brushing solution for 10 consecutive days. Clinical parameters included gingival index, periodontal bleeding index, gingival recession and pocket depths. In addition, bacterial mass of periodontopathogenic specimen was quantified and pictures before and after 10 days PerioTabs treatment were taken.

**Results:** A significant improvement of the overall clinical parameters was observed in all patients. Also a substantial reduction in bacterial count was observed. No side effects were recorded.

**Conclusion:** The use of NitrAdine as a brushing solution for teeth and gums (PerioTabs) after SRP proves to be a safe and efficient solution to help reduce gingivitis, periodontitis, peri-mucositis and peri-implantitis.
Cold atmospheric pressure plasma significantly reduces polymicrobial cariogenic biofilm - Figueira L.W

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Introduction: Less invasive methods for caries treatment has been studied, aiming to avoid unnecessary loss of dental tissue. Low temperature atmospheric pressure plasma (LTAPP) shows promising antimicrobial activity.

Hypothesis and aims: The hypothesis is that LTAPP is an effective and non-invasive tool for the disinfection of carious cavities previous to restoration procedures. The aim of this study is to evaluate the application of LTAPP in the control of multispecies cariogenic biofilms.

Methodology: The effective parameters of LTAPP against polymicrobial biofilms composed by Candida albicans, Lactobacillus acidophilus and Streptococcus mutans were established. The microorganisms were activated in BHI agar and incubated at 37 °C for 48h (and 5% CO₂ for S. mutans). Standardized suspensions were prepared (10⁷ cells/ml) using a spectrophotometer and C. albicans suspensions were diluted to 10⁵ cells/ml. Equal volumes of standardized suspensions were mixed up. Aliquots of 40 μL of the multi-species inoculum and 160 μl of TSB broth supplemented with 20% sucrose were added to the wells of microdilution plates. Plates were incubated (37 °C, 5% CO₂, 48h). After 24h, the biofilm was washed with sterile saline and the culture medium was refreshed. Subsequently, multispecies biofilms were exposed to LTAPP for 1, 3, 5 and 7 min. The number of viable cells were determined by culture method. LTAPP was generated by helium (99.5% purity, 2.0 SLM flow rate) and electric discharge (32 kHz signal frequency, 1.0 W mean power). Non-exposed control was included. Data were analyzed by ANOVA/Tukey's test (5%).

Results: Significant reductions in viable counts of C. albicans (54.0±15.0, 59.0±17.0, 79.0±15.0 and 74.0±7.0), L. acidophilus (81.0±16.0, 96.0±15.0, 98.0±14.0 and 99.0±14.0) and S. mutans (74.0±9.0, 67.0±13.0, 65.0±13.0 and 81.0±16.0) were observed after exposure for 1, 3, 5 and 7 min, respectively.

Conclusion: LTAPP showed significant inhibitory effect on cariogenic multispecies biofilms.
**84: Listeria monocytogenes** and **Salmonella Typhimurium** dual-species biofilm: development and inactivation with Cold Atmospheric Plasma - Govaert M

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**Introduction:** Biofilms are omnipresent on industrial abiotic food contact surfaces and are an unquestionable source of contamination. To date, most biofilm-related studies have been focusing on single-species biofilms. However, in natural environments, biofilms consist of multiple species. Due to the interspecies interactions within these biofilms, they can become even more resistant towards different inactivation methods (such as Cold Atmospheric Plasma, CAP) than single-species biofilms. Therefore, the promising efficacy of CAP observed for single-species biofilms should be validated for multi-species biofilms in order to comment on a possible application on an industrial level.

**Hypothesis and aims:** The presented study aims to develop a strongly adherent and mature dual-species model biofilm consisting of *L. monocytogenes* (Gram positive) and *S. Typhimurium* (Gram negative). Once the optimal biofilm formation procedure is developed, the biofilm is treated with optimal CAP conditions previously determined and the results obtained within the presented research are compared with those previously obtained for the single-species biofilms (Govaert et al. 2019).

**Methodology:** To obtain the optimal dual-species biofilm, the effect of different growth media, incubation temperatures, and incubation times on the adherence and the maturity of the biofilm was investigated. These characteristics were quantified by means of optical density measurements following crystal violet staining and viable plate counts, respectively. For the inactivation with CAP (Dielectric Barrier Discharge, helium gas flow, 7.0 W dissipated power), samples were treated for different treatment times (0-30 min) and the remaining cell density was determined by means of viable plate counts. Predictive models were finally applied to determine the inactivation kinetics.

**Results:** This study indicated that the CAP efficacy for inactivation of dual-species biofilms was similar to its efficacy for inactivation of single-species biofilms, although different inactivation mechanisms might be involved.

**Conclusion:** CAP treatment could be applied for inactivation of multi-species biofilms growing on industrial surfaces.
Introduction: Bacterial biofilms are known to have high antibiotic tolerance which directly affects clearance of bacterial infections in cystic fibrosis (CF) patients. Current antibiotic susceptibility testing methods are either based on planktonic cells or do not reflect the complexity of biofilms in vivo. Consequently, inaccurate diagnostics affect the treatment choice, preventing bacterial clearance and developing antibiotic resistance.

Hypothesis and aims: To employ the ex-vivo pig lung model to study antibiotic tolerance in CF to develop better diagnostics. The study also aims to demonstrate that the model can be used for realistic determination of antibiotic resistance profiling in biofilms. Thus, we will employ the CF representative model for studying polymicrobial communities.

Methodology: Sections of pig bronchiole were prepared and infected with lab strains and clinical isolates of *Pseudomonas aeruginosa* and incubated in artificial sputum media to form biofilms. Then, lung-associated biofilms were challenged with antibiotics and their bacterial load was quantified. All isolates were also tested for antibiotic susceptibility using standard planktonic and biofilm methods. Replicates of tissues were processed for staining and imaged to study the spatial structure of CF polymicrobial communities.

Results: The results showed increased antibiotic tolerance/resistance of *P. aeruginosa* at >500-1000-fold MIC against tested antibiotics demonstrating a persistent phenotype in the biofilm model, while all tested bacterial isolates showed a sensitive phenotype by the standard antibiotic susceptibility testing. This confirmed that the ex-vivo biofilm model is a realistic model for CF. We are also investigating the polymicrobial spatial location and structure of CF pathogens, such as *P. aeruginosa, Stenotrophomonas maltophilia* and/or *Haemophilus influenzae*, to better understand polymicrobial interactions.

Conclusion: We demonstrate a realistic model for antibiotic susceptibility testing clinically and in anti-biofilm drug development to help understanding antibiotic resistance and persistence in biofilms. We also demonstrate the use of the model for studying cell-cell interactions in polymicrobial communities of CF.
86: Microbial communities of central venous catheters - *Hola V*

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**Introduction:** Catheter Related Blood Stream Infections is group of common nosocomial infections. The use of central venous catheters (CVC) is associated with a risk of microbial colonization and subsequent potentially severe infection. Microbial contamination of the catheter leads to the development of microbial consortia embedded in biofilm. Adhesion of bacteria to the catheter depends on many factors, e.g. surface charge, hydrophobicity or hydrophility of the catheter and bacterial cell, on specific genes for adhesion etc. The longer has the patient catheter, the higher is the chance to find the poly-microbial biofilms.

**Methodology:** The aims of this study were to find out the species composition of the mixed biofilm communities formed on the central venous catheters and to assess the importance of particular microbial species as a biofilm-formers in the microbial community. We examined 380 CVCs from well-defined groups of patients. All catheters were sonicated and the microbes were quantified. The quantification of the microbes was followed by their MALDI-TOF identification.

**Results and Conclusion:** From the 366 CVCs we isolated 216 strains of microbes. Most of the catheters were not colonized 57.3%; 28.7% showed mono-microbial colonization and 14% showed poly-microbial colonization. We isolated 29 different microbial taxa. Most often we isolated coagulase-negative staphylococci (S. epidermidis, S. warneri, S. hominis, S. haemolyticus) and propionibacteria (P. acnes, P. granulosum, P. acidifaciens), in many cases in significant numbers (<10³ CFU/catheter), other microbial species (e.g. E. coli, P. aeruginosa, K. pneumoniae, K. oxytoca, Str. oralis, Str. mitis, Str. vestibularis, Str. agalactiae) were isolated much less often. Among the microbes isolated from the CVCs, there were significant differences in the biofilm-forming ability and therefore we conclude that particular microbial species have greater potential to cause biofilm-based infection, where others can be only passive members of biofilm community. Regarding the numbers of CFUs, we conclude that the role of some bacteria as etiological agent sis underestimated.
Introduction: Periodontitis is a bone destructive inflammatory disease arising due to an aggravated immune response to a sub-gingival biofilm (dental plaque). The condition affects the majority of the population, with severe forms affecting 10% of individuals globally. Tooth loss resulting from disease progression significantly compromises oral health, appearance, quality of life, and drives increased dental care costs. Likewise, the chronic systemic inflammation associated with severe periodontitis compromises general health, increasing the risk and severity of cardiovascular disease, diabetes and autoimmune disease. Periodontitis has been historically treated via non-surgical therapy, where clinicians use hand and/or ultrasonic instruments to physically remove the biofilm from below the gum line. Despite well-documented clinical success, speculation remains as to whether NST alone is sufficient to induce long-term microbiological improvements in patients (particularly in deep pockets), thereby preventing disease recurrence.

Hypothesis and aims: The aim of this study was to investigate whether non-surgical treatment alone was sufficient to achieve and sustain clinical and microbiological improvements up to 3-months following treatment.

Methodology: Samples of biofilm were removed from below the gum margins of 37 patients with periodontitis before and after treatment. DNA was extracted from the samples and 16S rRNA sequencing performed using a MiSeq analyser (Illumina). Clinical measurements of periodontal status were recorded at baseline and 3-months following treatment.

Results: In patients, there were significant alterations in biofilm composition following treatment, including reductions in alpha-diversity and shifts in overall composition. Assessing conventional and novel disease associated organisms revealed significant reductions following treatment, compatible with periodontal health. Co-occurrence networks at genus level revealed dynamic changes in the sub-gingival plaque community following treatment, however disease associated organisms remain.

Conclusion: Overall, our results suggest that NST alone is sufficient to induce clinical and microbiological improvements after 90 days, although remaining disease associated networks suggest there is potential for recurrence.
88: Antibiotic susceptibility patterns of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis strains isolated from subgingival biofilm samples in Switzerland in the last 37 years - Karygianni L

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Introduction: Development of microbial antibiotic resistance is a major concern worldwide. Oral bacteria may also carry several resistance genes.

Hypothesis and aims: To determine the antibiotic susceptibility patterns of 57 Aggregatibacter actinomycetemcomitans and 56 Porphyromonas gingivalis strains isolated from subgingival biofilms of periodontitis patients in Switzerland from 1980 to 2017.

Methodology: After specific 16S rDNA-PCR analysis, the minimal inhibitory concentrations (MIC) of common antibiotics used in periodontal therapy (amoxicillin, metronidazole, azithromycin, doxycycline) and of those applied in other body infections (amoxicillin/clavulanic acid, clindamycin, ertapenem, moxifloxacin) were determined by a commercial microbroth dilution test. High MIC values were additionally confirmed by the agar dilution technique and the strains were screened for beta-lactamase activity and for presence of selected resistance genes (fructose-bisphosphate-aldolase (cfxA), genes responsible for erythromycin resistance protein (ermF) and tetracycline resistance protein (tetQ), respectively.

Results: Overall, an increase of MIC values over time was not observed. The MIC90 of ampicillin was 2 µg/ml against A. actinomycetemcomitans and 0.5 µg/ml against P. gingivalis, while the MIC90 of doxycycline was 1 µg/ml against both species. Beta-lactamase-positive strains and strains containing the cfxA gene were not detected. Two recently (2010) isolated P. gingivalis strains showed the highest MIC values. In particular, the first strain was ermF positive and showed MIC values higher than >8 µg/ml, 2 µg/ml and 0.25 µg/ml for clindamycin, azithromycin and moxifloxacin, respectively. The second strain also presented a high MIC value of 4 µg/ml for moxifloxacin.

Conclusion: The absence of an enhanced antibiotic resistance among the tested periodontopathogens in the last 37 years can be attributed to restricted use of antibiotics against severe periodontitis at the dental universities in Basel, Bern and Zurich. However, the detection of resistant strains like P. gingivalis should raise concern about the optimization of antibiotic therapeutic protocols against periodontitis and other oral diseases.
89: The use of raman technologies for oral bacteria - Kriem L

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

Hypothesis and aims: The theme of the research is whether it is possible to see differences in Raman spectra between five different common oral bacteria (Fusobacterium nucleatum, Streptococcus mutans, Veillonella dispar, Actinomyces naeslundii and Prevotella nigrescens) in monoculture and how these differences can be used as an indicator of their role in the oral mouth flora.

Methodology: Confocal Raman Microscopy (ThermoFisher DXR2xi) was used for analysis. Different planktonic monocultures were concentrated by centrifugation and spread onto glass slides for Raman analysis. The obtained spectra were then compared with each other using least square analysis.

Results: The Raman spectra show a specific fingerprint region where differentiation between different strains are apparent. While it is not possible to use the full Raman spectra (50-3400 cm⁻¹) it is possible to choose a specific 'fingerprint' region to see differences in the composition of bacterial cells. Statistical analysis also supports the idea that bacterial spectra can be differentiated based on Raman spectra

Conclusion: It was possible to differentiate the oral bacteria from each other by using confocal Raman microscopy showing the potential of this technology in biofilm assessment and bacterial differentiation.
90: A potential role for \textit{Fusobacterium nucleatum} c-di-nucleotides production in (multispecies) biofilm formation - Kuehne S.A

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\textbf{Introduction}: \textit{Fusobacterium nucleatum} is an anaerobic species primarily found in dental plaque biofilm, where it is seen as a key orchestrator in the emergence of a dysbiotic pathogenic microflora in periodontitis (gum disease). Plaque biofilms consist of a complex microflora, aggregating in an organised manner. While this aggregation of different species is well documented, their means of communication and in particular their mechanisms of recruitment are less well understood. Cyclic di-nucleotides (CDNs) have been identified as means of communication in bacteria, regulating multiple cellular functions, including biofilm formation. Previous studies revealed the importance of CDNs in the virulence of other dental pathogens, but their function in \textit{F. nucleatum} virulence remains elusive.

\textbf{Hypothesis and aims}: C-di-nucleotides are regulators of dysbiotic biofilm formation, co-aggregation and pathogenicity in \textit{F.nucleatum}. Our aims are: i. to identify candidate genes involved in CDN synthesis/degradation. ii. to analyse their role in dysbiotic biofilm formation including gene expression, biomass production and biofilm architecture.

\textbf{Methodology}: Bioinformatics has been employed to identify candidate genes. Single and multispecies biofilms were grown over a variety of days. Gene expression was quantified using qPCR. Biofilm mass under the different growth conditions was quantified and viability assessed using live/dead staining. Biofilm architecture was investigated using scanning electron microscopy.

\textbf{Results}: We have shown that \textit{F. nucleatum} is producing CDNs when grown \textit{in vitro}. Furthermore, several genes have been identified, which could be involved in CDN synthesis/degradation in \textit{F. nucleatum}. Using qPCR, transcription analysis of these will be presented and transcription levels will be correlated to biofilm growth, viability and architecture.

\textbf{Conclusion}: CDNs are of critical importance in a variety of pathogenic bacteria, with involvement ranging from biofilm formation to host-bacteria interactions. Our data show that \textit{F. nucleatum} also produces CDNs, which could be important in the modulation of virulence, such as biofilm formation.
The role of quorum sensing in the development of *Microcystis aeruginosa* blooms: gene expression - *Lamas-Samanamud G*

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Introduction: Microcystin is a cyanotoxin produced by *Microcystis aeruginosa*. This toxin affects aquatic plants, fish and even human health (as a hepatoxin). *M. aeruginosa* growth is favored by excess of nutrients in the water systems and warmer temperatures. Therefore, water contamination and warming conditions could potentially increase the frequency and the scale of events associated to cyanobacteria. In addition, the mechanism associated to algal bloom and toxin production could also be related to quorum sensing. In other words, the cell-cell communication may be responsible for algal bloom, and consequently, microcystin production.

Hypothesis and aims: This project focuses on the expression of the gene *luxS* in *M. aeruginosa* and how it affects the development of algal bloom.

Methodology: The *luxS* gene in *M.aeruginosa* PCC7806 is believed to be associated to quorum sensing. Another gene, *mcyB* is believed to be responsible for microcystin production. Herein, both *luxS* and *mcyB* were tested by q-PCR throughout *M. aeruginosa* growth for 35 days to determine their concentration in the growth media. Samples were grown in 1L of BG 11 media and collected on specific days. The research strives to provide a specific answer to whether quorum sensing is a potential mechanism expressed by gene *luxS* or gene *mcyB* in *M. aeruginosa* growth based solely on gene expression.

Results: Results revealed that *luxS* is directly associated to algal bloom and it is mostly found during the log phase. *mcyB* is directly associated to the toxin production and it is mostly found under stressful conditions, early log phase and early stationary phase.
92: Polymicrobial denture-associated biofilms: in vitro interactions and infection modelling - Morse D.J

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Introduction: Denture-associated stomatitis (DS), which presents as areas of erythema on the palatal mucosa, is a frequent infection affecting approximately two-thirds of denture-wearers. DS is caused by biofilms that exist on the fitting-surface of dentures, containing the fungus Candida albicans. The contribution of co-existing oral bacteria toward the infection remains unclear.

Hypothesis and aims: This research investigated the in vitro modulatory effect of oral and probiotic bacteria toward C. albicans virulence in biofilms, and compared the bacterial microbiota of patients with and without DS at various relevant sites within the oral cavity to determine associations with infection.

Methodology: In vitro biofilm studies assessed expression of C. albicans virulence factors (morphological transformation, expression of selected virulence genes), and their impact on pathogenesis in an infection model. In clinical studies, microbiological samples were obtained from the tongue, palate and denture-fitting surface of 19 denture-wearing patients (DS n=8, non-DS n=11). The presence of Candida was ascertained by PCR. Bacterial DNA was extracted and subjected to next generation sequencing using bacterial 16S rRNA gene targets, and differences in the bacterial microbiota determined.

Results: Certain bacterial species, significantly (P<0.05) modulated the expression of C. albicans virulence factors compared with mixed-species biofilms of enhanced virulence. Biofilms with enhanced virulence also resulted in increased tissue damage and invasion when modelling infections. Candida was detected in clinical samples of 14 patients (DS n=6, non-DS n=8). Metataxonomic analyses revealed differences in relative abundance of bacterial species, and importantly, a significant (P=0.007) increase in the number of bacterial species was evident for the tongue microbiome of non-DS patients.

Conclusion: The in vitro modulating capacity of bacteria toward Candida virulence, and the observed species-level differences in bacteria between DS and non-DS patients highlight the need for consideration of the bacterial composition of oral biofilms in the pathogenesis and management of DS.
**93: PRESENCE OF AHL-TYPE QUORUM SENSING MOLECULES IN ORAL SAMPLES - Andrea Muras**

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**Introduction:** Although Quorum sensing (QS) plays a central role in bacterial biofilm formation, its role in dental plaque formation remains unclear. So far, the Autoinducer-2 (AI-2) and the peptides produced by oral Streptococci are the only QS signals described in pure cultures of oral pathogens, while acyl-Homoserine Lactones (AHLs) have not been reported so far. However, several indirect evidences indicate a role of AHL signals in the oral cavity.

**Hypothesis and aims:** The detection of AHLs in oral samples to a better understanding of the role of these type of QS molecules in dental plaque formation and in development of bacteria-associated oral pathologies.

**Methodology:** Saliva (12) and extracted teeth (12) from patients presenting different oral pathologies were obtained and used for the AHL extraction with organic solvents. Detection and quantification of AHLs was performed using HPLC-MS. Additionally, the analysis of 700 oral genomes and 137 oral metagenomes were performed in order to identify genes related to AHL-mediated QS and quorum quenching (QQ) enzymes.

**Results:** The QS molecules C8, C14 and C18-HSL could be identified in saliva and extracted teeth samples. Furthermore, the analysis of oral genomes and metagenomes showed a high prevalence of QS and QQ genes (99,27%) in these samples.

**Conclusion:** The presence of AHLs in dental plaque and extracted teeth indicate that the QS network in the oral cavity may be much more complex than the accepted paradigm open a new opportunity to find alternative therapies in order to prevent and control oral infections. Further studies are required in order to evaluate the role of AHLs in Gram-negative oral pathogen prevalence and in the equilibrium of oral microbiome.
94: Quorum sensing-inducing subpopulation specific eDNA production in *Streptococcus mutans* - Nagasawa R

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**Introduction:** *Streptococcus mutans*, an etiological agent of dental caries, causes tooth decay through biofilm formation on tooth surfaces. Extracellular DNA (eDNA) is an important factor for the biofilm formation that mechanically enhances the biofilm structure in *S. mutans*. The eDNA is believed to be released from dead cells as a consequence of cell lysis. However, no direct observation of eDNA production in *S. mutans* is documented, and how eDNA is released within the biofilm population remains unclear.

**Hypothesis and aims:** In this study, we focus on the cell death induced by quorum sensing (QS) and analyze the eDNA production at the single-cell and population level by using flow cytometry, time-lapse imaging, and confocal laser scanning microscope (CLSM).

**Methodology and Results:** Since secretory peptide signal (competence-stimulating peptide, CSP) was known to induce cell death in *S. mutans*, we added to CSP the medium to induce cell death and eDNA release. We confirmed that cell death was induced in a sub-population of the cells by the addition of CSP by flow cytometry analysis. Time-lapse imaging at the single-cell level revealed that a subpopulation of dead cells was lysed and releases DNAs to the extracellular milieu. The cell death and eDNA production was reduced in the deletion mutant of *lytF*, a QS-controlled putative autolysin. In addition, we found that *lytF*-expressing cells and dead cells were localized at the bottom of the biofilm by using the promoter reporter strain of *lytF* with CLSM.

**Conclusion:** Accordingly, our results suggest that *S. mutans* produces eDNA by cell lysis, which controlled by QS-dependent autolysin, at the bottom of the biofilm. This can contribute to enhance attachment to the surfaces and mechanical strength of the biofilm.
Introduction: Pancreatic cancer is the fourth leading cause of cancer death worldwide. The most common sign of presentation of pancreatic cancer is obstructive jaundice, which prevents the drainage of bile into the intestines and it is often associated with decreased survival in patients. The role of biliary stents in achieving biliary decompression has been well established; yet, biliary stenting disrupts the natural anatomic barrier between the biliary and the gastrointestinal tract, strongly increasing the risk of a bacterial infection. Still, very little is known about the growth of antibiotic-resistant biofilms on the stents and their role in infectious post-operative complications.

Hypothesis and aims: The biliary system is an inherently fluid mechanical environment, where the gallbladder provides the driving pressure: our hypothesis is that the mechanical stress induced by the bile flow in the stent is likely to play a significant role in the formation of biofilms.

Methodology: To test this hypothesis we exploit the power of microfluidics – i.e., the technology of driving fluids at the micrometer scale – coupled with advanced imaging and image analysis, which enable exquisite control of the physical and chemical environment while allowing high-resolution observations of biofilm formation in space and time. Six clinically relevant isolates from preoperative biliary stents were selected to be grown inside microfluidic channels at different flow rates, in which bacterial attachment and biofilm dynamics were recorded and quantified.

Results: We found that fluid flow largely influences biofilm formation in all the isolates, for which the conditions of flow and shear stress that maximize microcolony formation and extracellular matrix production have been determined.

Conclusion: These results will help us to improve our understanding of biofilm formation in the presence of fluid dynamic environments and eventually consider optimal parameters of flow in the design of medical devices.
96: The impact of smoking on subgingival biofilms and host antimicrobial peptides in patients with chronic periodontitis - Soldati K.R

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Introduction: Periodontitis is resulted from the interaction between subgingival biofilms containing periodontal pathogenic bacteria and the host response. The host produces antimicrobial peptides such as hBD1 and hBD2 upon bacterial challenge.

Hypothesis and aims: to compare the influence of smoking on the levels of periodontal pathogens, hBD1 and hBD2 in patients with chronic periodontitis.

Methodology: Sixty patients with chronic periodontitis were allocated into: NS (n = 30, non-smokers); S (n = 30, smokers). Subgingival biofilms and gingival crevicular fluid (GCF) samples were collected from 5 healthy sites and 5 diseased sites. Biofilm samples were analyzed by quantitative PCR for the levels of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Parvimonas micra, Fusobacterium nucleatum and Prevotella intermedia. While hBD1 and hBD2 in GCFs were quantified by enzyme-linked immunosorbent assay.

Results: Regarding periodontal pathogens, smoking enhanced the counts of P. micra and T. forsythia in healthy sites (P < .05). When comparing between healthy and diseased sites of the same patient, higher concentration of P. gingivalis, T. denticola and T. forsythia in diseased sites was observed in NS patients (P < .05) whereas in S patients only the level of P. gingivalis was higher in diseased site (P < .05). Regarding hBD1 and hBD2, smoking reduced the levels of both peptides in diseased sites but enhanced their levels in healthy sites. In NS patients, the levels of hBD1 and hBD2 were significantly higher in diseased sites than in healthy sites.

Conclusion: In non-smokers, the levels of both hBD1/hBD2 and periodontal pathogens were higher at the sites with chronic periodontitis. Smoking changed the expression pattern of the tested peptides, but it did not affect the levels of examined periodontal pathogens at the diseased site.
Prebiotic potential of native plaque DNase enzymes to control oral biofilms - *Rostami N*

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**Introduction:** Dental plaque is a multispecies biofilm formed on the surface of teeth. Extracellular DNA (eDNA) is an integral component of dental plaque and is required for attachment and colonization of certain oral bacteria. Some oral bacteria express deoxyribonuclease (DNase) enzymes, but their function in oral biofilms is not yet known. Here, we identified and characterized SsnA, a DNase enzyme produced by the pioneer coloniser *Streptococcus gordonii*.

**Hypothesis and aims:** The aims of this study were (i) to determine the regulatory mechanisms underlying the expression of *S. gordonii ssnA*, and (ii) to investigate the activity of SsnA enzyme against oral biofilms.

**Methodology:** An *S. gordonii ccpA* null mutant was constructed, along with a genetically complemented strain. Gene regulation by sugars was assessed by RT-qPCR. A microfluidic (BioFlux) microcosm model was employed to test SsnA efficacy in controlling mixed species biofilm formation.

**Results:** Using RegPrecise, a CRE motif was identified in the promoter region of SsnA suggesting carbon catabolite-mediated regulation of SsnA. The presence of glucose, sucrose and maltose, but not galactose, during growth inhibited SsnA expression. The carbon catabolite-mediated regulation of SsnA was lost upon deletion of *ccpA* in *S. gordonii*. SsnA exhibited a strong antibiofilm activity against oral microcosms grown under flow in natural human saliva using BioFlux. However, the presence of sucrose inhibited the antibiofilm activity of SsnA.

**Conclusion:** *S. gordonii* SsnA is an extracellular DNase that inhibits oral microcosm formation in the absence of sugars and at neutral to high pH. Strategies to modulate expression of DNases such as SsnA in dental plaque, may help to control the formation of plaque or the transition from health to disease.
Introduction: Healthcare settings’ surfaces and equipments can represent a reservoir of microbial contamination given the contact with MDRO and their particularities on environmental cleaning. The presence of microorganisms in biofilm form can represent a challenge for infection prevention. In the endoscopy unit scenario, the focus has been on endoscope reprocessing. However, evaluation of other factors in endoscopy units should be further addressed.

Hypothesis and aims: In this study, we aimed to identify areas with high bacterial contamination on endoscopy unit surfaces and equipment.

Methodology: Samples for microbial analysis were collected from the nurse’s station and two procedure rooms after routine decontamination process. Initially, an ATP test was performed to detect areas with high soil load, using a 100 RLU threshold. If above this threshold, a microbial sample from an adjacent area was collected, using sterile PBS wetted sterile gauze and vigorous rubbing movements (necessary to collect biofilm). For culture, each sample was sonicated, vortex and cultured. Bacteria isolated were identified by morphology using selective media and biochemical characteristics were assessed by Analytical Profile Index system.

Results: A total of 24 areas were initially sampled for soil by ATP assay. Sequentially, a total of 8 areas were tested for bacterial contamination by microbial culture. The areas with higher soil loads were the telephone at the nurse’s station and the sink pipe in the procedure room. The culture results show area with uncountable bacterial growth and areas from 5 to 165 CFU/cm². All culture positive results had multispecies bacterial growth and the microorganisms isolated included Shigella spp., S. aureus, E. coli, Pseudomonas spp., Pantoea spp. and Enterococci.

Conclusion: These findings suggest that not only the procedure room, but endoscopy unit’s nurse station area is also subjected to bacterial contamination with pathogenic organisms and therefore should be carefully addressed for more rigorous decontamination.
Introduction: Communication between bacteria in the environment affects their ecological functions and roles. Communication can happen via physical and/or chemical signaling. It is thought that the complex polymicrobial aggregates that comprise granular sludge, found in wastewater treatment systems, enables efficient waste processing. The use of these microbial granules, which colocalize organisms with different functions, such as nitrifying and/or denitrifying, has enabled more compact reactor designs. However, the relationship between physical location and activity remains unclear.

Hypothesis and aims: The bacteria found in activated sludge naturally self-segregate depending on specific needs. Gaining control over encapsulated distributions of bacteria, to mimic this segregation and control interspecies communication, using core-shell microcapsules may lead to methods for improving nitrogen reducing activity.

Methodology: We encapsulate bacteria in alginate microcapsules generated from monodisperse water-in-oil (w/o) emulsions formed at a microfluidic cross-junction. The distribution of different bacteria in the core-shell microcapsules is controlled by the laminar flow in the microchannels. *Pseudomonas aeruginosa* and *Nitrosomas europaea*, both Gram negative bacteria, are used as a model denitrifying and nitrifying organisms, respectively. After encapsulation we culture and assay the nitrogen removal activity.

Results: We generated 50 µm diameter microcapsules (C.V.~5%) where we control the distribution of encapsulated bacteria by modulating flow rates. The presence of oxygen typically decreases the denitrifying efficiency; however, we confirm that the denitrifying activity is not detrimentally affected by encapsulation.

Conclusion: We show that encapsulation of *P. aeruginosa* naturally creates an environment conducive for denitrification despite exposure to oxygen. Using the present system, it is possible to localize populations of bacteria either in the core and/or shell. Furthermore, substituting the bacteria used in the current study for others to test bioproduction and bioremediation efficiencies is easy. We aim to quantify activity of co-encapsulated species within the microcapsules to optimize the process.
100: Cross-domain signaling: bacterial response to archaeal quorum sensing - Thompson T.P


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Introduction: Quorum sensing (QS) is a cell-cell signalling mechanism used by microorganisms to regulate and coordinate community behaviour through the release of autoinducer molecules into the extracellular environment. In recent years, N-acyl homoserine lactone (AHL)-based QS mechanisms similar to the well-characterised AutoInducer-1 system used in Gram-negative system have been discovered in the domain Archaea.

Hypothesis and aims: Investigation into the potential of haloarchaea to induce bacterial QS systems.

Methodology: The crude extract of the haloarchaea from the genus *Halorubrum* was screened for QS induction using the AHL reporter strains *A. tumefaciens* ATCC BAA-2240, *C. violaceum* CV026, and the *E. coli* luminescence reporter JM109 pSB536, pSB401, and psB1142. LC-MS (QTOF) analysis was employed to determine preliminary structural elucidation of the autoinducer. The effect on virulence factor production was assessed using the *Pseudomonas aeruginosa* mutant strain PAO-MW1.

Results: The *Halorubrum* extract was found to induce β-galactosidase activity in *A. tumefaciens*, suggesting the production of AHL-like molecules typically associated with bacterial QS. TLC-overlay using the same reporter strain revealed structural similarity between this halophilic archaeal extract and the bacterial signalling molecule butyryl acyl homoserine lactone (C4-AHL). This activity was confirmed by a positive response from the luminescence based short-chain AHL reporter JM109 pSB536. QTOF analysis of the extract revealed the presence of a compound with comparable retention time and fragmentation pattern to that of a C4-AHL. Importantly, the extract restored the production of virulence factors, pyocyanin and pyoverdine, in PAO-MW1 emphasising the capacity of an archaeal autoinducer to be sensed by bacteria.

Conclusion: Our findings raise new questions concerning the evolution and role of QS-systems in bacteria and archaea, on the evolutionary relationship that exists between these microorganisms and on the ability of the halophilic members of these two separate domains of the tree of life to interact.
101: A novel rapid prototyping tool for suspended biofilm growth media - Tsagkari E

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Introduction: Biofilms play an essential role in treating water in biofiltration systems. The biofilm morphology and function are inextricably linked to the hydrodynamics of flow through a filter and yet engineers rarely explicitly engineer this interaction.

Hypothesis and aims: We develop a system that links computer simulation and 3-D printing to optimize and rapidly prototype filter media to optimize biofilm function with the hypothesis that biofilm function is intimately linked to the flow passing through the filter.

Methodology: A computational model that numerically solves the incompressible time-dependent Navier Stokes equations coupled to a model for biofilm growth and function is developed. The model is imbedded in an optimization algorithm that allows the model domain to adapt until criteria on biofilm functioning are met. This is applied to optimize the shape of filter media in a simple flow channel to promote biofilm formation. The computer code links directly to a 3-D printer and this allows us to rapidly prototype the design. Its validity is tested in flow visualization experiments and by microscopy.

Results: As proof of concept the code was constrained to explore a small range of potential filter media, where the medium acts as an obstacle in the flow that sheds a von Karman vortex street that was found to enhance the deposition of bacteria on surfaces downstream. The flow visualization and microscopy in the 3-D printed realization of the flow channel validated the predictions of the model and hence its potential as a design tool.

Conclusion: Overall, it is shown that the combination of our computational model and the 3-D printing can be effectively used as a design tool to prototype filter media to optimize biofilm formation.
102: Developing commercially relevant complex biofilm models: limitations for standardisation - Young T

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Introduction: The need for standardised biofilm assays to streamline industry testing is paramount. The reliance of simplistic mono-species biofilms can often be uninformative, as in medical and industrial settings biofilms are often polymicrobial. Biofilm testing against these biofilms can be less responsive to antimicrobial action, thereby providing a tougher and more realistic challenge for antimicrobial screening. These systems are more difficult to implement and standardize given the time and effort needing in creating these complex biofilms; hence there remains a requirement for robust and reproducible complex biofilm assays in a commercial setting.

Hypothesis and aims: The aim of this study is to assess the robustness and repeatability of 3 bulk-created inocula oral biofilm models.

Methodology: To facilitate commercialisation, biofilms were created and stored in a bulk inoculum format. Using simple and easily reproducible metabolic assays such as a resazurin dye, the metabolic profile of these polymicrobial biofilms reconstituted after freezing was established. The biomass has been semi-quantified using dyes to stain the biofilms. Quantitative PCR has also been utilised to assess the composition of these inocula created biofilms. A shelf life study over 3 months has been used to show the feasibility of such methodology in a commercial setting.

Results: Results indicate that there is good reproducibility in biofilms formed from the inoculum biofilm in a 96 well format when compared to fresh. The metabolic assays indicate some changes in the profile over the 3-month study but are still comparable under treatments. Biomass is shown to be a robust measurement of these biofilms over the 3 months shelf life. Composition remains mostly similar between sets with the main species still heavily identifiable, but limitations have been identified in the overall reduction in diversity shown in the qPCR results.

Conclusion: Limitations exist in this inoculum biofilm testbed, but this study identifies a proof of concept. Testing of actives in an industrial setting require simple outputs that can be relayed to the consumer quickly and effectively and this study provides this. In order to facilitate the advancement of biofilm research and industrial testing, there is the requirement for standardized tests of more complex biofilm research.
103: Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis - Artini M

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Introduction: Cystic fibrosis (CF) patients manifest a variety of multi-organ problems due to the alteration of sodium and chloride secretion across cell membranes. The impairment of muco-ciliary clearance leads to the production of a thick and dehydrated mucus in the CF lung, which promotes the airway chronic bacterial colonization. In the early stage of life, it is characterized by the prevalence of S. aureus. In early adolescence, Gram-negative bacteria chronically infected it. Among these, P. aeruginosa is the most relevant and recurring. Recently, several reports indicated in vitro efficacy of natural compounds as promising treatment to reduce the development of the CF associated infections. Among these, essential oils (EOs) seemed to be the most promising agents. Recently machine learning (ML) has been proved as tool to enable the deep investigation on EOs chemical components modulation role against both P. aeruginosa and S. aureus.

Hypothesis and aims: In this study an extensive analysis on 61 commercial EOs against a panel of 40 bacterial isolates from CF patients is reported.

Methodology: Clinical bacterial isolates were classified on the basis of phenotypic and genotypic features (descriptors). To speed-up the in vitro procedure, classification algorithms allowed the strains clusterization in to select representatives to be subjected to EOs antimicrobial evaluation.

Results: Some EOs, showing a strong efficacy to impair the growth of microorganisms, were promptly assayed against all the clinical isolates. Among them three EOs demonstrated their ability to inhibit all bacterial growths. The potent EOs were analyzed for by means of gas chromatography coupled with mass spectrometry to investigate on the likely chemical components mainly responsible for the antibacterial activity.

Conclusion: Investigation of the most important components by means of feature importance and partial dependence plots allowed us to indicate the chemical components mostly related to antimicrobial activity of three active EOs.
Introduction: Bacteria in biofilms present a collective behavior able to divert the immune system in case of infections. Their response to antibiotherapy differs between the planktonic or the biofilms forms. In the aim to take into account the biofilm growth, Antibiofilmogram was developed. It allows to study the bacterial behavior in presence of several antibiotics concentrations, and to determine the biofilm Minimal Inhibition Concentration (bMIC). It corresponds to the smallest concentration of antibiotics, able to prevent the bacterial adhesion.

Hypothesis and aims: Data are missing as antibiograms allow the determination of the antibiotic activity only on the plaktonic form. Antibiofilmograms should help to reconsider the antibiotherapy.

Methodology: The method derived from the BioFilm Ring Test was applied on a clinical strains panels of S. aureus from Joint and bones Infections. The bMIC relevance was validated for Cloxacillin and Gentamicin with confocal laser scanning microscopy. To go further, the efficiency of an adapted antibiotherapy was evaluated on 2 murine models, one with an infected catheter and the second one on wound to follow the healing of infected skin.

Results: Data from microscopy clearly indicate the absence of viable bacteria in the microwells with cloxacillin or gentamicin applied at the bMIC in contrast to the MIC. Gentamicin injected at a concentration corresponding to bMIC and MIC in a mouse model (BALB\C) with an infected catheter induces a decrease of 3 log of bacterial load with the bMIC whereas MIC dose was totally unefficient. The second murine model was developed with immunocompromised mice to avoid a fast spontaneous healing of the C57Bl/6 skin. The evolution of the bacterial load on the wound was better with cloxacillin applied at the bMIC than the MIC with a decrease of 4 log CFU/skin.

Conclusion: The Antibiofilmogram offers the opportunity to adapt the antibiotherapy with a new set of relevant data, when a biofilm development is suspected.
Introduction: Colonisation and biofilm formation on medical devices is driving an urgent need for novel antibacterial strategies. In this respect, biocide-releasing materials are a powerful tool to prevent the initial bacterial colonisation that eventually leads to medical device failure.

Hypothesis and aims: In this work, biocompatible polydimethylsiloxane (PDMS) was functionalised with salicylic acid (SA), a biocide approved for use on humans, to inhibit biofilm formation and proliferation of planktonic cells.

Methodology: We developed a novel post fabrication-modification method to incorporate SA within the PDMS bulk without altering the physicochemical properties of the surface. Changes in surface morphology and chemistry were assessed by atomic force microscopy, contact angle measurements and Raman spectroscopy. Vibrational and UV-vis spectroscopy allowed us to monitor the release of SA from the material's bulk into the surrounding media. The antimicrobial performance of the materials was tested on *S. aureus* and *E. coli* strains associated to medical device biofilms, using viability assays and high-resolution SEM characterisation of bacterial attachment.

Results: Our functionalisation method had minimal effect on the roughness and chemistry of the PDMS surface, while the release of SA into the surrounding media reached bactericidal concentrations within the first 24h for both tested bacterial strains, which caused strong inhibition of biofilm formation and planktonic cell death.

Conclusion: We demonstrated that our post fabrication-modification of PDMS led to effective biocide incorporation within the material's bulk while preserving the surface properties. This approach could be translated to existing medical devices and be used to prevent surface colonisation by Gram-positive and Gram-negative bacteria.
106: New antimicrobial peptide disrupts mono- and dual-species biofilms of Pseudomonas aeruginosa and Staphylococcus aureus - Bessa L.J

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Introduction: Pseudomonas aeruginosa and Staphylococcus aureus are two major pathogens involved in a wide variety of infections, namely chronic wounds and pneumonia in cystic fibrosis patients. The co-occurrence of those pathogens from the same site of infection has been frequently reported and it is linked to enhanced virulence and consequent difficult eradication.

Hypothesis and aims: Herein, antimicrobial and antibiofilm activities of an intragenic antimicrobial peptide (IAP) uncovered by the software Kamal from the human unconventional myosin 1H protein, named Hs02, were investigated against P. aeruginosa and S. aureus mono- and dual-species biofilms.

Methodology: Minimum inhibitory concentrations (MICs) and minimum bacterial concentrations (MBCs) of the peptide Hs02 were determined against reference strains and multidrug-resistant (MDR) isolates of P. aeruginosa and S. aureus (including methicillin-resistant S. aureus – MRSA). The ability of peptide Hs02 to interfere with the biofilm formation of P. aeruginosa and S. aureus strains was evaluated through the crystal violet assay. Subsequently, 24-h preformed biofilms (single and dual-species) of MDR P. aeruginosa and MRSA were treated with Hs02 at concentrations above the MIC and analyzed through several methods, including confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM).

Results: MIC values of peptide Hs02 ranged from 2 to 16 μg/mL against all strains and MDR isolates. Though Hs02 did not have ability to hamper the biofilm formation, it clearly affected 24-h preformed biofilms, especially by significantly reducing the viability of the bacterial cells within the single and dual-species biofilms as shown by CLSM and AFM images.

Conclusion: Peptide Hs02 presents great bactericidal activity on both Gram-negatives and Gram-positives and, additionally, it affects preformed biofilms of two major pathogens such as P. aeruginosa and S. aureus, both in single and dual-species biofilms.
107: Biofilm-related antimicrobial cross-resistance: lesson learned from an old hydroxyquinolines - Bidossi A

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Introduction: Persistence of skin and wound infections is widely recognized to be associated to bacterial biofilms, which are highly recalcitrant to treatments thus contributing to maintain a constant inflammation state and to prevent a correct healing. Topical antibiotics are the most common first line self-medications, however treatment failure is not uncommon and emerging resistance to antibiotics is alarming. Development of new antibiotics is drastically slowing down and old antimicrobials, are increasingly re-proposed. Furthermore, classical routes of drug testing might fail in predicting efficacy against biofilm-associated infections. Chlorquinaldol is a topical antimicrobial with a wide spectrum of activity, whose ability to prevent or eradicate Staphylococcus aureus and Pseudomonas aeruginosa biofilms was evaluated in this study in comparison to classic topical antibiotics like gentamicin and fusidic acid.

Methodology: Clinical strains isolated from skin and wound infections were chosen from the collection of our institute and the effect of the chosen antibacterial compounds on their in vitro biofilms was assessed by means of spectrophotometry and confocal laser scan microscopy.

Results: Though subinhibitory concentrations of chlorquinaldol generally displayed a good antibiofilm activity comparable to that of gentamicin, resistance to methicillin in staphylococci and impermeability to carbapenems phenotype in P. aeruginosa impaired its effect, while no difference in antimicrobial susceptibility between the groups was observed when tested on planktonic cells.

Conclusion: These observations strengthen the need to expand the tests routinely performed to biofilm bacteria and to include in the tests pathogenic isolates of the same species with different resistance patterns, since acquired genes and mutations might reflect in global changes that are not identifiable in the planktonic state.
**Introduction:** Oleanolic Acid (OA) and Maslinic Acid (MA) are pentacyclic triterpenic compounds that abound in the industrial olive-oil waste. These compounds have known antimicrobial properties and lack cytotoxicity in eukaryotic cells as well as resistance mechanisms in bacteria. Despite these advantages, their antimicrobial activity has only been tested in vitro, and derivatives improving this activity have not been reported.

**Hypothesis and aims:** In this work, a set of 14 OA and MA C-28 amide derivatives have been synthesized and analysed for their in vitro and in vivo antibiofilm and antimicrobial activity.

**Methodology:** Catheter infection and continuous-flow biofilm models were performed to test the derivative compounds’ antibiofilm activity against *S. aureus*. Also, their antimicrobial activity against several bacterial strains was examined. Moreover, different microscopic analysis was performed to identify a putative mechanism of action of these derivatives. Finally, the efficacy and toxicity of the compounds were probed in a *Galleria mellonella* invertebrate animal model of infection.

**Results:** Two of these derivatives increase the antimicrobial activity of the parents’ compounds in most of the Gram-positive bacteria tested, including a methicillin-resistant *Staphylococcus aureus*-MRSA. They are also capable of increasing the antibiofilm removal activity in a catheter and in a continuous-flow *S. aureus* biofilm as well as their efficacy in vivo in *G. mellonella*. Their mechanism of action shows that these compounds can damage the bacterial cell membrane.

**Conclusion:** These two derivatives highlight the benefits that natural feedstock has as an easily accessible source of bioactive molecules for the development of new antimicrobial agents.
109: Evaluation of novel XF-Drugs - Board-Davies E.L

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Introduction: A global action plan for antimicrobial resistance was issued by the World Health Organisation due to the increasing incidence of antibiotic resistance. Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus pneumoniae are amongst the most commonly reported antibiotic resistant bacteria, and globally, an estimated 4.6-6.4 billion bloodstream infections are caused by third-generation cephalosporin-resistant E coli and K pneumoniae. As traditional antibiotics become redundant, development of novel antimicrobial agents is urgently required. Destiny Pharma plc have developed a series of XF-drugs which exhibit a dual mechanism of antibacterial action. An intrinsic mechanism occurs through drug binding to bacterial membranes and coupled to this is a light-activated, photo-dynamic mechanism via a porphyrin ring, which results in the generation of reactive oxygen species.

Hypothesis and aims: We hypothesise that XF-drugs will exhibit an antibacterial spectrum of activity against a range of pathogenic bacteria which will be enhanced through light activation of the porphyrin ring structure of these novel small molecules.

Methodology: A collection of 114 bacterial isolates were used in this study, including clinical strains of E coli, K pneumoniae and S aureus. Minimum inhibitory concentration assays (MICs) were performed with 3 different XF-drugs. MICs for XF-73 and XF-70 were performed with and without light. DPD-207 was tested only in the absence of light activation as it is designed to lack photo-dynamic activation.

Results: All Gram-positive isolates were sensitive to XF-70 and XF-73 (DPD207 studies on-going), with lowest MICs of 0.25µg/ml. Light activation of XF-73 and XF-70 resulted in elevated bacterial susceptibility.

Conclusion: The XF-series of drugs have a broad-spectrum of antimicrobial activity, with light activation enhancing the sensitivity of some isolates. Further studies will ascertain the antibiofilm activity of XF-drugs and whether synergistic effects manifest with conventional antibiotics. Based on these findings, XF-drugs demonstrate significant promise as new agents in combatting antimicrobial resistance.
Introduction: P. aeruginosa and S. aureus are opportunistic pathogens that cause a wide range of infections. Their increasing resistance to antibiotics is a serious concern and making them susceptible to treatments is now more essential than ever. There is a need to discover new biofilm inhibitors to increase the susceptibility of these bacteria to antibiotics.

Hypothesis and aims: To evaluate the antibiofilm activity of heather honey, propolis and medicinal plant extracts against P. aeruginosa and S. aureus.

Methodology: Determination of optimum biofilm growth was carried out using a time-course assay over 24 h intervals, using P. aeruginosa PA14 and S. aureus NCTC 4135 strains. The inhibitory effects of all extracts were determined by biofilm inhibition assay in 24-well plates, with biofilms stained with crystal violet and de-stained with ethanol:acetone; OD were measured at 550 nm. Planktonic growth was measured at 600 nm and samples from the wells were streaked to determine bactericidal effects.

Results: Heather honey extracts inhibited both P. aeruginosa and S. aureus by 68%. At 60 µg/mL, one of the propolis extracts promoted biofilm growth of both pathogens. Two other propolis extracts also promoted growth in P. aeruginosa but inhibited biofilm formation in S. aureus by 76.5% and 13.8%, respectively. Three plant extracts inhibited S. aureus biofilm by 7.5%, 10.2% and 87.6% and inhibited P. aeruginosa by -34.9%, 34.7% and 19.4%, respectively.

Conclusion: All samples showed varying biofilm inhibition capabilities, but biofilm formation seemed to be more easily inhibited in S. aureus than in P. aeruginosa.
Introduction: Biofilms allow bacterial consortia to live attached to surfaces, with the protection and shielding from harsh environmental conditions and antimicrobial agents provided by the polymer matrix. This is of particular concern in food processing environments, where biofilms can facilitate the spread of pathogenic strains and contamination of food products, thus posing a risk to food safety and human health. The risk is further accentuated in the context of antimicrobial resistance, which can be heightened by the shielding effect of the biofilm matrix, which can also act as a potential reservoir of antimicrobial resistance genes.

Hypothesis and aims: The formation of biofilms is facilitated by quorum sensing processes between micro-organisms and, thus, inhibition of this communication can find applications in prevention of biofilm formation or in facilitating its destruction through enhanced antimicrobial sensitivity. The aim of this study is to identify and characterize bacterial isolates and products capable of interrupting quorum sensing processes and hence biofilm formation.

Methodology: A pre-existing collection of ~3000 bacterial isolates from dairy products and processing environments was screened for inhibition of AHL-mediated quorum sensing processes using agar overlays containing the indicator organism Chromobacterium violaceum DSM 30191.

Results: 50 presumptively positive isolates for inhibition of AHL-mediated quorum sensing were identified with a view to evaluating their ability to inhibit biofilm formation using static biofilm assays.

Conclusion: Presumptive inhibitors of quorum sensing have been identified through high-throughput screening. These isolates will be characterized using a functional genomics approach to elucidate the gene products responsible for the quorum sensing inhibitory phenotype and will be further evaluated for potential applications in prevention of the formation or dispersal of biofilms in industrial settings.
**112: In-vitro effect of antibiotic loaded calcium sulfate beads on bacterial growth from infected diabetic foot ulcer tissue - Julie Fletcher**

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**Introduction:** Diabetic foot infection (DFI) is the main reason for diabetes-related hospitalisation and is a major cause of diabetes-related amputation. Recent figures published by Public Health England show that there are more than 163 diabetes related amputations in England every week.

**Hypothesis and aims:** This study investigates the effect of antibiotic loaded calcium sulfate (Stimulan® Rapid Cure) beads on *in-vitro* bacterial growth from tissue taken from diabetic foot infections.

**Methodology:** Patients were recruited from the Macleod Diabetes and Endocrine Centre at the Royal Devon and Exeter Hospital. Inclusion in the study was based on the presence of an infected foot ulcer requiring wound debridement. Debrided tissue was homogenised and 50 µl spread over the surface of Columbia blood agar and fastidious anaerobe agar. Calcium sulfate beads containing a combination of vancomycin and gentamicin were then placed on the surface of the agar in triplicate. Each bead contained approximately 3.4 mg and 1.6 mg of vancomycin and gentamicin respectively. Plates were incubated aerobically or anaerobically as appropriate. Zones of inhibition were recorded at 1 and 4 days.

**Results:** Calcium sulfate beads containing vancomycin and gentamicin were able to inhibit bacterial growth in all tissue homogenates tested with zone diameters ranging from 12 – 40 mm.

**Conclusion:** Local release of antibiotics could have the benefit of achieving greater local concentrations which could lead to a more efficient clearance of infection. By improving treatment of DFIs, it may be possible to prevent amputation, maintain mobility and conserve quality of life.
Introduction: Plants are widely used to treat various diseases and have been widely recognized as a rich source of phytochemicals with antimicrobial potential. In fact, plants have received considerable attention by researchers being their biological properties widely explored.

Hypothesis and aims: Medicinal and aromatic plants are known to have a wide range of uses and health benefits, and should be exploited concerning their bioactivity. Therefore, the antimicrobial activity of *Satureja montana* L., *Origanum majorana* L., *Allium schoenoprasum* L. and *Anethum graveolens* L. were evaluated and its phytochemical composition was profiled.

Methodology: The antimicrobial susceptibility of Gram-positive and Gram-negative bacteria to four decoction and hydroethanolic (80:20, v/v) extracts, obtained from medicinal and aromatic plants (*S. montana*, *O. majorana*, *A. schoenoprasum* and *A. graveolens*), was assessed aiming to identify the active extracts and the most effective were then tested against biofilms. Furthermore, the decoctions were characterized in terms of phenolic compounds by HPLC-DAD-ESI/MSn.

Results: Overall, *S. montana* and *O. majorana* extracts were the most effective against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus dysgalactiae*) and Gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria, with decoction presenting the most pronounced effects. *O. majorana* and *S. montana* decoction, at minimum inhibitory concentrations, were significantly effective against planktonic cells of *S. aureus* ATCC 25923. Concerning biofilm cells, *S. montana* promoted a slight antimicrobial activity against *S. aureus* ATCC 25923. A total of twenty-four phenolic compounds (9 phenolic acids and 15 flavonoids glycosides) were identified in *S. montana* and *O. majorana* decoctions, being rosmarinic acid the main molecule in the extracts.

Conclusion: This study confirmed the bioactive potential of the medicinal and aromatic herbs, being *S. montana* and *O. majorana* decoction extracts those that showed the most promising applicability for the development of novel formulations with antimicrobial properties.
The potential use of probiotics to control biofilm formation in urinary catheters -
Gomes L.C


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Introduction: Urinary catheters (UC) are particularly susceptible to bacterial contamination, which can lead to the development of biofilms on the inner and/or outer surfaces of the device. The main problems associated with the microbial infections related with UC are a poor response to antibiotic therapy, recurrence of infections and selection of resistant pathogens. Therefore, new approaches are needed to treat and prevent such type of infections.

Hypothesis and aims: Previous studies have demonstrated that probiotics can displace adhering uropathogens from catheter materials and block bacterial adhesion to human uroepithelial cells. The aim of this work was to evaluate the impact of two different probiotics (Lactobacillus plantarum and Lactobacillus rhamnosus) on the development of E. coli biofilms pre-formed on silicone rubber.

Methodology: E. coli biofilms were grown on silicone coupons placed inside 12-well plates containing artificial urine medium. The plates were incubated at 37 ºC in an orbital shaker to mimic the hydrodynamic conditions found in UC. After 24 and 48 h of incubation, the E. coli biofilms were exposed to probiotic suspensions for 6 and 24 h. CFU (colony-forming unit) counts and the crystal violet staining method were used to determine the cell culturability and total biomass of the biofilms, respectively.

Results: This study showed that 24-h biofilms were more susceptible to the probiotic action than 48-h biofilms. Moreover, L. rhamnosus was responsible for higher reduction rates in culturable E. coli population (54% and 37% for 24-h-old biofilms after 6 and 24 h of exposure, respectively) than L. plantarum (13% and 18% after 6 and 24 h of exposure, respectively). Although L. rhamnosus caused significant E. coli killing, it did not reduce the biofilm mass.

Conclusion: These promising results suggest the potential of Lactobacillus strains to treat biofilms developed on polymeric surfaces, which will pave the way to further experiments on the topic.
115: Biofilm inhibiting activity of selenium compounds - Kincses A

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Introduction: Staphylococcus aureus and Salmonella Typhimurium have ability to form biofilms on both biotic (epithelial cells) and abiotic (plastic, glass, medical devices) surfaces. The biofilm is a community of microbes in which bacteria are more resistant to antibacterial agents compared to the sessile cells. Because of this fact treating the infectious diseases is often complicated. Consequently, the discovery of new antimicrobial agents or adjuvants is a major challenge for drug development, furthermore this is condescended to overcome the resistance.

Hypothesis and aims: The antibacterial activity of selenium-containing compounds has been found many studied in the literature. This is the reason why the aim of this study was to find selenium compounds which show anti-biofilm activity alone and combination with antibiotics on Gram-positive and Gram-negative model bacterial strains.

Methodology: Therefore, eleven (1-11) selenocompounds were evaluated for their antibacterial and anti-biofilm activity on Salmonella Typhimurium 14028s and Staphylococcus aureus ATCC 25923 strains. The combined anti-biofilm effects of antibiotics (tetracycline and ciprofloxacin) and compounds were also assessed by using the microdilution method in S. Typhimurium and S. aureus strains.

Results: The compounds 1, 6-8 and 10, 11 were the most potent inhibitor of biofilm formation on S. aureus. Compounds 4 and 5 were able to reduce the biofilm mass of S. Typhimurium. The combined effect of the tetracycline and compounds 4, 8 and 9 on the S. Typhimurium resulted in synergism when were used at 1.25 μM of tetracycline. On the S. aureus compounds 7 and 11 in combination with 1.6 μM of ciprofloxacin showed synergism.

Conclusion: These results suggested that selenocompounds could be effective anti-biofilm agents and adjuvants in the antibiotic treatment of infections caused by biofilm producing S. aureus and S. Typhimurium.
Introduction: Infections represent a major cause of morbidity and mortality, mainly due to the high antibiotic resistance (both genetic and phenotypic/tolerance of biofilm cells). Consequently, the interest of researchers was attracted toward complementary/alternative therapeutic strategies, able to replace or potentiate the antibiotics’ effect. The plant extracts/compounds represent a such alternative, empirically known, but now investigated to develop efficient and ecofriendly antimicrobial strategies, based on inhibitory biomolecules of pathogens’ growth, intercellular communication and virulence too.

Hypothesis and aims: To analyze the antimicrobial and antibiofilm effects of six widely used essential oils (EOs) (lavender, clove, eucalyptus, rosemary, thyme, oregano) on reference and clinical bacterial strains, recently isolated from Romanian hospitals.

Methodology: 60 clinical strains (Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa) were tested. Qualitative antimicrobial assay of the EOs was done by an adapted agar diffusion method, followed by a quantitative assay and determination of MIC. Bacterial adherence and biofilm formation in the presence of various concentrations of EOs was investigated by microdilution method, microscopy and dynamic (24, 48, 72h) crystal violet assay.

Results: The results demonstrated that all tested EOs have significant abilities to inhibit biofilm formation, depending on EO dose and bacterial strain. Rosemary, thyme and oregano EOs presented the lowest MIC values (0,01–0,1%). The most significant antimicrobial and antibiofilm effects were manifested against K. pneumoniae strains. Biofilm inhibition effect was observed on all tested strains, the efficient concentrations (0,001-1%) depending on EO type, thyme and oregano EOs showing the highest antibiofilm effect.

Conclusion: EOs represent valuable resources and could be further investigated for new pharmaceutic formulations, since they have great antimicrobial and antibiofilm effects, are not toxic and no resistance mechanism was reported yet. Recent studies performed by our and other research groups reveal they could be coupled with nanomaterials for a precise action and controlled release.
117: Elasnin, a bacteriostatic agent that has potent antibiofilm activities against Gram-positive bacteria - Long L

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Introduction: Biofilm is an existent form of microorganisms referred to the organized aggregates of microorganism community that is attached to a surface, which is responsible for the resistance and persistence of microorganisms in the environment. The infections it caused and the extra costs it resulted has become a real problem to human health and navigation industry. To tackle with it, efficient and sustainable approaches are urgently needed.

Hypothesis and aims: In history, natural products have always been the major source of drug discovery, and among them, the bioactive compounds isolated from Actinobacteria accounts for the highest portion. Therefore, through the screening of natural products from actinobacteria, we aim to discover strong antibiofilm compounds.

Methodology: Bioassay-guided isolation was employed to screen for candidate compounds.

Results: Elasnin was isolated from the secondary metabolites of Streptomyces mobariensis DSM40847 on the basis of bioassay-guided isolation, which showed potent antibiofilm activity specifically against Gram-positive bacteria with minimum biofilm inhibiting concentration (MBIC) lower than 1.25 μg/ml and minimum biofilm eradication concentration (MBEC) below 2.5 μg/ml. Minimum inhibiting concentration (MIC) assay and minimum bactericidal concentration (MBC) assay results indicated that Elasnin is a bacteriostatic agent which can inhibit the bacteria growth at a concentration lower than 1.6 μg/ml but does not kill the cells at 100 μg/ml. Elasnin’s antibiofilm activities were further assessed by confocal laser scanning microscopy (CLSM) observation and in situ anti-biofilm test which showed that elasnin can not only inhibit the multi-population biofilm’s formation in the sea water but also inhibit the attachment of large fouling organisms.

Conclusion: In summary, our results showed that Elasnin is a bacteriostatic agent with potent antibiofilm activities against Gram-positive bacteria and therefore demonstrated its potential applications in dealing with biofilm-related infections and biofouling problems.
Introduction: Antimicrobial peptides (AMPs) are naturally occurring macromolecules that demonstrate a potent antimicrobial activity against a broad range of microbes, including viruses, bacteria, and fungi. AMPs are part of every organism’s innate immune response and act as a first line of defence against infection. The mechanism of action of AMPs is dissimilar to that of current clinically used antimicrobial agent and therefore, they are not susceptible to developing resistance and hence there is interest in the development of AMPs for as therapeutics for multidrug-resistant infections. While the use of AMPs for infection control shows significant promise, efficacy can be hampered owing to proteolytic degradation and low bioavailability. Entrapment of AMPs into drug delivery vehicles can significantly increase the therapeutic index of AMPs by stabilising the peptide, increasing the residence time and potentially targeting it to the site of action.

Hypothesis and aims: This study investigates two potent and FDA approved AMPs (nisin and lactoferrin (LF)) encapsulated in gelatin microparticles in a facile one-pot synthesis for the treatment of infections. The influence of the choice of AMP, particle size, zeta potential value, drug loading and in vitro drug release was studied.

Methodology: Gelatin microparticles were prepared using an oil in water (O/W) emulsion technique. An aqueous solution of gelatin was added to corn oil to form the O/W emulsion, which was subsequently precipitated using acetone. A solution of nisin or LF was then left to incubate with the gelatin microparticles for 24 h to entrap the AMPs onto the microparticles. The microparticles were characterized with Fourier-transform infrared spectroscopy (FTIR), Zeta potential, particle size analysis and Scanning electron microscopy (SEM). The antimicrobial efficacy were tested against *S. aureus* using the spread plate technique and the colony forming units (CFU) were measured at 4 and 24 h time points.

Results: The influence of a number of experimental variables of microparticle synthesis and AMP used was investigated. The control particles sizes varied from 148.7 d.nm to 4893 d.nm, with a zeta potential of 3.34. After entrapment of the AMPs the particles sizes there was an increase in size and zeta potential. The antimicrobial efficacy of the AMP-loaded microparticles was tested against *S. aureus*. There was a 1-log reduction in the nisin and lactoferrin loaded microparticles compared with the control gelatin.

Conclusion: In this study we have developed a procedure to entrap antimicrobial peptides gelatin microcapsules using an oil-in-water emulsion method. Characterisation of the particles was performed using Zeta potential, particle size analysis FTIR and scanning electron microscopy. The AMP-loaded microparticles showed promise as a drug delivery vehicle to improve the delivery of the active agent for the treatment of infections.
119: Synthesis and evaluation of novel quaternary ammonium salts and development of bacterial biofilms by MBEC assay for future examination of these compounds - Markova A

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**Introduction:** Quaternary ammonium salts (QASs) are widely spread cationic surfactants applicable in various industrial branches. QASs 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 protozoa. Methods as the determination of MIC (minimal inhibitory concentration) are essential conventional methods for the susceptibility testing. However, regarding the important role of microbial biofilms in the development of persistent infections, the research is nowadays more focused on MBEC (minimal biofilm eradication concentration) evaluation. The anti-biofilm agents would be more valuable in clinical practice.

**Hypothesis and aims:** Our main research activity involves synthesis of novel compounds based on QASs and evaluating their eradication ability against pathogenic microorganisms. Thus, the aim was not only to evaluate the efficacy determination against the free-floating forms, but also introduction and optimization of the biofilm-based methodology.

**Methodology:** All novel prepared compounds were verified by Nuclear Magnetic Resonance (NMR) and High Resolution Mass Spectrometry (HR-MS). A set of eight bacterial strains was used for the MIC and minimal bactericidal concentration (MBC) evaluation by microdilution broth method. For the MBEC determination was selected the assay based on Calgary biofilm device.

**Results:** Series of novel QAS were prepared and verified by NMR and HR-MS. The MICs and MBCs were measured for all substances and several of them have shown antimicrobial potential. The MBEC assay was partly optimized for two bacterial strains and the eradication ability of some standard disinfections was determined as well.

**Conclusion:** Set of synthetized compounds underwent antimicrobial evaluation and several compounds have shown antimicrobial potential against the planktonic form of bacteria. The MBEC-based methodology was introduced and our contemporary interest is based on the determination of minimum biofilm eradication concentration (MBEC) of newly synthesized QAS.
Introduction: The ESKAPE pathogens are of great clinical significance, associated with high morbidity and mortality. These Gram-negative and Gram-positive pathogens demonstrate multiple drug resistance mechanisms and tolerate antimicrobial challenges via biofilm formation. The innate tolerance of bacterial biofilms poses a threat to public health and costs millions in colonised medical devices annually. The significant potential for antimicrobial applications of cold atmospheric plasma (CAP) has been recognised in recent years, including biofilm control and the potential for synergy with conventional antimicrobials.

Hypothesis and aims: The aim of this study is to investigate the influence of sublethal CAP exposure on the antimicrobial susceptibility of the ESKAPE pathogens in both planktonic and biofilm phenotypes.

Methodology:

Treatment of planktonic bacteria and biofilms with atmospheric non-thermal plasma, across a range of exposure times to determine sublethal exposure times resulting in a 1-log reduction in colonies.

Assessment of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum biofilm eradication concentration (MBEC) of conventional antibiotic/biocides against the ESKAPE pathogens.

Plasma-Antimicrobial Synergy: Determination of MIC, MBC and MBEC values of these antimicrobials following sublethal plasma exposure.

Results: Preliminary data indicate that pre-treatment of planktonic bacteria and biofilms with CAP reduced the MIC, MBC and MBEC values for tobramycin against P. aeruginosa. Ongoing studies aim to determine the mechanisms by which plasma increases antibiotic susceptibility among the ESKAPE pathogens, using a range of antimicrobial susceptibility assays, microscopy and thermochemical analysis.

Conclusion: The clinical application of cold atmospheric plasma may be limited in scenarios whereby long exposure times are needed to completely eradicate established biofilms. Synergy between antimicrobial treatments and plasma may offer a promising method of improving susceptibility to conventional antimicrobial agents, reducing both the exposure time of CAP and antibiotic concentrations necessary for complete eradication. In addition, this research will improve our understanding of the antimicrobial and antibiofilm mechanism(s) of action of CAP.
**Introduction:** WHO classified Quinolones and fluoroquinolones as hazardous to human health in 2017. In spite of their absence of use, the occurrence of Quinolone resistant *E.coli* (QREC) are high in broiler production according to the Norwegian monitoring program for antimicrobial resistance (NORMVET). Disinfectants are used as control strategy against microorganisms and aims at reducing all microorganisms on the surface by 99,9%. This proves difficult due to the formation of biofilms.

**Hypothesis and aims:** We studied biofilms from six QREC strains from the poultry industry, with three different morphotypes (BDAR, RDAR and PDAR) reflecting different matrix compositions. We assessed the log reduction effect in agreement with EN standard 13697 of three disinfectants along with differences between the morphotypes, age of biofilm and treatment time of the disinfectant.

**Methodology:** The biofilm was formed on stainless steel coupons for two and five days before being transferred to tubes with Virocid 0,25%, Virkon S 1% and TP990 1% and left for various application times. Further, the biofilms were scraped off the coupons and serial dilutions were spread on blood agar plates. The total no of colony forming units (CFU) were calculated.

**Results:** Our results show that the disinfectants studied had a reducing effect on biofilm, but it is inferior to that on planktonic bacteria. On 2-day old biofilm, satisfactory log reduction effect was seen with Virkon S and Virocid applied for 30 minutes and the greatest reduction on BDAR. On 5-day old biofilm, Virocid yielded the largest log reduction, however, none were sufficient according to EN13697 and no statistical difference was seen between the morphotypes.

**Conclusion:** A biocide needs to tackle biofilm matrix as well as the microbes and is reliant on their chemical composition and their mechanism of action. Hence, future susceptibility tests for disinfectants should include biofilms of different ages and with larger concentrations and longer contact times.
Introduction: A frequent and hard problem to treat in medicine is the formation of biofilms on biomedical surfaces such as implants or catheters. Opportunistic pathogens such as *Pseudomonas aeruginosa* can easily form said biofilms on medical devices. Efforts to prevent or treat these microbial infections are costly and often invasive. Therefore, there is a need for materials that can ideally prevent biofilm formation on medical devices but also to provide a mean for effective treatment.

Hypothesis and aims: Bacteria living in biofilms are able to tolerate higher antibiotic concentrations compared to planktonic bacteria and can survive long enough to evolve antimicrobial resistance (AMR). The aim of this research is to determine how bacteria adapt during biofilm formation to surfaces coated with antimicrobials, and what the effect of antibiotic treatment is.

Methodology: To better assess the best methods to counter biofilms and AMR, we first need to understand the influence of surface properties on bacterial adhesion. We study this by making use of atomic force microscopy. Through adhesion force measurements we can gain insight into the interplay between *P. aeruginosa* PAO1 on surfaces with different physico-chemical properties such as glass and polydimethylsiloxane (PDMS). By making use of microfluidics, we can also develop and study biofilms in a controlled environment where coatings and antibiotics can be tested.

Results: We have found that surface characteristics such as hydrophobicity play an important role on the adhesion of *P. aeruginosa* PAO1 to biomedical devices. By using especially developed microfluidic growth chambers, we have observed that this strain has a preference to form biofilms on PDMS rather than on glass and have managed a preliminary biofilm reduction by using antimicrobial coatings.

Conclusion: Surface properties of biomedical devices have an important effect on bacterial adhesion. Further research is needed to expand our knowledge about AMR development within mature biofilms.
**123: A new weapon against biofilm: a lipopeptide from Antarctica (ORAL) - Parrilli E**

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**Introduction:** The exploitation of the biodiversity remains the main road in the bioprospecting efforts and microorganisms able to thrive in harsh conditions, like in Antarctica, represent an untapped reservoir of biodiversity endowed with an interesting chemical repertoire. Antarctic bacteria living in adverse environmental conditions developed unusual survival strategies, such as an antagonistic activity that reduces the presence of competitive microorganisms. Such behaviour is particularly necessary when nutrients are limited or difficult to uptake, indeed, a preliminary characterization of molecules isolated from Antarctic bacteria revealed that these compounds display antimicrobial and anti-biofilm activity.

**Hypothesis and aims:** The aim of this work was to identify new anti-biofilm molecules against *S. aureus* and *S. epidermidis* produced by cold-adapted bacteria.

**Methodology:** Organic extracts obtained from cultures of different Polar marine bacteria were tested against different biofilm producers. Most promising samples were subjected to suitable purification strategies in order to identify the molecules responsible for the sought biological activity.

**Results:** A library of Organic extracts obtained from Polar bacteria was set up and screened looking for anti-biofilm agents acting against *S. aureus* and *S. epidermidis*. The most promising sample was fractionated and a cyclic lipopeptide was purified. This molecule resulted to be an effective anti-biofilm molecule able to strongly reduce both *S. aureus* and *S. epidermidis* biofilm formation.

**Conclusion:** Results obtained led to the identification of a novel lipopeptide from the Antarctic bacterium TAD1S able to effectively inhibit the biofilm formation of *S. aureus* and *S. epidermidis*, which may be potentially useful in biotechnological and medical applications.
124: Efficacy of Tetrasodium-EDTA alone and in a complex with metal ions against mono and mixed species biofilms - Percival S.L

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Introduction: Biofilms can form on abiotic and biotic surfaces, such as medical devices and wounds causing infection and delayed wound healing. Biofilms often show increased tolerance to antimicrobials and antibiotics in comparison to their planktonic counterparts and are therefore difficult to treat.

Hypothesis and aims: The aim of this study was to evaluate antibiofilm activity of several Tetrasodium-EDTA (TEDTA) complexes against mono and mixed species biofilms.

Methodology: The minimum biofilm eradication concentration (MBEC) was determined following an adapted version of ASTM E2799 17 against mono and mixed species biofilms of Acinetobacter baumannii ATCC 19606, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 29213 and Staphylococcus epidermidis ATCC 35984. The biofilm MIC and MBEC were determined visually as the lowest concentration of complex to inhibit biofilm growth/dissemination and that to eradicate the biofilm, respectively.

Results: Following MBEC testing of TEDTA alone and in complexes with various metal ions, increased antibiofilm potency was found with the complexes. The complexes demonstrated good antibiofilm activity against mono and mixed species biofilms.

Conclusion: The results demonstrate the potential for the TEDTA complexes to be used for treatment of biofilms, such as those that form in chronic wounds.
Introduction: Tetrasodium EDTA (t-EDTA) has been shown to be an essential tool in management of biofilm-related infections and should be considered as an anti-biofilm agent alone or in combination with other antimicrobials or technologies for increased antimicrobial performance in recalcitrant wounds.

Hypothesis and aims: T-EDTA can form different complexes with more than one metal ion by either covalent bond, chelation or ionic bond. In this study, we examined the cytotoxicity of a novel series of t-EDTA complexes in an in vitro model.

Methodology: Ten t-EDTA complexes (C1 to C10) were synthesized by the reactions of t-EDTA with one or two selected metal salts at pre-defined concentrations. The synthesized complexes were characterized by FTIR, MS and PXRD. The in vitro cytotoxicity of the complexes was measured by direct contact assay on fibroblasts (L929) and human dermal fibroblasts (HDFa). Briefly, L929 and HDFa were seeded into 24 well plates at a concentration of 5x10⁴/ml/well. After 24 h culture, t-EDTA complexes were added into pre-determined wells at a concentration equivalent to the MIC against P. aeruginosa ATCC 15442. After 24 h, cell images were taken, and the cells were washed with PBS and stored at -80°C. The cell viability of L929 and HDFa was measured using commercially available CyQUANT cell proliferation assay.

Results: The cell viability of HDFa was higher than 80% after C2, C3 and C4 treatment at MIC concentrations, which means these complexes are non-cytotoxic at an efficacious concentration. C5, C6, C7, C8, C9 and C10 complexes were cytotoxic (cell viability < 70%), however; cell viability after treatment with all the complexes was higher than cell viability following treatment with other commercial antimicrobial wound dressings.

Conclusion: Compared to some commercial wound dressings, t-EDTA complexes are less cytotoxic. The results indicate that t-EDTA complexes are novel, promising chemicals for next generation wound healing dressings, with anti-biofilm, anti-microbial and anti-inflammatory activities.
Introduction: Microbial biofilms are associated with major challenges in modern society. Biofilms are not only relevant in the healthcare industry, associated with the majority of bacterial infections, but furthermore are known to cause a variety of industrial problems leading to annual damages exceeding billions of USD. Unfortunately the mechanisms of biofilm formation vary between different biofilm forming strains, making the search for a globally active antibiofilm agent rather difficult. Therefore there is a high demand for new products for inhibiting and/or eliminating biofilm formation. Polyphenols are very large class of molecules with various functions, often hindered in their activity by their low water solubility, which can releaved upon glycosylation. However glycosylation does not only influence solubility but can also alter the bioactivity of polyphenols Fontaine et al. identified ellagic acid rhamnoside as an antibiofilm compound against Staphylococcus aureus, whereas the aglycone ellagic acid did not exhibit significant antibiofilm properties [1]. The large variety of polyphenols and their corresponding rhamnosides can lead the way to discover novel antibiofilm compounds.

Hypothesis and aims: This study aims to find new polyphenol rhamnosides with antibiofilm activities

Methodology: In order to find new polyphenol rhamnosides with antibiofilm activity we screened a platform of 18 glycosyltransferase for polyphenol rhamnosylation using HPLC. The created rhamnosides were purified using solid phase extraction on C18 columns. The purified rhamnosides were assayed for their antibiofilm activity using crystal violet assay in 24 well plates for Stenotrophomonas maltophilia, Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa.
Unlocking the full economic potential of converting plant materials via biorefineries into fuels is hampered due to the difficulty of breaking down lignocellulose, a major component. *Fibrobacter succinogenes*, isolated from the rumen of herbivores, is capable of robust lignocellulose degradation. However, the mechanism by which it achieves this is not fully elucidated. We substantially improve this understanding, through application of functionally validated proteomics. It is shown that when grown on cellulose, *F. succinogenes* strongly adheres to the microcrystalline cellulose surface and adopts a biofilm mode of growth. Furthermore, the cell envelope undergoes extensive rearrangements to accommodate multi-protein cellulolytic degradation machinery, as well as associated proteins involved in cellodextrin transport and metabolism. Molecular features of the lignocellulolytic enzymes indicate that the Type IX secretion system is involved in the translocation of these enzymes to the cell envelope. Finally, we demonstrate, for the first time, that cyclic-di-GMP may play a role in mediating catabolite repression, thereby facilitating the expression of proteins involved in the adhesion to lignocellulose and subsequent lignocellulose degradation and utilisation. The rich dataset we present not only advances our understanding of the cellulose degradation mechanism in *F. succinogenes*, but also contributes novel parts to the synthetic biology toolbox. This will facilitate future engineering of recombinant organisms capable of lignocellulose degradation and concomitant economically viable production of advanced lignocellulosic biofuels.
Introduction: Periodontitis is a prominent burden to overall public health. The disease is caused by pathogenic bacterial biofilms. Commensal species found in the oral cavity possess multifunction. One function is that they prevent pathogens from attachment, multiplications and invasion to epithelium. When the commensal bacterial communities are invaded by pathogenic organisms leading to dysbiosis within the system, disease is triggered.

Hypothesis and aims: The aim of this study was to investigate whether using a beneficial commensal biofilm can prevent or reduce the invasion of pathogenic microorganisms.

Methodology: Streptococcus sanguinis was used as a model strain to form the beneficial biofilm. The cell lysates of the known periodontal pathogens Fusobacterium nucleatum or Porphyromonas gingivalis were prepared and applied to block the beneficial biofilm. The blocked biofilm was then challenged with the pathogens to test the efficiency of the desired colonization resistance. Biofilm was quantified by crystal violet staining and characterized by FISH and CLSM.

Results: Both lysates of the F. nucleatum and P. gingivalis were found to block the beneficial S. sanguinis biofilm, suggesting that they are able to hinder the adhesion of pathogens onto the biofilm. In particular, the lysate of P. gingivalis resulted in around 50% reduction when either F. nucleatum or P. gingivalis was used for secondary adhesion. Additionally, the distribution of single species within the biofilms showed the blocking effect of the lysates on the secondary adhesion.

Conclusion: Our data demonstrate that beneficial biofilms can be an effective tool to prevent pathogen attack, creating a smart bioactive interface that acts as a bouncer for pathogenic species. However, it is important to note that oral microflora expresses multiple types of adhesins that are invoked when major receptors are blocked; therefore more complex beneficial biofilms and lysate mixtures must be evaluated.
**129: Bacteriophages for eradication of clinically relevant biofilms - Rice C**

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**Introduction:** *Proteus mirabilis* and *Stenotrophomonas maltophilia* are Gram-negative, opportunistically pathogenic, biofilm-forming bacteria. Multi-drug resistant strains of these pathogens are being reported at alarmingly increasing rates, highlighting the need for novel biocontrol agents. Bacteriophages are a unique type of virus that recognize a specific type of bacteria and then infect, replicate and kill the host via cell lysis. The application of phages and their enzymes for treating bacterial biofilms has recently gained significant interest due to a number of significant advantages compared to traditional antibiotics, including high specificity and efficacy, low immunogenicity and production costs.

**Hypothesis and aims:** We are isolating and characterizing novel bacteriophages to obtain key biofilm-degrading enzymes such as depolymerases and lysins. These will be assessed for their abilities to act as biocontrol agents against the respective bacterial biofilms via application on their own and in combination with other antimicrobials.

**Methodology:** Isolation of bacteriophages from environmental and clinical samples; Purification and characterization of phages via Nanopore sequencing; Genome analysis for novel antimicrobial enzymes; Obtainment of phage enzymes; Biofilm degradation assays.

**Results:** To date we have isolated 37 novel bacteriophages active against *P. mirabilis* and *S. maltophilia* and are in the process of characterizing each phage and extracting DNA for Nanopore sequencing. Some phages have already displayed potential depolymerase activity indicated by an additional zone of media degradation around the plaques of a plaque assay. Successful characterization of each phage will most likely result in the identification of novel biofilm degrading enzymes.

**Conclusion:** Bacteriophages and their enzymes are a promising option for treating bacterial biofilms. Preliminary data suggest that some of the phages in our currently expanding phage library could be a source of novel and highly active lysins and depolymerases, which will prove to be vital in the bid to treat biofilms for *P. mirabilis* and *S. maltophilia* in an age of increasing multi-drug resistance.
130: Improving the plasma activated liquids efficacy for the inactivation of *L. monocytogenes* and *S. Typhimurium* biofilms - SMET C

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**Introduction:** Most conventional disinfection processes to ensure the microbial safety of contact surfaces are based upon their potential to inactivate planktonic cells. However, as cells typically develop as resistant biofilms on abiotic surfaces, these traditional strategies do not suffice. The use of Plasma Activated Liquids (PAL), generated using the Cold Atmospheric Plasma (CAP) technology, is known to be a flexible and transportable innovative solution with antimicrobial potential. However, in order to be considered for use on a larger scale, the efficacy of the PAL often needs to improve.

**Hypothesis and aims:** PAL could be a worthy alternative compared to traditional strategies for the decontamination of biofilms. This work aims to improve the PAL antimicrobial efficacy by assessing the impact of different (activated) media, storage times, and temperatures.

**Methodology:** *Listeria monocytogenes* and *Salmonella Typhimurium* model biofilms were developed on an abiotic surface. PAL solutions were generated for 20 min using an air-based Surface Barrier Discharge electrode. For the creation of PAL, demineralized water, saline, phosphate buffered and phosphate buffered saline solutions were considered. Furthermore, solutions were stored up to 1 month at -80, -20, 4, and 20°C. Mature biofilms were treated with PAL up to 30 min, after which the remaining cell densities were determined by plate counts. Predictive models were finally used to describe the inactivation kinetics.

**Results:** The results indicate that both the type of media selected for PAL generation, together with the storage conditions significantly impact its inactivation potential.

**Conclusion:** PAL has proven its antimicrobial potential for decontaminating biofilms. If optimized, the innovative PAL treatment could be applied as an alternative technology to avoid or inactivate pathogenic biofilms growing on abiotic surfaces.
131: Novel polycationic photosensitizers for antibacterial photodynamic therapy -
Tiganova I.G

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Introduction: Infected long-term non-healing complicated wounds of the skin and mucosa, trophic ulcers, pressure sores, ulcers of diabetic feet represent serious problems for their treatment, especially in the case of multi-resistant pathogens. Evidences has been obtained of the association of chronicity of wounds with biofilms, as well as with the presence of polymicrobial communities in the wound. Antibacterial photodynamic therapy (APDT) is a promising method of treating local infected foci. Photodynamic inactivation (PDI) is able to effectively destroy bacterial cells without developing resistance in response to treatment. Gram-negative bacteria P. aeruginosa are often found in infected wounds, presumably in biofilm state, and characterized by rather low susceptibility to APDT, which is a problem.

Hypothesis and aims: The research is dedicated to the study of antibacterial properties of new photosensitizers (PS) based on polycationic phthalocyanines and synthetic bacteriochlorins for photodynamic inactivation of P. aeruginosa bacteria and their biofilms.

Methodology: The microbiological studies were carried out on P. aeruginosa 32 clinical isolate. The efficiency of PDI was estimated as decrease in viable bacteria after sensibilization, irradiation by near infra-red light, destruction of biofilms by multienzyme complex BFR and plating. Also the qualitative estimation of results of PDI was performed using fluorescent microscopy after Live/Dead staining.

Results: All photosensitisers tested have high efficiency against planktonic bacteria P. aeruginosa 32, which were completely inactivated after incubation with µMs of PS and irradiation. Inactivation of bacteria in biofilms depended on PS concentration and reached about 5 logs with ZnPcChol8 and PyrBC(EBr)4Br4. Fluorescent microscopy of biofilms after PDI and Live/Dead staining visualize the killing of bacteria, or increasing of permeability of membranes which is the first step of cell damage under action of reactive oxygen species.

Conclusion: Novel photosensitizers based on the derivatives of polycationic phthalocyanines and synthetic bacteriochlorins have a high efficiency in photodynamic inactivation of Gram-negative bacteria P. aeruginosa as well as their biofilms in vitro.
Introduction: Bacteriophage are viruses that infect bacteria, also known as phage. They are ubiquitous in nature found everywhere from sea water to the human gut. Phage are able to cause either lysogenic (phage integrates into host genome) or lytic (results in host cell death upon release of progeny phage) infections of bacteria. The tail fibres of phage have also been shown to have depolymerase effects against bacterial capsule and biofilm matrix. *Klebsiella* sp. are a major cause of nosocomial infections and an ESKAPE pathogen, a group of high-risk and increasing antibiotic resistance. They are also proficient biofilm formers, a phenotype which is well known to further increase tolerance to antimicrobial drugs. Lytic phage provide an alternative method of selectively targeting these species without any risk of developing antibiotic resistance mechanisms. Furthermore, non-lytic phage may sensitise bacteria to antibiotics, allowing them to be used synergistically.

Hypothesis and aims: Our aim is to investigate the synergistic and additive potential for bacteriophage in adjunct with antibiotic treatments to disrupt *Klebsiella* bacterial communities.

Methodology: Eleven *Klebsiella* isolates were screened for biofilm formation using a resazurin based viability assay and crystal violet biomass staining. Sensitivity to phage, with and without antimicrobial co-treatment, was assessed at different points of biofilm formation using the aforementioned techniques. To increase complexity, a number of phage and antibiotics were tested in more realistic biofilm models (Galleria and flow cell).

Results: Results have shown that phage and antibiotics work in conjunction to disrupt bacterial growth, even in strains of *Klebsiella* where lytic infection does not occur, highlighting its potential use in therapy.

Conclusion: We have shown how phage therapy is able to improve the activity of antimicrobials and have an important role in the approaching post-antimicrobial era.
133: The effect of fosmidomycin prodrugs against *Acinetobacter baumannii* biofilms - van Charante F

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**Introduction:** Isoprenoids can be produced through the mevalonate and/or the non-mevalonate pathway and multiple bacterial pathogens exclusively use the non-mevalonate pathway for isoprenoid synthesis. One of these pathogens is *Acinetobacter baumannii*, which is a responsible for various types of (nosocomial) infections. With increasing antibiotic resistance, also *A. baumannii* infections are becoming more difficult to treat, and novel drugs are needed. Fosmidomycin was originally developed as an antimalarial drug that targets the non-mevalonate pathway for isoprenoid synthesis and as *A. baumannii* uses this pathway fosmidomycin and its (prodrug) derivatives are interesting candidates for novel antimicrobial therapy.

**Hypothesis and aims:** The aim of this study is to verify the activity of the two fosmidomycin prodrugs (CC271 and CC366, identified in a previous screening) against biofilms of *A. baumannii*.

**Methodology:** The two prodrugs were evaluated for their ability to inhibit biofilm formation as well as for their biofilm-eradicating ability, using a 96 well microtiter plate model and nine *A. baumannii*. The activity of these compounds was subsequently also investigated in a more complex artificial dermis model that simulates the wound environment in vitro.

**Results:** The CC366 prodrug is able to fully inhibit biofilm formation 6 of the tested strains in the 96 well microtiter plate model at 16 µg/mL. This includes 4 strains that are resistant to ceftazidime at 16 µg/mL. CC271 shows a similar potency, but against 3 strains. Biofilm eradication by CC366 was similar to that achieved by aztreonam and ceftazidime at 16 or 32 µg/mL, up to about 60% eradication. In the artificial dermis model inhibition with CC366 resulted in a 3 log reduction in CFU at 32 µg/mL, whereas CC271 didn't show a significant effect.

**Conclusion:** The CC366 prodrug is an effective compound for inhibiting biofilm formation from *A. baumannii*. We are currently investigating the compound in further models and the possibilities of integrating it into wound dressings.
**134: Phage-encoded miniDNases as a novel source of enzyme-based antibiofilm strategies**  
*Marie Van der Gucht*

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**Introduction:** Despite the enormous diversity that exists in biofilm composition, extracellular DNA plays a pivotal role in structure and increased resistance of many microbial biofilms. Targeting this common denominator with DNA-degrading enzymes (DNases) is therefore an attractive and broadly applicable strategy to prevent and disrupt biofilms. While initially most research in this field focused on the use of mammalian DNases, their high production costs caused a shift towards non-mammalian sources of DNases. In this regard, we introduce a so far unknown family of homologous 9 kDa DNases (termed the miniDNases) produced by the natural enemy of bacteria, the bacteriophages.

**Hypothesis and aims:** The purpose of this study was to unravel the enzymatic properties of the miniDNases and to evaluate their potential to prevent and disrupt biofilms, using the opportunistic pathogen *Pseudomonas aeruginosa* as model organism.

**Methodology:**

- Recombinant protein production and purification
- Gel electrophoresis-based qualitative DNase assays
- Hyperchromicity-based quantitative DNase assays
- Static Calgary biofilm device assays

**Results:** The phage-encoded miniDNases were discovered to be non-specific DNases that are stable in a broad pH and temperature range. Showing no sequence homology to any previously characterized DNase, we believe to have introduced a completely novel DNase family. Based on the interaction of a miniDNase with *Pseudomonas aeruginosa* biofilms, it was shown to inhibit the formation of biofilms and disperse 24-h-old biofilms in a significant way.

**Conclusion:** The miniDNases are novel DNases that show potential to be used in the prevention and treatment of biofilms. In the future, dynamic biofilm models will be used to validate these findings, which can direct the research towards specific applications.
135: Nanocarriers with conjugated antimicrobials to eradicate pathogenic biofilms evaluated in vitro and in vivo - van der Mei H.C


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Introduction: Infection is predicted to yield more deaths than cancer and to become the number one cause of death by the year 2050, mainly because of the growing number of antibiotic resistant strains and the lack of new antibiotics being brought to the market. Conventional antimicrobials are becoming increasingly ineffective for treating bacterial infections due to the emergence of multi-drug resistant (MDR) pathogens. In addition, the biofilm-mode-of-growth of infecting bacteria impedes antimicrobial penetration in biofilms.

Hypothesis and aims: It was hypothesized that pH-adaptive micelles can efficiently eradicate infectious biofilms. It was the aim to investigate the penetration into biofilms and killing efficacy of poly(ethylene)glycol-poly(β-amino esters) (PEG-PAE) micelles with conjugated antimicrobials.

Methodology: The penetration and eradication of pH-adaptive PEG-PAE micelles with conjugated antimicrobials was investigated on biofilms of different bacterial strains in vitro and an in vivo subcutaneous murine infection model. In addition, the killing efficacy of ex vivo orthodontic biofilms was investigated with these micelles.

Results: In vitro, PEG-PAE micelles with conjugated Triclosan (PEG-PAE-Triclosan) yielded no inadvertent leakage of their antimicrobial cargo and showed a better killing of MDR S. aureus, E. coli and oral streptococcal biofilms than Triclosan in solution. In mice, PEG-PAE-Triclosan micelles with conjugated Triclosan yielded better eradication efficacy towards a MDR S. aureus infection compared with Triclosan in solution. Ex vivo exposure of multi-species oral biofilms collected from orthodontic patients to PEG-PAE-Triclosan micelles, demonstrated effective bacterial killing at 30-40 fold lower Triclosan concentrations than achieved by Triclosan in solution. Importantly, Streptococcus mutans, the main causative organism of dental caries, was preferentially killed by PEG-PAE-Triclosan micelles.

Conclusion: PEG-PAE-Triclosan micelles present a promising addendum to the decreasing armamentarium available to combat infection in diverse sites of the body.
Metal formulations have the potential for use as antimicrobials in controlling healthcare associated infections - Whitehead K.A

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Introduction: Healthcare associated infection has serious consequences since it severely compromises the health, recovery and psychological well-being of the patient. Antimicrobial resistance within a wide range of bacteria is a growing public health threat, thus the development of novel antibacterial agents is urgently needed. The development of novel antimicrobials is complex since there are a number of factors that influence the antimicrobial activity and biotoxicity of such compounds.

Hypothesis and aims: The hypothesis of this work was that metals in different forms and used in different conditions demonstrate a range of antimicrobial efficacies. The aim of this work was to determine the antimicrobial effects of metals, in different forms and combinations and in the presence of conditioning films against planktonic bacteria and biofilms. The biotoxicity pathways involved were also investigated.

Methodology: A range of methodologies have been used throughout this work, including minimal inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), crystal violet biofilm assays, fractional inhibitory concentrations, addition of conditioning films and biotoxicity assays.

Results: The results have demonstrated that although some metals are active in a coating, this antimicrobial efficacy may be significantly reduced or enhanced when used in different forms such as metal ions, or in the presence of a conditioning film. Synergistic effect of metals in different forms may also provide new antimicrobial remedies for use against bacteria in both planktonic and biofilm states.

Conclusion: To ensure that judicious use of metals as antimicrobials are used, consideration of the surrounding factors needs to be considered on a case by case basis. This work considers the effect of the above factors, and what it may mean in terms of antimicrobial design.
**137: Charge-reversible carbon dots for biofilm treatment - Wu Y**

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**Introduction:** Bacterial biofilms are difficult to eradicate because of reduced antibiotic sensitivity, as traditional molecular targets can be latent in the biofilm state, and the presence of the extracellular polymeric substance interferes with antibiotic localization. Significant efforts have been made to inhibit biofilm formation. However, studies about eradicating pre-established biofilms have been rarely reported; thus, the development of new approaches to biofilm eradication is urgently needed. Carbon dots, a class of emerging carbonaceous nanomaterials, have received considerable attention because of their excellent fluorescent properties, low cytotoxicity, good water dispersibility and ease of synthesis and modification. Recently, Studies demonstrated that positively charged carbon dots exhibit antibacterial properties on planktonic bacteria, and negatively charged carbon dots can inhibit biofilm formation without bactericidal activity.

The physiological pH of blood and normal tissues is about 7.4, but the microenvironment inside a biofilm is often acidic, pH values could reach 5.0 or even lower. So the difference in pH between biofilms and normal tissues can be used for specific biofilm targeting.

**Hypothesis and aims:** The aim of this study was to design pH-responsive and charge-reversible carbon dots which could disperse biofilms. In addition, the efficacy of antibiotics in dispersed biofilms by carbon dots was evaluated.

**Methodology:** Carbon dots were synthesized by one pot reaction in a Teflon-lined autoclave and modified with 2,3-dimethylmaleic anhydride by chemical reaction between the carboxyl and amino groups. The biofilms were grown for 24 h, and then treated with carbon dots in buffer pH 5.0 and pH 7.4, respectively. After 4 h, the biofilms were stained with live/dead stain and analyzed with confocal laser scanning microscopy (CLSM). The 24 h biofilms were also treated with antibiotics, or antibiotics together with carbon dots. After exposure for 72 h, the bacteria both in the suspension above the biofilm and in the biofilms were collected. Then, the number of colony forming units (CFU) were determined by serial dilution and plating on agar plates.

**Results:** CLSM images showed significant biofilm dispersion when treated by carbon dots. In combined treatment of carbon dots and antibiotics led to a further decrease in the CFU values of biofilms compared to biofilm that were treated with antibiotics alone.

**Conclusions:** The pH-responsive carbon dots could disperse established biofilm effectively. Moreover, disrupting the integrity of the biofilm by carbon dots increased the killing the biofilms by antibiotics. The synergistic effect of carbon dots and antibiotics offers a new strategy to kill biofilms.
**Thursday 5th September**

**Staphylococcus**

138: The human skin bacteria *Staphylococcus epidermidis* fermentation end product ameliorates UVB-induced ROS generation through production of free electron transfer - *Balasubramanian A*

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**Introduction:** UVB-induced skin damage results in various inflammatory disorders through the induced generation of reactive oxygen species (ROS) that quickly inundate tissue antioxidants and in severe cases it can lead to skin cancer.

**Hypothesis and aims:** To investigate efficacies of human skin commensal bacteria *S. epidermidis* (ATCC12228) with its specific skin fermentation inducer (SFI), named as Ncueh1 that on fermentation produces antioxidant electrons against UVB induced skin damage.

**Methodology:** In vivo affirmation on ICR mice has confirmed the photoprotective role and maintained sufficient Anti-4 hydroxynonenal (4-HNE), a major biomarker of oxidative stress and lipid peroxidation, has been recognized as an important molecule in UVB-induced skin damage. Results in the Western blot analysis using antibodies to 4-HNE demonstrate that 4-HNE is induced in mouse skin overexposure to UVB. On the topical application of *S. epidermidis* (ATCC12228) plus its specific prebiotic Ncueh1 onto mouse skin before and after UVB exposure significantly reduced the UVB-induced 4-HNE.

**Results:** Application of *S. epidermidis* (ATCC12228) alone or prebiotic Ncueh1 alone does not influence the level of 4-HNE in UVB exposed skin. Electrochemical behavior of *S. epidermidis* (ATCC12228) with and without Ncueh1 has been determined to produce electron transfer this result suggests that electrogenic and antioxidant property of *S. epidermidis* (ATCC12228).

**Conclusion:** We have also tested the similar efficacy from the human skin isolated *S. epidermidis* to prove the similar function of bacteria in compared to ATCC12228 Strain that can mediate prebiotic Ncueh1 to produce free electron which may effectively neutralize and scavenges the formation of free radical by UVB irradiation.
Introduction: We are facing a scenario where the current arsenal of antibiotics is no longer effective. Therefore, novel treatment options are required in a post-antibiotic era. Plant secondary metabolites (phytochemicals) are a recognized source of molecules with diversified bioactivity, including the action against specific biofilm targets. Moreover, phytochemicals are a natural and sustainable source of chemical diversity, with distinctive modes of action and low toxicity.

Hypothesis and aims: Furvina (FUR) (2-bromo-5-(2-bromo-2-nitrovinyl)furan) is a broad-spectrum antibiotic first developed in Cuba from sugar cane bagasse. This is already used in the formulation of ointments marketed in Cuba. FUR shown ability to inhibit quorum sensing (QS) in Pseudomonas aeruginosa, as well as interference on biofilm formation and downregulation of QS-controlled virulence factors. In this study, FUR and four tailored synthetic derivatives (FUR_DERIV_I, FUR_DERIV_II, FUR_DERIV_III, FUR_DERIV_IV) were selected to evaluate their ability to interfere with QS of Staphylococcus aureus, prevent biofilm setup and enhance biofilm susceptibility to fusidic acid.

Methodology: The effect of FUR and its derivatives in S. aureus QS was evaluated using bioreporter strains (ALC1742, ALC1743, ALC1745). The activity of the most prominent derivatives on the prevention of biofilm formation and antibiotic susceptibility was characterized in terms of biofilm mass, viability and membrane integrity.

Results: FUR_DERIV_II promoted the most significant QS inhibition with a reduction of approximately 50% in the early stages, while the other derivatives only showed some effect after 24 hours and with lower percentages. With this derivative it was also found a reduction in biofilm formation and antibiotic potentiation. It is important to highlight that almost of biofilm cells were not compromised and so the effect observed may be due to QS interference.

Conclusion: FUR can be explored as scaffolds for the design of new antibacterial drugs against S. aureus.
**140: Cutibacterium acnes** clinical isolates are able to modify the attachment, formation and structure of *Staphylococcus aureus* biofilms - Brown H.L

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**Introduction:** *Cutibacterium acnes* (formally *Propionibacterium acnes*) is co-isolated with other opportunistic pathogens for deep tissue infections. They are a particular problem for upper torso surgical procedures, such as shoulder arthroplasty. Studies have demonstrated that *C. acnes* is able to form biofilms and when co-cultured with *Staphylococcus* sp. both inhibitory and stimulatory effects have been reported across a number of studies when the bacterium are co-cultured.

**Hypothesis and aims:** We hypothesized that the presence of *C. acnes* and *S. aureus* together within biofilms may lead to modifications in behavior by one or both bacterium, our aim was to investigate these modifications.

**Methodology:** Biofilm formation of 100 clinical *C. acnes* isolates from various infection sites was measured. Crystal violet staining of biofilms was used to measure differences in biofilm biomass and microscopy was used to provide a more detailed view of how the presence of *C. acnes* supernatants altered the biofilm structure.

**Results:** All isolates were able to form biofilms and no correlation was observed between isolation site and biofilm biomass. Supernatant from five of the *C. acnes* isolates produced a statistically significant reduction in the biofilm biomass of *S. aureus* NCTC 6571 biofilms. The reduction in biofilm formation was not linked to the ability of the *C. acnes* supernatants to suppress *S. aureus* growth and biofilm maturation, not attachment was impacted. Currently we are screening the other *C. acnes* isolates to determine how wide-spread the effect is alongside carrying out whole genome sequencing of the *C. acnes* of the isolates in an attempt to identify responsible genetic factors.

**Conclusion:** This study suggests that complex interactions between *C. acnes* and other opportunistic pathogens are likely to exist during colonisation and infection events. Further investigation of these interactions may lead to increased treatment options and a better prognosis for patients.
PepR, a viral-derived peptide, is efficacious against Staphylococcus aureus biofilms - Sandra N. Pinto

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Bacterial infections are a major human health threat given both the increasing incidence of drug-resistant bacteria and the ability of bacteria to form biofilms. Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to conventional antibiotics. Given the increasing interest in the use of antimicrobial peptides (AMPs) as alternatives against bacterial biofilms, our work is focused on the key factors that govern the antibiofilm action of a model AMP at the molecular level. pepR, a peptide derived from the Dengue virus capsid protein, was selected as an AMP model because it abrogates biofilm formation and kills bacteria in preformed S. aureus biofilms. Using a combination of flow cytometry and confocal fluorescence microscopy assays, with quantitative imaging data treatment, we showed that the ability of pepR to prevent biofilm formation and act on preformed biofilms is directly related to bacterial membrane permeabilization. The effect of the peptide on biofilm-associated bacteria is dose and depth-dependent, and is controlled by its diffusion along the biofilm layers. Overall, our study contributes to shed light on the antibiofilm mechanism of action of AMPs, particularly regarding the importance of their diffusion through the biofilm matrix on their activity.
**142: Facing the in vitro challenge in Pseudomonas aeruginosa and Staphylococcus aureus coexistence - del Mar Cendra M**

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**Introduction:** Chronic bacterial infections rarely exist as single-specie events. Polymicrobial infections challenge the antimicrobial chemotherapy to administrate and often aggravates the disease’s outcome. The coexistence between species that has been seen occurring *in vivo* remains hard to achieve *in vitro*, since differences between bacterial fitness’s eventually lead with a single organism dominating the mixed culture. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are major pathogens found growing together in biofilms in disease-affected lungs or wounds.

**Hypothesis and aims:** We aimed to find optimal conditions to grow *P. aeruginosa* and *S. aureus* together in mixed biofilms, including stable populations of both microbes. Furthermore, we wanted to analyse the antimicrobial susceptibilities depending if the organisms were growing in mono- or in co-cultured biofilms.

**Methodology:** Bacterial co-culture biofilm growth has been studied in static conditions (over plastic and glass surfaces) and during continuous-flow. Analysis and quantification of *P. aeruginosa* and *S. aureus* have been done by confocal microscopy imaging and by colony forming units counting onto selective agar. Assessment of the oxygen consumption during the co-culture biofilm growth was performed with a genuine setup system involving a micro-optode oxygen sensor. Differential antibiotic susceptibilities were determined by treating mono- and co-cultured biofilms with gentamicin and ciprofloxacin and analysing the respective viability after viable counting onto selective agar plates.

**Results:** After testing different culture media, additives and environmental parameters we have designed a combination of conditions that allow *P. aeruginosa* and *S. aureus* to grow in mixed biofilms during, at least, three days. Additionally, we have detected a very marked oxygen stratification in the co-culture system that seriously compromises *Staphylococcus* viability and modulates the bacterial growth and distribution in the mixed biofilm. Furthermore, we demonstrate increased antibiotic resistance when both organisms are growing together in co-culture.

**Conclusion:** Proper culture of bacteria that provoke polymicrobial infections is crucial to optimise their treatment.
143: Modelling *Staphylococcus aureus* biofilm on infected chronic wounds - Yanyan Cheng

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**Introduction:**
Chronic wounds, for instance venous, pressure, arterial and diabetic ulcers, are a major health problem throughout the world. Compared with normal wounds, those that take more than four weeks to heal are defined as chronic. Interestingly, the numbers of patients suffering from chronic wounds and the cost for treatment have been increasing during the past two decades. There is increasing evidence that suggests that bacteria infect those chronic wounds and there exist as a biofilm, which affects the wound healing and success of wound treatment. To study biofilms in infected wounds, both *in vitro* and *in vivo* biofilm models have been developed.

**Hypothesis and aims:**
The aim of this project is to develop a dynamic *ex vivo* chronic wound biofilm model for *Staphylococcus aureus* using pig skin. In addition, this dynamic model will be used to determine drug delivery from wound dressing made with electrospinning technology.

**Methodology:**
In this project, both the colony biofilm assay and new designed dynamic flow system were used to determine antibiotics effect on removing mature *S. aureus* biofilm, using both commercial antibiotic discs and electrospun nanofiber.

**Results:**
The results of this study so far indicated that mature *S. aureus* biofilms were resistant to vancomycin treatment, which works effectively on killing planktonic cells. However, other antibiotics used topically for healing infected chronic wounds, for example, gentamicin, tetracycline, and fusidic acid, were more effective at killing mature biofilms in the colony biofilm model and dynamic model.

**Conclusion:**
From the results gathered so far, it indicated that this easy applied dynamic model can then be used to study the drug delivery and topical treatment of chronic wounds.
Introduction: *S. epidermidis* is one of the main causes of nosocomial infections associated with the use of medical devices, due to its ubiquitous presence in human skin and mucosae and capacity to form biofilms. Biofilms are a major concern in healthcare systems, since they present higher antimicrobial tolerance and ability to evade host immune defenses, leading to recurrent and relapsing infections. Moreover, the presence of dormant bacteria in biofilms increases their pathogenicity, by decreasing the effectiveness of both host immune response and antimicrobial therapy.

Hypothesis and aims: It was earlier found that a gene encoding a protein of the mazEF complex was upregulated in *S. epidermidis* biofilms with induced dormancy. Herein, we proposed to study the role of mazEF system in *S. epidermidis* biofilms dormancy.

Methodology: *S. epidermidis* strain 1457 was used to construct a mazEF mutant and its respective complemented strain. Then, we studied the influence of mazEF in biofilms dormancy, by assessing the number of viable and culturable cells and the optical density (OD) of the biofilms, under induced (excess glucose) or prevented (addition of MgCl2) dormancy conditions.

Results: All *S. epidermidis* populations tested (wild type, mazEF mutant and complemented strains) showed a higher ratio of culturable/live cells when dormancy was prevented, comparing to the dormant state. The mutant strain showed the higher ratio of culturable/live cells in the dormancy state.

Conclusion: Dormancy affects the number of culturable cells in biofilms, despite maintaining the number of total cells of the biofilm, as well as its OD. MazEF seems to impact biofilm dormancy, since the most significant difference in the number of culturable cells between the two conditions was found in the mutant strain.
**145: Virulence factor expression dominates the *S. aureus* transcriptomic signature of human infection (ORAL) - *Ibberson C.B***

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**Introduction:** Individuals with the heritable condition cystic fibrosis (CF) experience chronic bacterial lung infections that begin in early childhood and persist through their lifetime. Bacteria within CF lung infections are thought to grow as small dense biofilm-like aggregates. *Staphylococcus aureus* is a prominent CF pathogen, isolated from the lungs of ~70% of individuals with CF. Despite the prominence of *S. aureus* as a CF pathogen, *S. aureus* physiology during CF lung infection is poorly understood, partly due to the challenge of investigating organisms within their native environment. Addressing this knowledge gap is necessary to evaluate and improve models to study *S. aureus* lung infections.

**Methodology:** Here we perform RNA-Seq directly from expectorated human sputum to assess *S. aureus* physiology *in situ* within human cystic fibrosis lung infections.

**Results:** Through principal component and hierarchical clustering analyses, we found a remarkable conservation of *S. aureus* gene expression in the CF lung despite differences in the patient clinic, status, age, and therapeutic regimen. Examination of the genes that are most differentially expressed in the CF lung compared to the *in vitro* models indicate that many *S. aureus* virulence factors as well as genes involved in metal acquisition are significantly higher in expression in the CF lung than in the models. In addition, we used machine learning to identify a *S. aureus* transcriptomic signature in the CF lung and identified a set of 27 genes that can distinguish between *S. aureus* transcriptomes in the CF lung and *in vitro* samples with 100% accuracy in both leave-one-out and cross-validation assessments.

**Conclusion:** Ongoing studies are developing an accuracy metric, which will allow us to provide a quantitative assessment of each model system and define ways in which model systems, including biofilm models, do and do not recapitulate *S. aureus* functions in the human CF lung. Collectively, these results will advance our knowledge of *S. aureus* physiology during human CF lung infection.
146: Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in biofilm-related infections: insights through network reconstruction and creation of a new online database - Jorge P

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Introduction: Despite important advances in biofilm research, these consortia remain a critical concern for many biomedical applications. Their naturally occurring polymicrobial nature is characterised by the development of complex communities, where pathogen interactions can promote disease progression and severity. Intra- and inter-species communication within these consortia is majorly regulated by quorum-sensing, affecting the expression of virulence factors and biofilm formation, making it a promising target for new anti-infective strategies. *P. aeruginosa* and *S. aureus* are two major pathogens that co-occur in many biofilm-related infections and whose competitive interaction is highly related to infection resilience.

Hypothesis and aims: Information on *P. aeruginosa*-*S. aureus* interactions is currently scattered in the ever-growing scientific literature, making it difficult for researchers to grasp critical information. Therefore, this study aimed at systematically collecting and analysing experimental information presented in the biomedical literature on the molecular basis of *P. aeruginosa*-*S. aureus* interactions, identifying promising therapeutic targets, and making this data available to the research community.

Methodology: Full-text papers were optimally retrieved from PubMed and classified by their relevance. Interaction data was methodically annotated, reconstructed as networks to identify promising therapeutic targets, and integrated with specialized databases to identify promising antimicrobials. A new online database was created to deposit the gathered interaction data in searchable format.

Results: Network analysis revealed key entities regulating *P. aeruginosa*-*S. aureus* interactions, for instance the PqsABCDE/PqsR quorum-sensing system, which affects *S. aureus* growth and biofilm formation. By identifying the most reported *P. aeruginosa* virulence factors affecting *S. aureus*, e.g. HQNO and siderophores, a list of experimentally validated agents affecting those factors, ranging from synthetic drugs to natural plant extracts, was constructed.

Conclusion: The complex experimental data on *P. aeruginosa*-*S. aureus* interactions was for the first time thoroughly retrieved, systematized, and made publically available in the new Inter-Species CrossTalk Database (www.ceb.uminho.pt/ISCTD).
147: Dabigatran has anti-biofilm properties and enhance antibiotic efficacy of experimental *Staphylococcus aureus* endocarditis (ORAL) - Lerche C.J

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**Introduction:** *Staphylococcus aureus* infectious endocarditis (IE) is the most frequent and fatal cause of left-sided IE (30–40%). *S. aureus* colonization of cardiac valves promote biofilm formation by fibrin formation and platelet aggregation. Dabigatran is a direct thrombin inhibitor (Factor IIa), blocking the conversion of soluble fibrinogen to insoluble fibrin. Furthermore, is dabigatran known to have a direct anti-virulent effect towards *S. aureus*.

**Hypothesis and aims:** We hypothesized that by limiting fibrin formation and platelet aggregation involved in *S. aureus* biofilm formation in IE, we could enhance the antibiotic efficacy of *S. aureus* IE.

**Methodology:** In male Wistar rats high grade *S. aureus* aortic valve IE was established. Infected rats treated with gentamicin (20 mg/kg/day s.c.) were randomized into two groups, 1) receiving dabigatran etexilate (10 mg/kg i.p. BID) or 2) saline (control). Infected rats were treated for two days and evaluated day 3 post-infection by blood cultures, quantitative bacteriology of valve vegetations, myocardium, spleen, kidneys and valve vegetation size.

**Results:** Adjunctive dabigatran treatment significantly reduced valve vegetation size compared to controls (p<0.0001). A significant reduction of the bacterial load in aortic valves was seen in dabigatran group compared to controls (p=0.02), as well as expression of key pro-inflammatory markers keratinocyte-derived chemokine (IL-8), IL-6, ICAM-1, TIMP-1, L-selectin (p<0.04). Furthermore, was a reduced number of positive blood cultures seen in adjunctive dabigatran treated compared to controls (6 vs 11, p<0.02).

**Conclusion:** Dabigatran enhanced antibiotic efficacy by means of reduced valve vegetation size, bacterial load, and inflammatory markers. Our results indicate that, adjuvant dabigatran treatment might be a beneficial strategy to reduce biofilm formation and thereby augment antibiotic efficacy. Adjunctive dabigatran could potentially be a candidate for anti-biofilm development in patients with *S. aureus* IE.
Demonstrating the efficacy of cold atmospheric gas plasma against biofilm of *Staphylococcus aureus* (ATCC 6358) - Onuoha O

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Introduction: Biofilm existence and its clinical significance can never be over emphasised. Established biofilm can survive after exposure to several physiochemical invasions such as phagocytosis and antimicrobials; hence, there is need for alternative and more efficacious treatment. Cold atmospheric gas plasmas (CAPs) technology treatment is highly effective against bacterial cells. The treatment is based on electrical power supplied to maintain gas discharge and time exposure through generation of a wide range of nitrogen and oxygen reactive species.

Hypothesis and aims: This study was focused on investigating the use of CAPs to inactivate bacterial biofilm.

Methodology: *S. aureus* biofilm was used as the model in this study. Cells were inoculated in Tryptic soy broth overnight, centrifuged and washed twice in Dulbecco’s phosphate-buffered (DPBS). Pellets were re-suspended in DPBS, vortexed and the absorbance was measured at 600nm. Aliquots of organisms in tryptic soy broth-glucose (1 x 107 CFU/ml, n=3) were added into petri dishes and incubated at 37°C for 20 hrs. Biofilm formed was washed in DPBS thrice and then exposed to CAPs at different time intervals (0–10 mins) or treated with CHLX (31.3μg/ml, 0–24 hrs). Log10 reductions were calculated for both treatment options.

Results: Treatment of planktonic and biofilm *S. aureus* against Chlorhexidine (CHLX) (31.3μg/ml) showed -6.50 and -1.20 log10 reduction respectively. Treatment with CAPs for 1, 5 and 10 mins showed a significant log10 reduction of -0.4, -1.5 and -3.2 respectively. In contrast, the CHLX effects for 1, 2, 4 and 24 hrs with -0.23, -0.24, -0.40 and -0.75 respectively. Therefore, treatment with CAPs produced higher log reduction at a quicker time compared to treatment with antimicrobial.

Conclusion: This study has demonstrated high effectiveness of CAPs against *S. aureus* biofilm, indicating the potential for this technology in disinfecting surfaces, surgical instruments, targeting antibiotic resistant organisms and treating chronic wounds.
149: A systematic comparison of factors affecting the antimicrobial susceptibility of Staphylococcus aureus and Staphylococcus epidermidis - Regan H.C

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Introduction: Staphylococcus aureus and Staphylococcus epidermidis are well-known commensal organisms with propensity to cause chronic opportunistic infections of wounds, implants and medical devices. Chronic infections are associated with the biofilm phenotype and are recalcitrant to antibiotic therapy.

Hypothesis and aims: The aim of this study was to systematically elucidate the factors affecting the in vitro susceptibility of S. aureus SH100 and S. epidermidis RP62a to gentamicin, vancomycin and clindamycin, three antibiotics commonly prescribed for implant-related infections.

Methodology: The susceptibility of planktonic cultures, grown in Mueller-Hinton broth and cation-adjusted Mueller-Hinton broth, were tested using broth microdilution assays following BS EN ISO 20776-1:2006. Minimum biofilm inhibition concentration and minimum biofilm eradication concentration were investigated using biofilms cultured in the Calgary biofilm device and in standard tissue culture-treated microtiter well plates. The number of viable bacteria within biofilms were quantified via viable plate counts and the use of metabolic dyes. Since S. aureus and S. epidermidis are facultative anaerobes, baseline antibiotic susceptibility values established using standard culture conditions described above were then compared against planktonic cultures and biofilms grown under anaerobic conditions.

Results: The results indicate that, as expected, biofilms of both strains are more resistant to all tested antibiotics relative to planktonic cultures. The bacterial cells displayed differential susceptibilities depending on whether the cells were cultured in either Mueller-Hinton broth or cation-adjusted Mueller-Hinton broth. The antimicrobial susceptibility of planktonic cells and biofilms were significantly altered when the bacteria were cultured under anaerobic conditions.

Conclusion: In conclusion, the observations from this study suggest that the in vitro antibiotic susceptibility displayed by bacteria are dependent on the laboratory conditions used for culturing the cells. The observed susceptibility profiles, in the future, will be compared to those of clinical isolates and correlated to clinical outcomes. This would enable us to identify appropriate culture conditions in order to obtain clinically-relevant antibiotic susceptibility profiles.
150: New anti-biofilm PDMS-based coating reducing biofilm formation of *Staphylococcus epidermidis* - Ricciardelli A

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**Introduction:** *Staphylococcus epidermidis* represents the most common source of infections on indwelling medical devices. *S. epidermidis* is a permanent and ubiquitous colonizer of human skin, and consequently, the device contamination during insertion is highly probable. Its ability to colonize a surface and form a biofilm is an important virulence factor.

**Hypothesis and aims:** The complex and resistant structure of the biofilms protects bacteria from the most common therapeutic treatment, therefore the necessity of novel anti-infective strategies is becoming increasingly urgent. The discovery of antifouling or antimicrobial surfaces can be a possible approach to prevent biofilm formation. The aim of this study was to create a novel anti-biofilm strategy against *S. epidermidis* RP62A devices-associated infections.

**Methodology:** A coating system was developed through the adsorption on a polydimethylsiloxane (PDMS) surface of the novel anti-biofilm agents pentadecanal and pentadecanoic acid. The influence of the anti-biofilm molecules adsorption on the surface properties of PDMS was evaluated in terms of hydrophobicity and roughness. Then, the anti-adhesive and biofilm-inhibiting effects of the proposed coatings were studied using a parallel plate flow chamber.

**Results:** The pentadecanal adsorption increased the roughness and the hydrophobicity of the surface, whereas, after pentadecanoic acid adsorption, the PDMS surface resulted to be, albeit quite rough, more hydrophilic. Despite showing different physicochemical properties, the proposed coatings were not only able to affect the staphylococcal adhesion, but were also able to reduce the biofilm formation.

**Conclusion:** Two different anti-biofilm PDMS-based coatings were proposed and characterized. The biofilm formation of *S. epidermidis* RP62A on both uncoated and coated PDMS was performed in a parallel plate flow chamber system. Optical Coherence Tomography and Confocal Laser Scanning Microscopy analysis demonstrated the capability of the proposed anti-biofilm surfaces to avoid a strong attachment of the bacterial cells and to reduce the biofilm formation.
151: Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis - *Marco Artini*

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**Introduction:** Cystic fibrosis (CF) patients manifest a variety of multi-organ problems due to the alteration of sodium and chloride secretion across cell membranes. The impairment of mucociliary clearance leads to the production of a thick and dehydrated mucus in the CF lung, which promotes the airway chronic bacterial colonization. In the early stage of life, it is characterized by the prevalence of *S. aureus*. In early adolescence, Gram-negative bacteria chronically infected it. Among these, *P. aeruginosa* is the most relevant and recurring. Recently, several reports indicated in vitro efficacy of natural compounds as promising treatment to reduce the development of the CF associated infections. Among these, essential oils (EOs) seemed to be the most promising agents. Recently machine learning (ML) has been proved as tool to enable the deep investigation on EOs chemical components modulation role against both *P. aeruginosa* and *S. aureus*.

**Hypothesis and aims:** In this study an extensive analysis on 61 commercial EOs against a panel of 40 bacterial strains isolated from CF patients is reported.

**Methodology:** Clinical bacterial isolates were classified on the basis of phenotypic and genotypic features (descriptors). To speed-up the in vitro procedure, classification algorithms allowed the strains clusterization in to select representatives to be subjected to EOs antimicrobial evaluation.

**Results:** Some EOs, showing a strong efficacy to impair the growth of microrganisms, were promptly assayed against all the clinical isolates. Among them three EOs demonstrated their ability to inhibit all bacterial growths. The potent EOs were analyzed for by means of gas chromatography coupled with mass spectrometry to investigate on the likely chemical components mainly responsible for the antibacterial activity.

**Conclusion:** Investigation of the most important components by means of feature importance and partial dependence plots allowed us to indicate the chemical components mostly related to antimicrobial activity of three active EOs.
Repurposing metal chelators to combat staphylococci biofilms - Richter K

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Introduction: Staphylococcus aureus and Staphylococcus epidermidis play a major role in infectious diseases, such as hernia mesh infections and surgical site infections. Current medical care fails to effectively control these infections, in particular when bacteria established antibiotic-resistance. In addition, the formation of biofilms on hernia mesh and surgical sites frequently causes major clinical complications. Elevated healthcare costs and the lack of effective antibacterial treatments urgently call for improvements in antimicrobial strategies.

Hypothesis and aims: We hypothesise that the metal chelator diethyldithiocarbamate (DDC) can be repurposed as antibiofilm treatment. The aim is to investigate the antibacterial activity of DDC combined with copper(II) (Cu) against staphylococci.

Methodology: The minimal inhibitory concentration (MIC) of DDC and Cu was determined in 2 methicillin-resistant S. aureus and 2 S. epidermidis strains. To assess the potential synergy between the two compounds, checkerboard assays were performed with planktonic and biofilm bacteria. The fractional inhibitory concentration index was calculated and used to define synergism, near synergism or additive effects of DDC and Cu.

Results: The MIC of DDC was 64 and 32 µg/ml, respectively, for all MRSA and S. epidermidis strains. The MIC of Cu was >256 µg/ml in all strains tested. Synergistic effects of DDC and Cu (DDC-Cu) were observed in the 2 planktonic MRSA strains, however, in MRSA biofilms DDC-Cu reached near synergism in one strain and additive effects in the other strain. In all S. epidermidis strains, DDC-Cu showed an additive effect in both planktonic cells and biofilm.

Conclusion: Synergistic and additive effects against MRSA and S. epidermidis make DDC-Cu an interesting new treatment strategy against staphylococci. Future research will expand on the antibacterial and antibiofilm studies, determine the mode of action of DDC-Cu and develop drug-delivery approaches for applications in hernia mesh infections and surgical site infections.
Decontamination effect of the novel water vapor plasma generator prototype on staphylococcal biofilm - Růžička F

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Introduction: A non-equilibrium plasma can be used for generation of various agents with decontamination potential, such as reactive oxygen and nitrogen species, charged particles, free radicals or UV photons. These particles can be used for surface decontamination of medical devices.

Hypothesis and aims: Decontamination effect on microorganisms of a plasma generated by diffuse coplanar surface barrier discharge has been repeatedly demonstrated. The novel type of plasma generator based on surface barrier discharge utilizes low-cost water vapor and/or air, instead of relatively expensive noble gasses. Decontamination potential of particles generated in water vapor plasma on biofilms was investigated.

Methodology: Biofilms of Staphylococcus aureus ATCC 43300, Staphylococcus epidermidis CCM 7221 and clinical isolate Pseudomonas aeruginosa FB 45 were grown in BHI broth supplemented with 1 % glucose overnight on polypropylene discs with hydrophilic surface treatment. Remaining adherent cells were gently rinsed twice using normal saline. Subsequently, biofilm samples were treated by plasma-activated and not activated water vapor at distances 20, 30 and 40 cm from the plasma source and for two different times (30 and 150 seconds). Decontamination effect was determined as a log reduction in bacterial numbers by plating and CFU counting and novel high-throughput approach using Start of growth time method.

Results: Decontamination effect was strongly dependent on distance from the plasma source and time of decontamination. Samples at 20 cm distance (30 seconds) showed 0.5 log difference when treated with not activated plasma and 1 log difference in activated plasma. Similarly, 150 second treatment showed 1.5 log and 2 log difference on average. Longer distances from the source showed negligible effect.

Conclusion: The novel type of plasma generator utilizing water vapor and air has a potential for decontamination purposes with a prospect of design of novel types of decontamination devices.
**Introduction:** *S. aureus* is an important opportunistic human pathogen that causes a wide range of infections, including minor skin infections and life-threatening diseases. Treatment of infections caused by *S. aureus* has become an increasing challenge particularly due emergence of strains resistant to multiple antibiotics like Methicillin-Resistant *S. aureus* (MRSA) and further complicated when bacterial biofilm is formed, a phenotype associated to medical devices such as catheters and endotracheal tubes.

**Hypothesis and aims:** In this study, it was hypothesized that MRSA biofilms exposed to Cold Atmospheric Plasma (CAP) leads to biofilm disruption and bacterial cell death. We investigated the impact of the reactive chemistry generated by indirect CAP on MRSA biofilm decontamination.

**Methodology:** Biofilms of *S. aureus* USA 300 – the leading MRSA clone in America and an emerging MRSA clone in many European countries – were grown on polypropylene coupons using the CDC biofilm bioreactor and were further exposed to CAP from 0 up to 240 seconds. CFU count was used to determine bacterial kill efficacy. Multiple assays were performed to detect DNA, protein and cell membrane damage and also to detect intracellular Reactive Oxygen/Nitrogen Species (RONS).

**Results:** CAP was effective in the inactivation of *S. aureus* USA300 biofilm over a period of 240 seconds treatment. Investigation of treated cells shows that the stress conditions caused by ROS and RNS produced by indirect CAP exposure play a major role in inactivation of biofilm cells.

**Conclusion:** This study shows the potential of CAP treatment for eradication of MRSA biofilms grown on medical devices and give indications of interactions between bacterial cells and CAP reactive species.
Introduction: Bacterial biofilms possess complex and dynamic structures which demand orthogonal approaches studies in order to enable and maximize their clear understanding. Earlier investigations of our research group have shown the suitability of utilizing paper-based arrays for biofilm formation studies by Staphylococcus aureus. Despite these paper-based arrays being excellent tools for biofilm sensing purposes, they do not allow monitoring the biofilm formation by fluorescence imaging techniques nor studying the biofilm biomatrix (composed mainly for exopolysaccharides, proteins and lipids).

Hypothesis and aims: In this study, a set of newly developed latex-coated glass coverslips were characterized in-depth, particularly in terms of S. aureus biofilm attachment through two experimental platforms which were here optimized and compared to each other.

Methodology: The early and mature biofilm colonies of Staphylococcus aureus ATCC 25923 were quantify by viable plate counts, the biofilm matrix was determined by quantitating the poly-N-acetyl-β-(1-6)-glucosamine fraction. Protein amount changes in the matrix as well as proteome dynamics were also studied in detail. Changes in the composition of surface proteins (surfaceomics) were investigated via trypsin-shaving followed by protein identification. Furthermore, Fluorescence Microscopy (LIVE/DEAD® BacLight™ staining) successfully aided to visualize the biofilm adhesion process.

Results: The influence of the experimental conditions to the biofilm adhesion is discussed here. Different latex proportions in the coating gave particular modification which impacted in the levels of bacterial adhesion (viable colonies, biomatrix, proteins).

Conclusion: Overall, these latex-based glass coverslips promoted higher biofilm adhesion than bare coverslips, and offered the advantage of microscopy visualization as well, therefore, they can be regarded as suitable tools for orthogonal investigation approaches of bacterial adhesion.
156: Nanostructured surfaces prevent the formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms - Tolordava E

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**Introduction:** Biofilm bacterial infections are difficult to treat because of the potential resistance of organisms to widely used antimicrobial agents. Nanoparticles have a promising future in the development of new therapy due to their easy functionalization and unique mode of action. Nanoprint structures formed under the influence of laser radiation on surfaces made of various materials in recent years have shown high efficiency in preventing the formation of biofilms on nanostructured surfaces.

**Hypothesis and aims:** Study of antibacterial properties of nanostructures

**Methodology:** Se and Si nanoparticle-based coatings were prepared by nanosecond laser ablation of the corresponding solids in water and tested regarding their antibacterial properties. The antibacterial effect of Se, Si and reference Ag nanocoatings was tested in respect to Gram- positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* biofilms. To visualize live and dead cells the coloration set «Live/Dead Biofilm Viability Kit» was used and the results were analysed in the fluorescence microscope Nikon H600L with a fluorescent lens.

**Results:** Se and Si coatings have demonstrated a strong inhibitory effect on biofilm formation comparable to the cytotoxic effect of reference Ag nanocoatings. However, silver particles are toxic and have an unpleasant feature to accumulate in the internal organs. The advantage of Se and Si nanoparticles is their biocompatibility, biodegradability and high penetrating power. Nanostructured Si surfaces have also demonstrated good antibacterial properties. A number of studies have reported on mechanical stress on the bacteria which causes damage to the membrane and further loss. But also, it is necessary to consider that the nano-razors obtained as a result of laser femtosecond treatment consist of Si particles, which themselves have an antibacterial effect.

**Conclusion:** The antibacterial activity of laser-generated Si and Se nanoparticle-based surface coatings was demonstrated regarding biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. 
Thursday 5th September

*Candida*

157: Candida albicans biofilm heterogeneity modifies persistence following sodium hypochlorite and EDTA treatment - *Alshanta O-A*

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**Introduction:** *E. faecalis* and *C. albicans* are frequently co-isolated in polymicrobial communities in persistent endodontic infections, suggesting an interkingdom interaction between bacteria and fungi. Evidence from the gastrointestinal tract indicates that *C. albicans* has the ability to promote the growth of *E. faecalis*. These same nature of interactions may occur in the root canal, with *Candida* hyphae acting as a physical carrier for non-motile *E. faecalis* to penetrate into dentinal tubules.

**Hypothesis and aims:** We hypothesise that *C. albicans* and *E. faecalis* interact with one another in the form of complex biofilm communities, which has implications for pathogenicity and resistance.

**Methodology:** Dual species biofilms of *C. albicans* and *E. faecalis* were optimised using different concentrations of cells in RPMI/TSB. These were then quantified using culture (CFU) and molecular methods (qPCR), analysed microscopically, and transcriptional analysis performed. Biofilms were then tested for inhibition, regrowth and killing using a range of endodontic irrigants.

**Results:** We were able to demonstrate that *C. albicans* and *E. faecalis* grew together in dual-species biofilms, and this was influenced by the clinical strains tested. We demonstrated that key *C. albicans* adhesion and biofilm genes were differentially expressed in dual species biofilms. Treatment of these biofilms showed that despite initial killing, the dual species biofilms were able to regrow and recolonise the substrate.

**Conclusion:** These studies indicate that *C. albicans* and *E. faecalis* have the potential to co-exist in endodontically important biofilms, and that this interaction may explain their resilience and ability to withstand endodontic irrigants.
**Introduction:** Microbes employ chemical signaling (Quorum sensing, QS) mechanisms to communicate with each other within polymicrobial communities. However, the impact of QS in conferring antimicrobial resistance is largely unknown.

**Hypothesis and aims:** This study aimed to unravel the role of interkingdom QS interactions between the bacterium *P. aeruginosa* and the yeast *C. albicans* on exposure to the common antifungal fluconazole, in the presence of [N-(3-Oxododecanoyl)]-L-homoserine lactone, C12AHL – a QS molecule produced by *P. aeruginosa*. We hypothesize that C12AHL is likely to modulate candidal response to fluconazole.

**Methodology:** Changes in *C. albicans* growth, drug efflux, and multidrug efflux genes *CDR1, CDR2, MDR1* expression were evaluated under the foregoing conditions. Changes in the *C. albicans* proteome and transcriptome when exposed to fluconazole ± C12AHL were was also assessed by 2-dimensional gel electrophoresis/mass spectrometry and next-generation sequencing (RNA-Seq), respectively. Differentially expressed genetic pathways were then determined using the *Candida* Genome Database.

**Results:** *C. albicans* sensitivity to fluconazole was reduced 8-fold (0.15µg/ml vs 1.25µg/ml, P<0.05) when exposed to C12AHL (50µg/ml). Compared to fluconazole or untreated-controls, exposure to fluconazole+C12AHL or C12AHL alone significantly enhanced drug efflux from *C. albicans* (P<0.05). C12AHL+fluconazole upregulated genes coding for *C. albicans* multidrug-efflux pumps *CDR1* and *CDR2* (P<0.05). Exposure to fluconazole+C12AHL, led to under-expression of *C. albicans* proteins associated with antifungal sensitivity, oxidative stress, respiration, protein metabolism and nucleic acid synthesis (P<0.05). The ergosterol synthesis pathway was significantly upregulated in *C. albicans* when exposed to fluconazole, as opposed to fluconazole+C12AHL exposure (P<0.001).

**Conclusion:** Our data imply that *P. aeruginosa* quorum sensor C12AHL dampens the effectiveness of fluconazole via multiple mechanisms, but mainly by restoring the cell wall integrity of the yeast by fostering ergosterol synthesis. These findings demystify to some extent the antimicrobial resistance mechanisms operational within interkingdom, polymicrobial ecosystems.
159: Evaluation of the antifungal activity of chitosan against *Candida auris* using an *in vivo* infection model - *Brown J*

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**Introduction:** *Candida auris* is a nosocomial pathogen responsible for recent global healthcare outbreaks. Resistance to multiple antifungals and the ability of biofilm formation are challenges to its eradication.

**Hypothesis and aims:** The aim of this study was to evaluate the antifungal effect of a chitosan on aggregating and non-aggregating strains of *C. auris* using an *in vivo* infection model.

**Methodology:** Planktonic and sessile minimum inhibitory concentrations (PMICs and SMICs) of chitosan against 5 aggregating and 5 non-aggregating strains of *C. auris* were evaluated. In addition, an *in vivo* infection model using *Galleria mellonella* was employed to test the efficacy of chitosan against one aggregating and one non-aggregating strain of *C. auris*. Briefly, different groups (n=10) were infected at 5 \(\times 10^5\) cells/worm of each *C. auris* strain and after 2-h incubation before the worms were inoculated with chitosan at 50, 100 and 200 mg/Kg. Appropriate controls were included. Survival analysis was performed with mortality monitored every 24 h for 7 consecutive days.

**Results:** Non-aggregating strains PMICs were 10.9 mg/L while aggregating strains PMICs ranged between 5.46 to 21.8 mg/L. Sessile aggregating strains were more resistant than non-aggregating *C. auris* requiring 1 to 4 fold-change of chitosan concentration to reach the SMIC\(^\text{80}\). The *in vivo* results showed that chitosan significantly increased the survival rate of worms infected with non-aggregating *C. auris* (\(P<0.001\)). Treatment with chitosan at 200 mg/Kg reached a survival percent of 20% of the worms at the end of 7 days of infection while infection by aggregating *C. auris* was more resistant to chitosan treatment.

**Conclusion:** Given the well documented resistance of *C. auris* to conventional antifungals, there is an urgent requirement for alternative therapeutics. We have shown that chitosan is an attractive candidate, demonstrating antifungal activity both *in vitro* and *in vivo* against planktonic and sessile *C. auris* cells.
160: Employment of polyclonal antibody anti-CR3-RP Ab in the treatment of biofilm of Candida albicans and Candida auris resistant to common antifungals - Dekkerová J

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Introduction: Candida auris is currently considered as a serious emerging multidrug-resistant fungal pathogen. Recent studies have confirmed that C. auris shares similarity with Candida albicans in regards to virulence-associated proteins involved in adherence and biofilm development. Complement receptor 3-related protein (CR3-RP) is one of such key surface antigens expressed by C. albicans during biofilm formation.

Hypothesis and aims: Here, we documented presence of the CR3-RP on the surface of C. auris and potential of anti-CR3-RP polyclonal antibody (Ab) to inhibit biofilm formation. Additionally, we estimated relative gene expression of biofilm-associated genes in the presence of Ab during biofilm formation.

Methodology: The presence of CR3-RP on the C. auris surface was confirmed by indirect immunofluorescence and ELISA. The XTT reduction assay was used for determination of viability of biofilm in the presence of selected antifungals (fluconazole-FLC, amphotericin B-AMB and caspofungin-CAS) and anti-CR3-RP Ab (added at the adherence phase and the 24-h pre-formed biofilm). Quantitative real-time PCR was used for determination of changes in the ALS1, ALS3, ALS9, BCR1 gene expression in biofilms formed w/wo Ab.

Results: ELISA and indirect immunofluorescence confirmed the presence of CR3-RP in C. auris. The anti CR3-RP Ab was able to inhibit biofilm of C. auris and FLC-resistant C. albicans strains when added to the adherence phase. Moreover, Ab also demonstrated activity against 24-h pre-formed biofilms, which compared favorably to levels of inhibition achieved by treatment with selected antifungals. Real-time PCR showed decrease of gene expression of biofilm-associated genes (2x for ALS1, 4x for ALS9 and 3x for BCR1) in biofilm of C. albicans formed in the presence of anti-CR3-RP Ab.

Conclusion: Overall, our data point to the potential of anti-CR3-RP Ab in eradication of biofilms formed by resistant C. auris and FLC-resistant C. albicans. Additionally, administration of anti-CR3-RP Ab during biofilm formation led to relevant decrease in expression of biofilm-associated genes, resulting in less ability to adhere and form biofilm.
**161: Candida albicans enhances initial biofilm growth of Cutibacterium acnes under aerobic condition - Imbert C**

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**Introduction:** Candida albicans is the leading fungal cause of nosocomial bloodstream infection. Many of these infections are linked to the presence of implanted medical devices (IMD) and involve bacterial partners within polymicrobial biofilms. *Cutibacterium acnes* is a facultative anaerobic opportunistic bacterial pathogen responsible for IMD infections, frequently encountered in biofilms on prosthetic joints. Moreover, both microorganisms are commensal of the gastrointestinal and genito-urinary tracts and of the oral cavity.

**Hypothesis and aims:** As these microorganisms share common localization on the human body and are responsible of IMD infections, the objective of this study was to evaluate the capacity of *C. albicans* and *C. acnes* to interact and form a polymicrobial biofilm in different conditions.

**Methodology:** Dynamic adhesion assays were performed using the Bioflux Z1000 platform and mature biofilms were observed by SEM. Biofilm formation in both anaerobic and aerobic conditions of both microorganisms was evaluated using CFU counts.

**Results:** Results of adhesion assays and SEM imaging showed that *C. acnes* adhesion to *C. albicans* did not have a preference for a specific morphological state of the fungus. Bacteria were able to adhere to both hyphal- and yeast forms of *C. albicans*. Moreover, under aerobic condition, *C. albicans* had a positive influence on early *C. acnes* biofilm growth within the biofilm. This favorable impact of the fungal cells was not mediated by secreted compounds and required metabolically active *C. albicans* cells. This effect could be related to rapid oxygen consumption by *C. albicans* creating an “anaerobic local niche” favorable to *C. acnes* growth.

**Conclusion:** *C. acnes* and *C. albicans* are able to interact and form polymicrobial biofilms. These interactions within these structures under aerobic conditions are beneficial for bacterial growth and could modulate the physiopathology of infections linked to these microorganisms if present together.
162: *Spirulina* sustainable lipid extracts and their vectorization to combat *C. albicans* biofilms - Imbert C

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**Introduction:** Biofilms are involved in numerous skin, hair and dental inconveniences like acne vulgaris, dandruffs, or dental plaque. *Candida albicans* can be part of these biofilms. Our public/private consortium is interested by developing natural ingredients displaying anti-biofilm activity, and that could be easily introduced in daily-used cosmetic products.

**Hypothesis and aims:** Free fatty acids (FFA) are naturally occurring on skin and are in charge of the microbiota regulation; they exhibit wide antimicrobial spectrum depending on carbon chain length and number of double bonds. FFA are well known metabolites authorized in cosmetic field. Microalgal biomass, especially the blue cyanobacteria *Arthrospira platensis* (spirulina), represents a natural renewable source of FFA especially of polyunsaturated fatty acids (PUFA).

**Methodology:** In order to develop a biomimetic approach against *Candida* biofilms, FFA extracted from microalgae spirulina using four solvents were studied. Lipid and pigment amounts, combined with FFA profiles were analyzed to select the optimal conditions: 30 min of ultrasonic extraction using dimethyl carbonate or ethyl acetate. Anti-biofilm tests were performed.

**Results:** A vectorization using a macroalgal-alginate nanocarrier was successfully performed. Vectorized extracts were safe towards keratinocytes. Spirulina lipid-enriched extracts showed a high anti-biofilm growth activity at low concentrations (about 80% inhibition after 24h at 0.2 mg/mL). The combination of extracts in copper-alginate nanocarriers potentiated the anti-biofilm growth activity. As expected spirulina lipid-enriched extracts did not reduce preformed biofilms (*p* >0.2), certainly due to their hydrophobicity. However, interestingly once encapsulated, spirulina extracts inhibited preformed biofilm by 34% or 52% depending on the solvent used for FFA extraction. Unfortunately it was not clear if this activity was due solely to nanocarriers or partly due to its core (extracts).

**Conclusion:** The approach of encapsulated spirulina lipid extracts represents a relevant concept to develop all in one anti-biofilm weapons and offers a new perspective for algae biomass valorization.
163: Transcriptional profiling of biofilm formation by the emerging fungal pathogen Candida auris - Kean R

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Introduction: The emerging fungal pathogen Candida auris has drawn considerable attention as a source of healthcare associated infections, predominantly due to its ability to cause hospital outbreaks and its well documented antifungal resistance profile. Studies have shown that the organism can form antifungal resistant biofilms, yet the mechanisms conferring this resistance are unknown.

Hypothesis and aims: It was hypothesised that C. auris biofilms would exhibit resistance to antifungals and prolong the organisms’ survival within the environment. Using RNA-Seq, we aimed to understand the mechanisms which govern biofilm formation and the clinical implications of these communities.

Methodology: RNA-Seq was performed on biofilms formed by two phenotypically distinct C. auris isolates (aggregative[agg] and single cells). A C. auris transcriptome was assembled de novo with differential expression performed using DESeq2. Biochemical analysis was performed to confirm RNA-Seq findings. In addition, survival assays were performed across a period of 14 days, with and without treatment with sodium hypochlorite disinfectant.

Results: C. auris biofilms were shown to be resistant to all three classes of antifungals, irrespective of phenotype. RNA-Seq analysis revealed that 791 genes were differentially expressed between planktonic and biofilm cells. Specifically, a number of drug transporters were shown to be significantly upregulated (>2 log₂ fold change) in biofilms, with the function of these transporters further confirmed biochemically. In addition, an isolate exhibiting an aggregative phenotype demonstrated a prolonged survival capacity in comparison to the single cell isolate, with transcriptional analysis revealing significant, biofilm-associated changes within the cell wall of the agg isolate.

Conclusion: Collectively we have shown that biofilms formed by the emerging pathogen C. auris confer resistance to antifungals due to an increased expression of efflux pumps. In addition, the organism can employ phenotypic survival strategies that could enhance its persistence in within the environment.
Introduction: Mixed bacterial-fungal colonization of the endotracheal tubes is now evident, with microbial interplay withstanding common antimicrobial therapy and paying for persistent and severe VAP infections. While alternative therapeutic strategies effectively targeting inter-kingdom biofilms are required, the role of each microorganism need to be appraised to deliver effective treatments.

Hypothesis and aims: We earlier reported the combination therapy involving polymyxin B (PMB) and amphotericin B (AMB) as holding an attractive therapeutic option to treat dual-species biofilms. This study aimed to determine the “post-antimicrobial” phenomenon of PMB/AMB combined action in P. aeruginosa (PA) + Candida albicans (CA) biofilms, and to ascertain the events underlying biofilm growth restoration.

Methodology: Post-antimicrobial effect of PMB combined with AMB was assessed in 24-h dual-species biofilms. Cell culturability and viability were evaluated by CFU and Live/Dead staining, respectively. The gene expression profile was assessed by qPCR.

Results: Results showed that PA+CA biofilms lost their culturability straightaway being exposed to PMB/AMB combined solution. However, 24h was enough to both species recover their growth onto agar medium, with microbial counts approximating those observed for pre-treated biofilms. Following the subsequent treatment cycle, CFU estimation was only slightly disturbed. L/D results revealed that PA and CA populations displayed a compromised status at the end of the first PMB/AMB treatment cycle. Finishing the 24-h-regrowth cycle, most biofilm-encased species exhibited viability, which endured after the second treatment period. Transcriptional analysis of dual-species biofilms exposed to PMB/AMB combined action showed a high expression level in all PA resistance-encoded genes – anrB, galU, mexA and algD – and in ERG3 and ALS2 CA genes.

Conclusion: Our findings showed that PA+CA biofilms were able to escape to the combined action of PMB/AMB, and both species had a preeminent role while retaining adaptive resistance mechanisms that likely contributed for their recovery and adaptation on the ensuing treatments.
165: Identification of Candida biofilm-related genes and expression of inflammatory biomarkers in RVVC - McKloud E

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Introduction: Recurrent vulvovaginal candidiasis (RVVC) is a chronic and debilitating condition that is estimated to affect 138 million women annually. Despite this high prevalence and its associated economic burden, pathogenesis of disease is poorly understood. The biofilm-forming yeast Candida albicans is reported as the causative pathogen in up to 90% of VVC cases and around 50% in RVVC disease. Despite the identification of Candida biofilms on the vaginal mucosa, their associated therapeutic challenge in RVVC is still disputed.

Hypothesis and aims: Using a combination of proteomics and transcriptional analysis, it is hypothesised that Candida biofilm-related characteristics and markers of inflammation will be differentially expressed between clinical samples of disease (RVVC) and health.

Methodology: A panel of 100 cervico-vaginal lavage (CVL) samples were used in this study. Sixty CVL samples from healthy women and forty from women suffering from RVVC allowed for proteomic analysis to identify inflammatory biomarkers present in each group. Additionally, RNA was extracted from Candida positive CVL samples for subsequent transcriptional analysis for Candida biofilm-related genes.

Results: Proteomic analysis of clinical samples revealed significantly up-regulated inflammatory biomarkers in disease compared to health including chemotactic immune mediators such as IL-8, CXCL9 and CXCL10. Furthermore, Candida biofilm-related genes including those involved in adhesion and virulence, were shown to be differentially expressed between health and RVVC.

Conclusion: This work supports the hypothesis that formation of Candida biofilms on the vaginal mucosa could negatively impact clinical treatment and suggests a role of specific inflammatory biomarkers in VVC pathology. Further work to identify triggers for development and recurrence of VVC, and the pathogenesis of the microbes involved, could considerably improve prevention and treatment options for women with recurrent, azole-resistant infections.
Introduction: Surfaces in contact with physiological fluids, like medical devices (MD) for humans, are firstly covered by adsorbed proteins. This conditioning film is considered crucial regarding microorganism adhesion then biofilm formation. Among microbial cells implicated in infections, especially nosocomial infections linked to the colonization of MD surfaces, yeasts of the genus Candida, i.e. C. albicans, are frequently involved.

Hypothesis and aims: Regarding our previous results on the interactions between thin dielectric layers of SiO$_2$ with tailored by silver nanoparticles (AgNPs) properties and proteins, our aim was to evaluate the shear-induced detachment of C. albicans in contact with a thin silica layer, containing or not AgNPs, at low shear stresses using a shear stress flow chamber.

Methodology: The study focuses on the shear-induced detachment of the yeast Candida albicans IP48.72 in contact with a thin silica layer ± AgNPs covered by adsorbed Bovine Serum Albumin (BSA) or Fibronectin (Fn) at low shear stresses using a shear stress flow chamber. The experimental arrangement is designed to address large range of shear stresses, up to 80 Pa, with specific attention paid to the low shear stress domain (0.01 Pa).

Results: Regarding C. albicans IP48.72 and SiO$_2$ alone, 27% of the cells remained stuck to the surface. In the same time, BSA induced a significant reduction of adherent cells (14%), while Fn favored the adhesion (84%). In all the tested conditions, adhesion level was maintained to 5 Pa. AgNPs deposited on the SiO$_2$ surface induced an increase in C. albicans adhesion level.

Conclusion: We demonstrated the impairment of C. albicans adhesion forces regarding the protein adsorbed on silica layer. This was also the first demonstration of a positive impact of AgNPs on C. albicans adhesion.
167: In vitro polymicrobial bacteria-fungal biofilm model in the context of prosthetic joint infections - Ruiz-Sorribas A

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Introduction: The number of hip and knee arthroplasties in the developed countries is increasing.1,2 Prosthetic joint infections (PJIs) are one of the most severe complications, which require additional surgeries,3 due to the failure of antibiotherapies. The etiology of PJJ is diverse but associated with the formation of biofilms. Among them, an average of 20% are considered polymicrobial, which have an even further reduced sensitivity to antimicrobials due to interactions among species that reinforce the biofilms.3–6

Hypothesis and aims: To set up a three-species biofilm model pertinent of PJIs, including a representative Gram-positive, Gram-negative, and fungal species.

Methodology: A co-culture of Staphylococcus aureus ATCC25923 or Staphylococcus epidermidis ATCC35984 (most frequently isolated pathogens), Escherichia coli ATCC47076 (model for Enterobacteriaceae), and Candida albicans ATCC24433 (model for fungi), was performed in 96-wells plates. Biofilm formation was evaluated by assessing the total biomass (staining with crystal violet), and culturable cells (cfu counting on selective media). Fluorescence microscopy was performed on biofilms cultured on titanium coupons.

Results: The conditions for which biomass and culturable cells were the most stable are a 48-hours grown in RPMI + 1% glucose buffered with 50mM KH2PO4 / 74.1mM Na2HPO4, with inocula of 1.5*10⁷ : 6.0*10⁶ : 2.5*10⁶ cfu/mL for S. aureus or S. epidermidis : E. coli : C. albicans respectively. Based on fluorescence microscopy pictures, maximal thickness was 40 µm. The corresponding dual-species biofilms showed a higher biomass in the case of S. aureus or S. epidermidis : C. albicans and a lower biomass in the case of E. coli : C. albicans.

Conclusion: We have set up two 3-species models with representative species for the majority of pathogens isolated in PJI. Our models are stable and repeatable, and will be used to study the response to antimicrobials.
**168: Candida auris exhibits resilient biofilm characteristics in vitro: implications for environmental persistence - Bryn Short**

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**Introduction:** In the decade since its discovery, Candida auris has quickly established itself as a public health threat. This fungal pathogen is responsible for numerous nosocomial outbreaks around the globe and exhibits high levels of mortality and anti-fungal resistance. Environmental surfaces are thought to be exploited as a means for this pathogen to disseminate throughout hospital wards.

**Hypothesis and aims:** Given there is a lack of knowledge of the survival strategies utilised by C. auris, we herein investigated the impact C. auris phenotype has on biofilm formation, environmental persistence and survival.

**Methodology:** Firstly, a pool of 26 clinical C. auris isolates were monitored in real-time to assess their ability to form biofilms. The transcriptome of C. auris biofilms were then identified using RNA-sequencing. Following this, C. auris strains were introduced into a dry biofilm model to assess persistence and resistance to sodium hypochlorite (NaOCl).

**Results:** Contrary to previous findings, heterogeneity in biofilm forming abilities was observed between C. auris isolates. Transcriptional analysis of these biofilms showed significant up-regulation of genes associated with the fungal cell wall and anti-fungal resistance in aggregating C. auris. When introduced into a dry biofilm model, over 1x10^4 CFU/mL aggregating C. auris cells were recovered after 14 days of desiccation with significantly less single-cell C. auris persisting for the same period of time. This ability of aggregating C. auris to persist within the environment coincides with the up-regulation of key biofilm-associated genes such as als5 and kre6. Finally, C. auris displayed an ability to tolerate clinically relevant concentrations of NaOCl up to 10,000 ppm with over 1x10^3 CFU/mL recovered immediately following treatment.

**Conclusion:** These findings further highlight the need to better understand the biology of this continuously emerging pathogen and emphasize the importance of exposure time to active agents to prevent the spread of this deadly pathogen around healthcare environments.
169: Preliminary study into the effects of tobacco smoke on Candida albicans - Williams M

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Introduction: Denture-stomatitis (DS) affects approximately 65% of denture wearers and is one of the most common forms of oral candidosis. DS has increased prevalence in cigarette smokers and tobacco condensate has been shown to increase Candida albicans adhesion, growth, biofilm formation, expression of the virulence genes and hyphal production.

Hypothesis and aims: We hypothesise that the chemically characterised tobacco condensate on denture acrylic surfaces will modify C. albicans biofilm development and alter expression of virulence characteristics. Assessment of modulation involved investigating effects on adherence, biofilm quantity, virulence gene expression and hyphal transformation.

Methodology: Acrylic discs (tobacco condensate treated and controls) were incubated statically at 37°C in Yeast Nitrogen Base medium inoculated with C. albicans (n=6) for either 90 min or 24 h to facilitate adherence and biofilm formation, respectively. Candida were stained with calcofluor white and imaged by confocal laser scanning microscopy (CLSM). Expression of virulence genes (n=7) was assessed using qPCR.

Results: Preliminary CLSM results suggested that the effect of tobacco condensate was strain dependent in terms of adherence and biofilm coverage. All but one strain (C. albicans PTR/94) exhibited increased adherence to the tobacco condensate treated discs. The tobacco condensate also appeared to increase hyphal presence, although data analysis remains ongoing. Further investigation will establish whether there were significant differences between tobacco treated, artificial saliva treated and untreated acrylic discs with regards to the measured parameters.

Conclusion: Tobacco condensate altered adherence and biofilm coverage of C. albicans to acrylic surfaces and appeared to increase hyphal development. Work is ongoing to ascertain the significance of these effects upon C. albicans pathogenicity.
170: Control of Candida auris infections by using selected hospital surface disinfectant und new biocides - Zatorska B

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Introduction: Microorganisms living in biofilm communities are protected from detrimental influences including disinfectants. Candida auris represents a new challenge for treatment and infection control in hospitals.

Hypothesis and aims: To investigate the efficacy of surface disinfectants and new biocides on planktonic and biofilms forms of Candida auris reference strains with regards to control of hospital infections.

Methodology: The efficacy of: A. alcohol based, B. quaternary ammonium compounds based, C. aldehyde, quaternary ammonium and surfactant based surface disinfectant, two new biocide D. a micelic based formulation containing 17\% v/v hydrogen peroxide and E. a hypochlorite based formulations were tested in vitro against planktonic forms and biofilms of different Candida reference strains. The antifungal sensitivity; minimum inhibitory concentrations of 100\%, 50\%, 25\%, and 12.5\% of the initial biocide concentrations were performed using a modified microtiter plate assay for planktonic forms. The log10 reduction of yeast strains under disinfectant exposure was investigated for both, planktonic and biofilms forms. Finally, the effect of disinfectant on Candida biofilms was established by recording the reduced metabolic activity measured using the tetrazolium salt (XTT) method. The biofilm forming capacity of the yeast was established using Crystal violet staining.

Results: All tested planktonic forms were easily eradicated using manufactures recommendations and showed > 5 log10reduction. Growth of C. auris NCPF 8971 and NCPF 8977 was fully inhibited by 12.5\% concentrations of A, B and C disinfectants. By following the commercial recommendation for the use of the tested surface disinfectant with the exception of biocide E, the growth of all Candida biofilms (> 9 log 10 reduction) could be eradicated. XTT assay confirmed 90\% reduction of metabolic activity in biofilms after treatment.

Conclusion: Surface disinfectants used in adequate concentrations are effective against biofilms and planktonic forms of C. auris and C. albicans.
Thursday 5\textsuperscript{th} September

\textit{Surfaces & Methods}

171: Nucleating biofilms using polymers for use in biotechnology - Adoni P

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\textbf{Introduction:} We have shown that \textit{Escherichia coli} biofilms can be used as effective biocatalysts for the generation of pharmaceutical intermediates. In order to use E. coli biofilms in this way, it would be advantageous to be able to stimulate and control biofilm formation. Chemically synthesized polymers offer the opportunity to be able to achieve this goal.

\textbf{Aims and hypothesis:} We wanted to investigate the relationship between physicochemical properties of synthetic poly(acryloyl hydrazide) polymers and their ability to drive cluster formation in E. coli strain PHL644 which overproduces the adhesin curli. We hypothesized that there would be correlations between polymer physicochemical properties and cluster formation.

\textbf{Methodology:} Poly(acryloyl hydrazide) was functionalized with a range of functionalities predicted to interact with E. coli cells. Clustering of \textit{E. coli} by poly(acryloyl hydrazide) was characterized using spectrophotometric settling and crystal violet assays and Mastersizer particle size analysis. Bacterial physiology was assessed using LIVE/DEAD staining and formation of components of the biofilm extracellular polymeric substances (EPS) was measured using lectins and gfp reporter gene assays.

\textbf{Results:} Hydrophobic interactions between the polymers and E. coli PHL644 cells is the main driver towards polymer induced clustering with results showing a direct correlation between polymer hydrophobicity and cell cluster sizes. Our data suggests that day-old cell clusters have gone on to develop many of the traits of a biofilm, with the expression of specific EPS components being directly correlated with polymer hydrophobicity and/or cell cluster sizes. Similar trends were observed for the non-curli overexpressing parental stain E. coli MC4100.

\textbf{Conclusions:} We have shown that hydrophobic polymers can be used to cluster bacteria in a predictable manner. These clusters then develop into biofilms with good control over phenotype.
172: Biocidal performance of a metal oxide coating on surface treated polyethylene - Alemi F

Alemi F

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Introduction: Polymers are commonly used for their inert, malleable and durable nature, however their inert nature can make them highly desirable for biofilm growth. Hence finding a good way of reducing bacterial cell viability at the surface of polymers is of great interest.

Hypothesis and aims: Metal oxides are known for their antimicrobial activity due to their unique chemical properties. This project investigates a proprietary colloidal metal oxide coating on surface treated polyethylene to determine whether surface adhesive treatment improves coating adhesion and to test the performance of the metal oxide in reducing bacterial viability.

Methodology: The metal oxide colloidal coatings (SOL A-C) on polyethylene has been investigated against Pseudomonas aeruginosa (NTC12924) using Confocal Laser Scanning Microscopy to investigate the change in viability and surface adhesion. The oxide coating has been probed using contact angle goniometry to determine changes in surface wettability and surface energy, alongside X-ray photoelectron spectroscopy for surface chemical characterization.

Results: Surface treatment shows to improve adhesion of the coating, this is most likely due to the increase in adhesive polar groups at the surface with water contact angle reducing from 84° to <47° after surface treatment. Surface treatment also reduces bacterial viability on its own by 71 - 98% depending on the treatment time. However, with the addition of the metal oxide coating, bacterial viability is reduced even further by 95 - 99.9% compared to uncoated control surface when looking at the change in surface area occupied in ratio of syto-9/propidium iodide in an aqueous environment which was validated with a T-test with all data achieving P<0.05.

Conclusion: The metal oxide coating provides a simple coating system for polyethylene and aids in reducing bacterial cell viability of surface bound Pseudomonas aeruginosa. Surface treatment is beneficial for the increase of adhesion of the sol and reducing bacterial surface attachment.
173: Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates - Allkja J

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Introduction: The lack of reproducibility of published experimental studies is one of the major issues facing science today, and the field of biofilm research is no exception. While many factors contribute to this phenomenon, selective or insufficient reporting of experimental details in the published literature is one of the more significant causes.

Hypothesis and aims: One effective strategy to improve reproducibility is the use of minimum information guidelines. These can be defined as a guide for authors and reviewers on the necessary information that a manuscript should include for the experiments in a study to be clearly interpreted and independently reproduced. We propose a guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates.

Methodology: The guideline was created through a literature review of articles related to the methods included in the guideline and articles on factors that affect biofilm formation and properties. Furthermore, several discussions among international groups working in the area of biofilms provided a more balanced view on what were reasonable and relevant requirements to include in the guideline.

Results: The final guideline has been divided into 5 main sections (Experimental design, Biofilm formation, Biofilm assessment, Statistical assessment and Bioinformatics), each presenting a comprehensive set of recommendations. This outline is designed to follow the chronological order in which the assays are typically performed and described.

Conclusion: We believe that the implementation of this minimum information guideline will improve the quality of scientific communication leading to better reproducibility in biofilm microplate assays.
Modulating an antimicrobial release approach by dopamine chemistry to fight infections associated to orthopedic implants - Alves D

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Introduction: Alongside with orthopedic implants contribution for modern healthcare improvements, there's the risk associated to their microbial colonization and biofilm formation, compromising the performance of the implant itself and representing niches for infection.

Hypothesis and aims: This study aimed to engineer an antimicrobial release coating for stainless steel surfaces (SS) to empower them with the ability to prevent Staphylococci colonization.

Methodology: Surface modification was based on dopamine chemistry, which self-polymerization results in the deposition of a thin, adhered film called polydopamine (pDA). Chlorohexidine (CHX) was chosen to confer the antimicrobial features. Its immobilization was performed through a 2-step approach, including pDA formation and immersion in CHX solution, and 1-step strategy, in which dopamine and CHX were dissolved together and SS coupons were immersed in this solution. An additional layer of pDA was also performed for both strategies.

Results: SEM and AFM confirmed pDA coating by the presence of self-polymerized pDA particles without altering the roughness of SS surfaces. Immobilization of CHX using a 1-step approach yielded surfaces with a more homogenous coating than the 2-step approach. Different pDA-based strategies yielded different CHX release profiles: the amount of CHX released was higher for the 2-step approach and the addition of another pDA layer reduced the amount of CHX released. The antimicrobial performance of the modified surfaces was evaluated against S. aureus and S. epidermidis and the results showed that all the strategies caused a significant reduction (more than 3 LOG) in the number of cells adhered to the surfaces and in suspension, after 24 h. The 2-step approach was able to impart SS surfaces with antimicrobial activity even after 10 days of exposure.

Conclusion: In conclusion, dopamine chemistry can modulate CHX release from the surfaces to obtain an antimicrobial coating strategy with great potential to fight infections associated with orthopedic implants.
Flow dynamics and material surface properties influence ureolytic biofilm development and encrustation - *Blood N*

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Introduction: Catheters are essential for the management of urinary incontinence, a condition currently affecting 5 million people in the UK. The commonly-used Foley urinary catheter has a number of drawbacks including a propensity for blockage due to the formation of bacterial biofilm-mediated encrustations. These blockages are often linked to the urinary pathogen Proteus mirabilis. Efforts at reducing catheter blockage have so far yielded disappointing results.

Hypothesis and aims: We contend that a better understanding of the factors influencing biofilm formation and catheter encrustations will increase the efficacy of treatments & preventative strategies. As such, we aim to develop platforms to test materials under physiologically-relevant conditions in order to investigate how cell-environment interactions influence the development of ureolytic biofilms. We postulate that the phenotype of Proteus mirabilis biofilms is dependent on both the surface properties (charge & hydrophobicity) and the flow rate in the growth environment.

Methodology: Fluorescent strains of *P. mirabilis* were used to measure adhesion to glass/PDMS, and characterizing the surface hydrophobicity & zeta potential of glass & PDMS surfaces and *P. mirabilis* cells lead to an xDLVO model of cell-surface interactions. Microfluidic reactors were used to model a variety of physiologically relevant hydrodynamic conditions, enabling the generation of time-lapse-epifluorescence & confocal images of biofilm formation. The impact of encrustation on adhesion and biofilm development was studied using urease-negative mutants.

Results: The morphology of Proteus mirabilis biofilms under flow in artificial urine is markedly different when growing on glass/PDMS. Results indicate this is due to increased interactions of bacterial cells with PDMS, as evidenced by the greater rate of adhesion to this material. Urease activity does not appear to influence adhesion but significantly alters biofilm development.

Conclusion: Surface characteristics strongly influence *P. mirabilis* adhesion and biofilm formation. Alternative catheter materials will now be tested to further define the relationships between adhesion, biofilm formation and encrustation.
Introduction: Porphyrins are naturally occurring, red fluorescing intermediates of the heme synthesis pathway, essential to bacterial survival. Clinically, a handheld fluorescence imaging device is able to visualize moderate-to-heavy bacterial loads in wounds based on porphyrin fluorescence. Prior in vitro work has demonstrated this device’s detection of porphyrin fluorescence from the majority of common wound pathogens (planktonic growth) when a key substrate required for porphyrin production (aminolevulinic acid (ALA)) is present.

Hypothesis and aims: This study investigated the device’s capability to detect biofilm using two polymicrobial in vitro biofilm models, which is more representative of the chronic wound environment.

Methodology: Cultures of S. aureus, E. cloacae, and E. coli were inoculated into two established biofilm models: (1) Bolton’s broth with bovine plasma and a plastic tip to act as scaffold and (2) Bolton’s broth, bovine plasma, and red blood cells allowed to form a coagulated mass. ALA was added after seven days to induce porphyrin production. Fluorescence images (405 nm excitation) were acquired on day 8 pre and post wash steps to remove planktonic bacteria. Experiments included at least three replicates and an ALA-negative control. Monomicrobial biofilms (S. aureus) were also tested.

Results: Red fluorescence was readily detected from these biofilm models, both pre-wash and post-wash, indicating that fluorescence imaging can detect porphyrin-positive bacterial species in vitro encased within biofilm matrix in monomicrobial and polymicrobial communities.

Conclusion: These data demonstrate the ability of a fluorescence imaging device to detect porphyrin-positive species of bacteria growing as a biofilm with monomicrobial and polymicrobial communities. These data further validate the clinical capability and relevance of the device for use in wound care. Future work will extend these in vitro biofilm findings to an in vivo murine chronic wound model.
Introduction: Touch surfaces as room door handles are a likely reservoir for potential pathogens, which can play a role in the acquisition of healthcare associated infection. Microorganisms, such as methicillin-resistant Staphylococcus aureus (MRSA) or Enterococcus faecium from contaminated surfaces, may then be transmitted to hands of the next user and quickly spread across entire healthcare units. Moreover, an important part of healthcare touch surfaces are colonized by bacterial biofilms, increasing the persistence of potential pathogens and the risk for users.

Hypothesis and aims: To reduce the bacteria spreading, several healthcare facilities were equipped with door handles, door push plates and handrails made of copper alloys. While the antimicrobial activity of these components is known, a question was raised regarding the biofilm formation: is it possible to form a biofilm on copper alloys? The aim of this study was to evaluate the anti-biofilm properties of different copper alloys.

Methodology: Static MRSA biofilm cultures on these materials were established in different environmental conditions with a low organic soiling (peptone water) or with a high density of organic soiling (tryptic soy broth) on control surfaces and compared to copper alloys surfaces. Biofilm formation on surfaces was evaluated by classical culture methods and by scanning electron microscopy.

Results: Although these environmental conditions are extreme, our results demonstrated that the biofilm formation is directly dependent to soiling. The biofilm formation is reduced in presence of peptone water, whereas the biofilm formation is favoured in tryptic soy broth. Moreover, we observed that the biofilm formation is also dependent to the composition of the copper alloys.

Conclusion: These artificial contaminations and extreme environmental conditions highlight the anti-biofilm properties of the copper alloys tested. However, as cumulative soiling and cleaning procedures can alter the anti-biofilm efficiency of materials, this anti-biofilm effect will also be evaluated under tarnishing conditions.
178: Optimising phosphate treatment in UK drinking water systems to prevent plumbosolvency: evaluation of its impact on biofilm development - del Olmo G

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Introduction: Phosphate is added to drinking water to minimise lead dissolution from household pipes to protect public health. However, phosphate can favour microbial biofilm formation in Drinking Water Distribution Systems (DWDS). 98% of biomass in DWDS is present as biofilms attached to pipes. These biofilms are linked to problems of corrosion, discolouration and dispersal of pathogens. Besides, it has been reported that biofilms potentially act as a reservoir and source of lead release.

Hypothesis and aims: To optimise phosphate addition by water utilities, it is necessary to understand the impact on biofilm formation and on overall water quality and safety.

Methodology: Evaluation of biofilm-mediated phosphate consumption and lead dissolution dynamics in DWDS is explored with Bio-inLine biofilm reactors using lead coupons. The reactors are set up to study different doses of phosphate concentration in water (2mg/L and phosphate limiting concentrations) and compare with UK normal water phosphate concentrations. Phosphate rate consumption is measured, and lead dissolution monitored at regular intervals over 30 days using ICP-MS.

Results: Differences in the microbial characteristics of drinking water will be explored by quantitative PCR, using the rpoA gene as target. It is expected that the addition of phosphate will increase biofilms development and alter the biofilm’s structural stability, facilitating its mobilization. Presence of lead in bulk water is hypothesized to be higher in both experimental conditions, since phosphate limiting concentrations won’t prevent lead corrosion, and abundance of phosphate (2 mg/L) will increase biofilm growth and therefore the accelerated deterioration of metal. It is possible that the measurements of lead, phosphate and biomass suggest an accumulation of phosphate and lead in biofilms.

Conclusions: This study will provide information on the effect of phosphate on biofilm development and lead stability, which will facilitate to adjust an optimal phosphate dose to prevent plumbosolvency in DWDS.
Discovery of a polymer resistant to biofilm, swarming and biomineralization for the prevention of catheter-associated urinary tract infections - Dubern J-F

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Introduction: Indwelling urinary tract catheters are the most commonly employed prosthetic medical devices with some 15-25% of patients requiring bladder catheterization during hospitalization. However, they promote catheter-associated urinary tract infections (CAUTIs) which are responsible for some 75-80% of the ~150 million urinary tract infections occurring annually worldwide. The most common CAUTI-associated Gram-negative pathogens are Escherichia coli and Proteus but Pseudomonas aeruginosa, Enterobacter and Klebsiella species as well as Gram positives including Staphylococcus aureus are frequently found.

Hypothesis and aims: Catheter-associated urinary tract infections (CAUTIs) are the commonest healthcare associated infections worldwide. Consequently, there is an urgent requirement to discover novel materials that resist bacterial biofilm formation, biomineralization and surface migration on urinary catheters.

Methodology: A microarray screen consisting of 496 (meth) acrylate homo- and co-polymers was employed with Proteus mirabilis to discover novel copolymers capable of preventing Proteus biofilm formation, inhibiting swarmer cell differentiation and swarming motility and reducing biomineralization while also preventing biofilm development by other CAUTI-associated pathogens in both single and mixed species communities.

Results: From this screen we selected tert-butyl cyclohexyl acrylate (tBCHA) which exhibited <5% bacterial surface coverage after scale-up. However, poly(tBCHA) failed to inhibit Pr. mirabilis swarming. 2-hydroxy-3-phenoxypropyl acrylate (HPhOPA) was identified as an inhibitor of swarming that blocked differentiation of Pr. mirabilis into hyper-flagellated cells. A partial least square (PLS) regression model suggested that the interplay between molecular rigidity and hydrophilicity influences polymer swarming inhibition. Since poly(HPhOPA) did not prevent biofilm formation by other uropathogens, we synthesized tBCHA:HPhOPA copolymers.

Conclusion: A tBCHA:HPhOPA 2.4:1 copolymer retained multi-species biofilm and swarming inhibitory properties and prevented biomineralization making it an potential candidate material for coating urinary catheters to the prevent CAUTI.
**Introduction**: Biofilms are responsible for many human infections, including catheter-associated urinary tract infections (CAUTIs). The high tolerance of biofilms to physical and chemical removal necessitates research into novel agents and surfaces to inhibit their development.

**Hypothesis and aims**: The objective of this research was to evaluate the antimicrobial and antibiofilm potential of novel agents with potential for incorporation into biomaterials.

**Methodology**: Minimum inhibitory concentrations (MICs) of the novel antimicrobial compounds and triclosan were established for 7 bacterial species and Candida albicans using a broth microdilution approach. Minimum biocidal concentrations (MBCs) were determined by culture on agar following MIC assessment. Antibiofilm assessment involved exposing preformed biofilms in 96-well plates to the antimicrobials. Regrowth of biofilm post treatment was then determined by optical density. Silicone formulations with 1% (w/w) triclosan, triclosan acetate or a novel imidazolium compound incorporated into the bulk of the silicone material or as surface coatings were evaluated for antimicrobial efficacy against a panel of CAUTI causing microorganisms. Briefly, following inoculation of silicone coupons with CAUTI causing microorganisms and subsequent attachment and post wash periods, propidium iodide staining of adhered cells allowed quantification of dead microorganisms. Viable cells were recovered from the silicone coupons and colony forming units were determined by the Miles & Misra method (Miles, Misra and Irwin, 1938).

**Results**: Triclosan was inhibitory against 6 out of 8 microorganisms (MIC 0.39 – 3.125 g/ml), whilst 3 novel imidazolium compounds (3-hexadecyl-1-methyl-1H-imidazole-3-ium bromide, MIC 0.78 – 100 g/ml), 1-(2,3-dihydropropyl)-3-hexadecyl-1H-imidazole-3-ium bromide, MIC 1.58 – 3.125 g/ml) and 3-hexadecyl-1-isopropyl-1H-imidazole-3-ium bromide, MIC 1.58 – 100 g/ml) demonstrated inhibition of 5, 2 and 7 microorganisms respectively. 1% triclosan containing silicone demonstrated biocidal action against S. aureus, E. coli, P. mirabilis, K. pneumoniae, P. stuartii and C. albicans. Serratia marcescens and Pseudomonas aeruginosa were resistant to triclosan containing silicone.

**Conclusion**: Triclosan, triclosan acetate and the novel imidazolium compound 3-hexadecyl-1-isopropyl-1H-imidazole-3-ium bromide represent potential compounds for use as coatings on urinary catheters to prevent catheter associated urinary tract infections.
181: Multi-mode microscopy to elucidate early stages in biofilm formation - Farthing N.E

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Introduction: Every year 700,000 people die from drug resistant infections. With this figure predicted to rise to a staggering 10 million, costing 100 trillion USD by the year 2050, there is a pressing need to develop new ways of treating resistant infections and to limit the use of antibiotics to prevent further resistance developing. Many resistant infections are associated with biofilms and this has driven an interest in developing methods to prevent or limit biofilm growth without antibiotics. A key area of investigation is the treatment/coating of surfaces to either prevent the initial attachment stage of biofilm formation or discourage attached cells progressing into a full biofilm. Using high-throughput polymer arrays and monomer libraries, polymers that either promote or prevent biofilm formation, with no effect on bacterial growth, have been identified. Having discovered polymers that inhibit biofilm formation, the mechanisms involved are now investigated. Utilizing a novel multimode microscope, capable of employing the complementary imaging techniques of digital holographic microscopy (DHM), differential interference contrast (DIC) and total internal reflectance microscopy (TIRM), bacteria may be observed directly above the surface as well as in the bulk. This allows the influences of the polymers on bacterial behavior to be investigated.

Hypothesis and aims: The aims are to identify the key bacterial regulatory pathways and surface components responsible for the observed differences in polymer interaction.

Methodology: In this work, motility mutants of Pseudomonas aeruginosa were used to investigate the role of different aspects of the bacterial motility apparatus in how cells approach, and attach to either biofilm-promoting or biofilm-inhibiting polymer surfaces. 3D tracking data from holographic microscopy is interlaced with 2D surface data obtained from DIC and TIRM to observe differential surface and bulk behavior.

Conclusion: This information will inform the further development of surfaces that resist bacterial biofilm formation.
Characterization of microcosm biofilm regrowth on titanium surfaces after various decontamination treatments - Jiang Y

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Introduction: Peri-implantitis is a biofilm-related oral infection. Although implant surface decontamination is a key step in all treatment strategies, no strategy is clinically effective.

Hypothesis and aims: We proposed that the biofilm regrowth after decontamination might be a cause for treatment failure. Hence, we aimed to investigate viability and composition of the biofilms regrown after antimicrobial treatments in an in vitro model.

Methodology: Saliva-derived microcosm biofilms were grown on titanium discs in an active attachment model. Treatments including hydrogen peroxide (HP), citric acid (CA), chlorhexidine (CHX) and distilled water (control), at different concentrations, were applied to 2-day biofilms for 1 or 5 min. The viability, metabolic activity (lactic acid production) and the composition of the biofilms were followed for 3 days. The biofilm composition was analyzed by 16S rDNA amplicon sequencing.

Results: The short treatments of CA, CHX and HP resulted in a 2-3 log reduction in biofilm viability and a considerable reduction in lactic acid production. However, both parameters in the biofilms returned to pre-treatment level within 2 days due to the regrowth of the biofilms. The microbial diversity of the regrown-biofilms in antimicrobial treated groups tended to decrease 2 days after treatments. The composition of the regrown-biofilms altered compared to those before antimicrobial treatments. Streptococcus oralis/mitis and Klebsiella were enriched in the regrown biofilms.

Conclusion: Our findings confirmed that the multi-species biofilms were able to regrow within a short period of time even though the antimicrobial treatments were efficient. The altered microbial composition in the regrown-biofilm might increase clinical treatment difficulties.
183: Bioactive glass granules inhibit mature bacterial biofilms on the surfaces of cochlear implants - Kirchhoff L

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Introduction: The formation of bacterial biofilms on medical devices, such as cochlear implants, can lead to chronic infections resulting in the need for implant removal. This is associated with extended length of stay, increased morbidity and costs. In this study, bacterial biofilms formed on cochlear implant (CI) kits from different manufacturers were treated with bioactive glass for the evaluation of antibiofilm effects on pre-existing biofilm.

Hypothesis and aims: Bacterial pre-existing biofilms formed on the surface of s CIs can be reduced by the application of S53P4 bioactive glass.

Methodology: Biofilms of Pseudomonas aeruginosa (ATCC9027) and Staphylococcus aureus (ATCC6538) were formed for 24 hours at 36°C on the surfaces of CI implants, being from silicone, platinum and titanium composition. The preformed biofilms were treated with S53P4 bioactive glass and reduction in viable biofilm biomass after application was compared to the non-treated control. P. aeruginosa and S. aureus biofilm reduction after application of S53P4 bioactive glass was additionally evaluated in imaging processes using scanning electron microscopy (SEM).

Results: Application of S53P4 bioactive glass resulted in a significant reduction of P. aeruginosa and S. aureus mature biofilm on all CI surface. Additionally, a morphological change of biofilm was visible in SEM.

Conclusion: The results show that bioactive glass can reduce bacterial biofilm formation on CI materials in vitro. Future studies are necessary to confirm the results in vivo.
184: Scalable cell factories in membrane-based bioreactors - Leonov P

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Introduction: Whole-cell biocatalysis is increasingly used for the industrial production of chemicals due to its high catalytic efficiency, mild operational conditions, and comparatively low environmental impact. However, this technology has not yet been harnessed for the production of toxic or growth inhibiting chemicals, due to the lack of robust biocatalysts and efficient fermentation processes.

Hypothesis and aims: The aim of this project is to utilize the inherent characteristics of biofilms, such as self-immobilization and their robustness towards toxic environments, for opening up new possibilities for the manufacturing of biochemicals.

Methodology: As proof of concept, we want to optimize the production of rhamnolipids, which are non-toxic biosurfactants, by a genetically modified Pseudomonas putida strain grown in a biofilm. During this project, membrane-based bioreactor prototypes are being designed for overcoming obstacles related to biosurfactant production such as their strong foaming activity in liquid media. To increase the efficiency of the fermentation process, the experimental work will be combined with a model-based approach in search for optimizing the reactor design and process conditions to achieve a controlled biofilm growth and a high rhamnolipid yield. The model should also allow evaluating the effect of biofilm-relevant variables, such as biofilm thickness, on the system performance. Eventually, the most suitable set-up will be further investigated in scale-up studies.
Introduction: According to statistics, approximately 70% of orthopaedic implant infections are caused by the biofilm-forming bacterial species Staphylococcus. Many localized methods, including antibiotic loaded coatings, have been reported to address these infections but the risk of bacteria developing antibiotic resistance is of great concern. Nitric oxide (NO) is an attractive alternative as it exhibits broad spectrum antimicrobial activity without contributing to antimicrobial resistance. However as NO is a reactive gas, with a relatively short half-life, delivery of this antimicrobial is challenging.

Hypothesis and aims: The use NO donors such as diazeniumdiolates, allow the controlled, sustained release of NO at physiological pH. In this study, we have synthesized NO releasing coatings on Ti with varying antimicrobial payloads. We have developed a mechanistic understanding of NO donor formation, NO release kinetics and determined biofilm inhibition on NO releasing surfaces.

Methodology: XPS, AFM and contact angle measurements were used to characterize the functionalized surfaces and confirmed the formation of diazeniumdiolates. Biofilm CFU assays were performed to determine the inhibition of biofilm formation on NO releasing surfaces after 6h and 24h incubation. In addition, SEM was used to determine S. aureus morphology after 6h incubation on NO releasing surfaces.

Results: Results confirmed the formation of diazeniumdiolates on Ti surfaces prepared using different silane precursors which produced varying NO payloads. The kinetics of NO release were dependent on pH, with acidic conditions (pH 4) resulting in the release of higher NO concentrations than pH 7.4 and pH 8.5. The surface releasing the highest concentration of NO showed more than 1 log reduction in S.aureus biofilm formation.

Conclusion: Diazeniumdiolates were successfully tethered using four selected silane precursors to produce NO releasing surfaces with varying NO payloads. These surfaces have the potential to be used as antimicrobial surfaces for orthopaedic applications.
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Introduction: Bacteria in chronic infections typically consist of relatively small (~20-200 µm wide) cell aggregates embedded in host material and surrounded by polymorphonuclear leukocytes that impose strong O2 depletion and slow growth of the pathogenic bacteria. However, most in vitro studies of biofilms involve use of microtiter plate assays or other surface associated assays, which do not reproduce the mentioned in vivo growth patterns. We recently introduced growth of pathogenic bacteria (Pseudomonas aeruginosa) in alginate beads as a simple model system for studying embedded biofilm aggregates. The model shows in vivo-like growth of pathogenic bacteria, and enables application of a wide range of experimental methods at high reproducibility and replication.

We now have a method for accurately controlling the size distribution of aggregates within the alginate beads by modulating the inoculation density which alters the resource availability during biofilm formation thereby shaping the size and spatial distribution of aggregates.

By implementing these findings in mathematical models, we have developed a framework for addressing questions about the growth patterns observed in chronic infections regarding growth limitation of aggregates, susceptibility toward antibiotics etc.

Conclusion: We present the model system and the newest findings showing various metabolic activity profiles of aggregates grown with different access to electron acceptors and how the size and spatial distribution of embedded aggregates change the susceptibility toward antibiotics.
187: Characterization of microbial diversity in Czech mineral springs - Maťátková O

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Introduction: Deep mineral springs were historically utilized as a source for spa water all round the world, however, in the present day, their potential is explored also as a source of unique microbial communities. In the Czech Republic, the diversity of such environments ranges from radon waters to thermal, highly mineral springs. Such an extreme environment has presented a unique niche for evolution of indigenous microbial communities, often bound to mineral materials in the form of biofilm. Microorganisms in these communities have adapted to the specific conditions and present a unique research opportunity with regards to their composition and lifestyle.

Hypothesis and aims: The aim of our work was to examine biofilm microbial communities found in Czech mineral springs, as well as to find cultivation approaches that would allow to determine their biotechnological potential.

Methodology: Radon springs from Jáchymov and thermal springs from Karlovy Vary were chosen as study subjects. Total metagenomic DNA was isolated and 16S rRNA genes were amplified and sequenced using Illumina MiSeq platform to characterize the microbial community structure. Modified cultivation strategies to promote the growth of oligotrophic microorganisms were employed to select culturable microorganisms, which were further characterized.

Results: The analysis of Karlovy Vary spa springs show that phylogenetic structure of all communities differs substantially. While the colder springs were dominated by Proteobacteria, in the warmer spring the majority of reads were not classified at the genus or higher taxonomic level. From the Jáchymov radon springs, four bacterial isolates were identified as Kocuria, which were remarkable with respect to their metabolic profile, specifically for their potential for the production of polyunsaturated fatty acids.

Conclusion: Deciphering the biodiversity, physiological ecology and potential of microbial biofilm communities from extreme environments is important for furthering our understanding of these unique ecosystems and consequently for exploiting their biotechnological potential.
Introduction: A critical issue in medical environments is the dissemination of bacterial colonies across biotic and abiotic surfaces, presenting major problems to human health. Currently, novel antimicrobial surfaces are considered expensive and difficult to produce. Sulfur, an abundant by-product of the petroleum industry, has previously been shown to have antimicrobial properties, however this has only been demonstrated in spin-coated polymers rather than bulk material.

Hypothesis and aims: The study aimed to evaluate the antimicrobial properties of bulk sulfur polymers bonded by two crosslinking materials (A&B), using Escherichia coli (DSM1576) and Staphylococcus aureus (DSM346). We will assess bacterial survival after 24h exposure to these sulfur polymers in both the planktonic and sessile states.

Methodology: Using fluorescence microscopy (Live/Dead staining) and an international standard (ISO 22196:2011) [5], we will assess antimicrobial activity of bulk sulfur polymers (A&B). Colony counting will determine if the materials are stable and initiate leaching of sulfur into the environment.

Results: Microscopy results of the two cross-linked sulfur polymers showed that both polymers exhibited a significant decrease in live cells after 24h incubation (E.coli). After undergoing rigorous antimicrobial testing using the ISO22196:2011, polymers A&B both exhibited a decrease in bacterial viability after 24 hours, with polymer A reducing viability to below 0.1%. For S.aureus, only polymer A reduced bacterial cell viability (<0.1%), however no significant changes were observed for polymer B or in the planktonic state.

Conclusion: Sulfur polymer (A) showed a substantial decrease in live cells, presenting a greater than 99% reduction in bacterial survival. Polymer (B) only showed a reduction in the presence of E.coli, but no effectiveness against S.aureus. Surface leaching data indicated negligible release from the material that would influence bacterial survival, suggesting the antimicrobial capacity is at a surface interaction level, highlighting the role that sulfur can play in the fabrication of antimicrobial surfaces.
Using *in vitro* models of the farm environment to assess the biofilm-forming abilities of pig and poultry production associated *Salmonella enterica* serovars - Oastler C

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Introduction: *Salmonella*, which was the second most commonly reported zoonosis in the European Union in 2017 (EFSA, 2018), has been found to survive in the environment of pig and poultry production premises. Ultimately, this poses a risk to humans through the consumption of infected pig and poultry products through the food chain. One of the contributing factors for *Salmonella* survival in the environment is biofilm-formation. Biofilms act as a protective mechanism against environmental stresses, and so may facilitate the persistence of *Salmonella* in the environment. The most widely used method for assessing biofilm-formation is the crystal violet microtiter plate assay. However, this method does not accurately replicate conditions under which biofilms form in real-life farm environments.

Hypothesis and aims: Biofilms are hypothesised to develop differently on a variety of surfaces. Work aimed to assess *Salmonella* biofilm-formation under realistic conditions using farm environment surfaces.

Methodology: The biofilm-forming ability of *Salmonella* isolates recovered from commercial pig and poultry farm environments were evaluated by aerobic incubation for up to 72 hours, at realistic farm temperatures of 20 or 25°C, using a crystal violet microtiter plate assay. A proportion of non, weak, moderate and strong biofilm-forming isolates were selected for further testing. Biofilms grown on PVC, stainless steel, wood and concrete coupons, by aerobic incubation, at 25°C, were evaluated by staining using crystal violet and metabolic or fluorescence dyes. SEM microscopy allowed for further visualisation of the biofilms on the surfaces.

Results: Varying biofilm-forming abilities were seen by the isolates across the different surface types; with the correlation between biofilm formation on the surface coupons and the conventional crystal violet plate method also investigated.

Conclusion: Assessing biofilm-formation using realistic *in vitro* methods enables greater understanding of biofilm-formation on surfaces, and the behaviour of *Salmonella* biofilms in the farm environment. This information can be used to inform biofilm prevention and control.
190: Structure and metabolism of engineered enhanced current-producing biofilms - Otero F.J


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Introduction: Current-producing biofilms produce power, serve as inexhaustive oxidant to redox-dependent industrial reactions, and guide selective ion flow across membranes. Such biofilms are formed by organisms able to respire extracellular terminal electron acceptors up to tens of cell lengths away, but anodes are artificial substrates for this biological system and present current densities are insufficient for most industrial applications.

Hypothesis and aims: Recently, the model organism Geobacter sulfurreducens was engineered to produce 140% of wild type current. Here, we present a single-cell to biofilm wide analysis of the characteristics that enable this strain to generate higher current densities.

Methodology and Results: Using microscopy engineered extABCD+ biofilms were found to be 40% denser than wild type at the biofilm:electrode interface but of similar total thickness. Single-cell metabolic activity tracked through heavy isotope incorporation shows metabolic activity is highest within 5 μm of the anode surface and decreases linearly with distance in all biofilms, however, extABCD+ cells within the first few microns from the anode are also ~30% more active compared to wild type. Using surface plasmon resonance we show that all electron carriers within 100s of nm from the anode in extABCD+ biofilms are able to interact with the anode compared to only a third of those in wild type biofilms. Finally, charge transport parameters were found to differ between extABCD+ and wild type biofilms through electrochemical measurements of biofilms grown on interdigitated array electrodes.

Conclusion: While it is yet unclear if the metabolic activity of current producing biofilms can be extended beyond 10 μm from the anode surface, our results show that engineering key steps of the G. sulfurreducens extracellular electron transfer pathway resulted in denser biofilms with higher per-cell respiration rates able to interact more efficiently with the anode surface.
Micro-scale topographies instruct bacterial attachment to surfaces - Romero M

Romero M1, Carlier A2, Carabelli A3, Vermeulen S2,4, Cámar M4, de Boer J2,4,5, Alexander M.R3 and Williams P1

Introduction: Studies using topographical features fabricated with precise dimensions in the micro and nanoscale have shown some degree of success in reducing adhesion and bacterial biofilm formation. Unfortunately, the interplay between bacterial cells and topographical landscapes is still poorly understood. Moreover, most studies focused on a limited number of topographical designs, an approach that has restricted exploring model-based methods to draw correlations and predict bacterial responses on the basis of surface properties.

Hypothesis and aims: We sought to increase our understanding of the interplay and response of bacterial cells to surfaces to allow a more rational biomaterials design.

Methodology: A high throughput process assessing bacterial adhesion on 2,176 distinct combinatorial generated micro-topographies and real-time imaging were used to gain insights into the interface between micro-topographical landscapes and bacteria.

Results: Results revealed that the surface parameter “fraction covered by primitives” (FCP) negatively influences bacterial adhesion in a reproducible mode. This predictor provides information on the size and density of the micro-pillars. FCP impact is modulated by the intricacy of the features as indicated by the relevance of the Fourier transformation of the patterns. Moreover monitoring of the spatio-temporal surface colonisation provided insights into the resistance mechanism of lead topographies, which can have wide application where bacterial biofouling is problematic such as biomedical device centered infection.

Conclusion: The high number of micro-topographies assessed and the remarkably strong correlation found between local landscape and bacterial attachment, allowed a detailed analysis on the relevance of surface parameters on adhesion and exploring an innovative approach for antifouling surface engineering, which predicts attachment based on surface design criteria rather than single traits such as surface energy or water contact angle. This illustrates the strength of unbiased screenings to reveal previously unperceived cell–surface interactions and provide insights towards the rational fabrication of new bioactive surfaces.
Introduction: UK water companies use the addition of phosphate to the drinking water supply as a treatment to prevent corrosion, metal leaching and plumbosolvency in pipes. However, contradictory results have been reported regarding the effect of phosphate dosing on the microbial ecology of Drinking Water Distribution Systems (DWDS) and particularly on biofilms attached to pipes.

Hypothesis and aims: This study will investigate the effects of phosphate addition on biofilm formation and its consequences for drinking water quality using a multi-looped experimental pipeline facility representative of live DWDS.

Methodology: Biofilms will be developed over one month with a phosphate dosing (2 mg/L) and compared with biofilms grown under average UK normal water phosphate concentrations. After the growth phase, the system will be flushed to assess the risk of mobilisation using gradual increases in flow. During the experiment, physico-chemical analysis of water and microbial analysis of both water and biofilms will be carried out. Sequencing analysis of the 16s rRNA gene, from extracted DNA obtained from biofilms and water samples, will provide information on any bacterial changes.

Results: It is expected that additional phosphate will increase biofilm growth, if limited in the system, and that will shape the structure of microbial communities, particularly of those inhabiting biofilms. Changes might be found in the bacterial community structure in phosphate-added water samples even if bacterial growth is not reported to be different. Furthermore, biomass and phosphate measurements may reveal a phosphate accumulation in the biofilms. Phosphate might cause changes in biofilm physical stability, promoting a higher risk of mobilisation into the bulk water of bacteria and therefore affecting water quality.

Conclusion: The study will provide new insights and information to understand the progression of biofilm formation in phosphate-added water and to optimise phosphate dosing by water utilities to prevent plumbosolvency and subsequent downstream removal to protect the environment.
Propidium iodide staining underestimates viability of adherent bacterial cells - Rosenberg M

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Introduction: Intact membrane impermeable DNA-binding stain propidium iodide (PI) is widely used for bacterial viability staining in combination with membrane-permeable counterstains. Extracellular nucleic acids (eNAs) are abundantly present in biofilm matrixes and carry potential to interfere with viability staining results.

Hypothesis and aims: Our objective was to quantitatively assess possible eNA interference with PI-based viability staining. This possible caveat in in situ biofilm diagnostics was investigated in the framework of COST Action CA15114 (AMICI).

Methodology: Monolayer aggregates of 24h Staphylococcus epidermidis or Escherichia coli biofilms on glass in phosphate buffered saline were stained with PI and Syto 9 in situ or harvested, stained and visualized with epifluorescence (EM) or confocal laser microscopy (CLSM). Fluorescein diacetate staining and plate counts were used as controls and Congo Red (CR) to stain surface-associated amyloid fibers (SAFs).

Results: In situ stained biofilms presented 75.69±18.44% (S. epidermidis) to 96.35±5.3% (E. coli) PI-positive red cells, even though 68% of the bacteria of bot species were metabolically active. Higher biofilm viability estimates with 19.56±8.93% (S. epidermidis) to 43.50±5.30% (E. coli) PI-positive cells were achieved after harvesting adherent cells possibly due to partial removal of extracellular matrix during ultrasonication. 82% of harvested E. coli and 89% of S. epidermidis cells were cultivable. CLSM revealed that this false dead layer of red cells consisted of green cells with diffuse red PI corona confirming extracellular PI signal. Amyloid-binding Congo red and Syto 9 co-staining demonstrates similar staining pattern to PI and Syto 9 indicating that SAF-bound eDNA may be involved in this extracellular PI-signal.

Conclusion: In this study we show that PI-based viability staining can significantly overestimate dead cell counts in biofilms. We conclude that eNAs and their possible impact on PI-based viability staining outcome must be considered and controlled for to avoid significant overestimation of dead cells in biofilms.
194: Can anammox surface (S-) layer protein nucleate biofilm formation? - Seviour T

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Introduction: The tendency of anaerobic ammonium oxidizing (anammox) bacteria to form biofilms is exploited to achieve high nitrogen removal rates from wastewaters. However, the identities and functions of the extracellular polymers supporting anammox biofilm formation are unknown. Surface (S-) layer proteins have been observed on the surface of Ca. Kuenenia stuttgartsiensis cells and in the extracellular matrix of Ca. Brocadia caroliensis-enriched biofilms. S-layer proteins are thought to promote cell-cell adhesion and biofilm formation, although how has not been described. Anammox bacterial S-layer proteins are intrinsically disordered at their C-terminal (unpublished), suggestive of a role as molecular adhesives.

Hypothesis and aims: We hypothesize that the presence of an intrinsically disordered domain (IDD) enables S-layer anammox proteins to adhere cells. We aim to describe the ability of anammox S-layer proteins to promote cell adhesion and determine whether it is a consequence of their ability to undergo phase transition (i.e. solution, liquid phase condensate, crystalline).

Methodology: We isolated S-layer protein Brosi_A1236 from Candidatus Brocadia sinica-enriched granules and described their crystallization by electron microscopy, liquid phase condensation by phase contrast microscopy, adhesive properties in terms of microsphere attachment, and ability to coalesce cells using non-motile Pseudomonas aeruginosa (PAO1). These assays were repeated on the IDD, which was expressed recombinantly using E. coli.

Results: When incubated with 20 mM CaCl2 (HEPES, pH 7.4), isolated putative S-layer protein Brosi_A1236 formed aggregates with an ordered structure. Further high-resolution electron microscopy will determine whether the isolated protein displays the lattice pattern characteristic of S-layer proteins. In the absence of CaCl2 and in the presence of polyethylene glycol (PEG), the IDD of Brosi_A1236 undergoes liquid phase condensation, as indicated by increased turbidity, and the appearance of ≈2 µm spherical droplets including those that fuse. This was additionally observed for full-length Brosi_A1236, which stained positive to protein-specific Alexa Fluor 647 dye further supporting our assertion that the droplets are the result of protein phase separation. In the presence of CaCl2, relative to the controls, the IDD of Brosi_A1236 promoted the aggregation of red and green cell-like microspheres to form yellow clusters indicating that the protein is acting as an adhesive. Liquid phase condensates of Brosi_A1236 formed in the absence of CaCl2 promoted the coalescence of GFP-tagged P. aeruginosa cells.

Conclusion: We provide evidence that Brosi_A1236 can exist as either crystals or liquid phase condensates. Calcium promotes S-layer crystallization and droplets promote the coalescence of cells. Ongoing work will aim to determine whether liquid phase condensation of Brosi_A1236 is a requirement for them to adhere, as observed to date, which would indicate that coalescence precedes crystallization. Elucidating factors contributing to phase transitions and cell coalescence may thus inform the sequence of and mechanisms underpinning anammox biofilm nucleation, which would enable rapid start-up of full-scale anammox bioprocesses.
Bacterial adhesion and early biofilm formation on soft surfaces: towards a better understanding of bacteria mechanosensing abilities and physico-chemical interactions at play - Straub H

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Introduction: Bacterial adhesion to surfaces is the first step toward biofilm formation. Substrate mechanical stiffness has been recently shown to influence bacterial adhesion behavior. However, the mechanisms used by bacteria to respond to surface stiffness are still elusive.

Hypothesis and aims: We aim at understanding the roles played by physicochemical interactions between substrates of different stiffness and bacteria as well as shedding light on the active mechanosensing abilities of bacteria.

Methodology: Bacterial adhesion on polydimethylsiloxane (PDMS) samples, having four different degrees of stiffness with Young’s modulus ranging from 0.06 to 4.52 MPa, is investigated. Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus are employed as model organisms and their adhesion on PDMS is quantified by optical microscopy under static condition. To determine whether the observed adhesion behavior is caused by bacteria-specific mechanisms, abiotic carboxylate-modified and amine modified PS polystyrene (PS-COOH and PS-NH2, respectively) beads are employed as bacteria substitutes.

Results: E. coli and P. aeruginosa are found to adhere in greater numbers on soft PDMS (7- and 27-fold increase, respectively) than on stiff PDMS, whereas S. aureus adheres in similar numbers on the four tested surfaces. PS-COOH beads exhibit the same adhesion pattern as E. coli and P. aeruginosa with four times more adhered beads on soft PDMS than on stiff PDMS. In contrast, PS-NH2 beads adhere in similar numbers on all tested samples, reminiscent of S. aureus adhesion.

Conclusion: This work demonstrates for the first time that the intrinsic physicochemical properties associated with PDMS substrates of different stiffness strongly influence bacterial adhesion and complements the previously reported theory on active bacterial mechanosensing, which provides new insights into the design of antifouling surfaces.
**196: Bacterial film formation at oil-water interfaces - Subbiahdoss G**

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**Introduction:** Biofilms are predominantly investigated at solid interfaces. However, microorganisms also adhere to liquid-liquid interfaces, which triggers cell aggregation and subsequent biofilm formation. Such biofilms strongly impact ecology, human health and industries that include pharmaceuticals, food and oil recovery.

**Hypothesis and aims:** The mechanics of biofilm formation at liquid-liquid interface related to bacterial physical properties is not well investigated. Our aim is to study the mechanism of biofilm formation of *P. aeruginosa* ATCC 15692 (PAO1), *S. aureus* ATCC 12598, and *S. epidermidis* ATCC 12228 at the decane-water interface and its effects on interfacial properties.

**Methodology:** Adhesion capacity of bacteria to decane-water interface was characterized using bacterial adhesion to hydrocarbons (BATH). The structure of the biofilm was investigated using scanning electron microscopy. Pendant drop was used to study the evolution of the mechanical properties of the decane-water interface as the biofilm formed.

**Results:** The BATH test revealed that PAO1 is hydrophobic compared to other strains. PAO1 formed biofilms, remained intact at the interface up to 10 days. The structure of PAO1 biofilm revealed an interconnected network of bacteria, secreted polymeric matrix and depressions formed where bacteria grew into and degraded decane droplets. When a decane drop aged in a bacterial suspension for 48 h, PAO1 adhered to the interface forming bacterial film and the interfacial tension decreases. As the drop volume decreased, film wrinkles indicating its elastic property.

**Conclusion:** We demonstrate that the hydrophobicity of PAO1 translates into stable, elastic film formation at the interface and promotes degradation of oil droplets.
Construction of the analysis method for monitoring the physiological properties of individual cells in biofilm - Takabe K

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Introduction: Phenotypic heterogeneity within a biofilm population has been often quoted as a mechanism that confers resilience to stress factors such as antibiotics. Fluorescent protein reporter constructs have been widely used to monitor development of such phenotypic heterogeneity. Here, we propose a novel tag-free approach to probe the spatiotemporal dynamics of the phenotypic heterogeneity within live biofilm at the resolution of single-cell level.

Hypothesis and aims: A cell’s innate cellular fluorescence signature is known to reflect various cellular properties and physiological statuses. Studies have demonstrated that the analysis of fluorescence signatures allows tag-free analysis of physiological statuses within live and intact microbial colonies. However, the analysis at single-cell level has remained rare. We have recently developed a minimally-invasive method, which we call confocal reflection microscopy-assisted single-cell innate fluorescence analysis (CRIF). In this study, we developed this technique to evaluate the spatiotemporal changes in heterogeneity of the innate fluorescence signature from each individual live cell within biofilm.

Methodology: We combined the CRIF and advanced image processing that allowed us to detect individual cells even within a highly noisy and complex biofilm structure, to probe into the heterogeneity of innate fluorescence signature within a Pseudomonas aeruginosa PAO1 biofilm population.

Results: We performed PCA and t-SNE analyses with 500+ single-cell innate fluorescence signatures at various stages of biofilm development. We found that, at any stage of its development, biofilm harbored rich diversity in fluorescence signature, which is reflected in the distinct cluster formation on the PCA and t-SNE. Further, we found multiple cases where cells with specific innate fluorescent signature at the specific depth in a biofilm structure.

Conclusion: Taken together, above results and the fact that the CRIF does not require any tagging point that single-cell innate fluorescence signature is a promising tool to simplify the analysis of phenotypic heterogeneity within biofilms.
Introduction: Nowadays, biofouling on immerged surfaces is an important issue causing economic and ecologic concerns. This phenomenon is caused by diverse organisms, beginning with bacteria and diatoms. These last ones are still less studied than bacteria and needs more information to understand the different mechanisms allowing their colonization.

Hypothesis and aims: In this way, adhesion and biofilm formation were studied for different surface properties. It is supposed that diatoms react differently and produce different adhesive molecules regarding the coating they tend to colonize.

Methodology: To answer these questions, adhesive molecules from diatoms were extracted and identified by their amino acid and monosaccharide compositions. Comparison were made between organisms and surfaces. Moreover, dynamic attachment was studied under flow cell system in artificial sea water to determine whether hydrophilic or hydrophobic area were more suitable for microalgae adhesion. Finally, biofilm formation was studied under these conditions.

Results: Final results showed a high diversity of biomolecules compositions implicated in diatom adhesion. Moreover, some molecules seem more suitable for the adhesion on hydrophobic surfaces once amino acids compositions are different. Concerning the dynamic adhesion, diatoms display more affinity to hydrophobic interfaces than hydrophilic ones. These results can be reliable with adhesives compositions studied above.

Conclusion: To conclude, a technique for the study of the adhesion behavior of diatoms has been studied and can be linked with further analysis concerning adhesives molecules. This technique allows the biofilm formation and its quantification after the adhesion step. Then, use of fouling release coating as surfaces could be done to test their capacity inhibiting biofouling.
Introduction: A synthetic furanone, F202, has earlier been shown to inhibit biofilm production by several bacterial species, including Salmonella. The mechanisms behind this action in Salmonella have not been known. However, we have previously shown that Salmonella serovars Typhimurium and Agona respond differently to F202 regarding biofilm formation.

Hypothesis and aims: The aim of the present study was to investigate and compare gene responses to furanone F202 in these two serovars to elucidate mechanisms involved in biofilm inhibition by this furanone in Salmonella.

Methodology: One Salmonella ser. Typhimurium strain (ATCC 14028) and one Salmonella ser. Agona strain (from a Norwegian feed factory) were used in the present study. Total RNA was isolated from cultures after incubation in LB broth wo/NaCl with or without 50 µM F202 at 20 °C (±1 °C) until an optical density at 600 nm of 0.35 (± 0.05). Microarray slides including 4679 Salmonella genes were prepared and used as described by Porwollik et al. (2001), and validated by RT qPCR on selected target genes. Isogenic mutants with depletions in the flagellar gene fliA, the quorum sensing genes lsrA and sdiA, and the sigma factor gene rpoS, were constructed and compared with their respective wild type strains regarding growth and biofilm production.

Results: Gene expression of 1145 genes in Salmonella ser. Agona 2168-10 and 592 genes in Salmonella ser. Typhimurium 14028 were significantly affected under furanone exposure (p<0.05). The results clearly showed that biofilm inhibition was associated with reduced stringent response and reduced transition into the stationary phase. All mutants, except ΔfliA, displayed the same growth rate as their wildtype counterparts. All mutants except ΔsdiA responded to F202 in the same way as their respective wildtype strains.

Conclusion: Results indicate that F202 affects the bacteria’s ability to properly sense and/or react to the changes in the environment.
Assessing the role of pharyngeal cell surface glycans in Group A *Streptococcus* biofilm formation - Vyas H.K.N

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**Introduction:** Group A *Streptococcus* (GAS), causes 700 million infections, and accounts for half a million deaths per year. GAS pharyngitis costs the U.S economy $224 to $539 million per year. Antibiotic treatment failure rate of 20-40% has been observed. This may be due to the capacity of GAS to form biofilm. The role of biofilm formation in GAS pathogenesis during pharyngeal colonisation and antibiotic treatment failure has not been fully elucidated. Moreover, it has long been known that glycans present on eukaryotic cell surfaces are important for bacterial attachment and adherence, however their role in GAS biofilm formation is yet to be investigated.

**Aims:** The aim of this work is to assess how pharyngeal cell surface glycans impact biofilm formation by pharyngitis associated GAS emm-types.

**Methodology:** GAS biofilms were formed for 72 h on Detroit 562 pharyngeal cell monolayers, with or without pre-treatment with exoglycosidases α1-6 Mannosidase, α1-2, 3 Mannosidase, or Sialidase A. Resultant GAS biofilms were assessed for: i) biofilm biomass via crystal violet staining, ii) biofilm cell viability, and iii) initial planktonic GAS adherence. Future experiments will assess GAS biofilm EPS production via EPS staining, and biofilm EPS/architecture via scanning electron microscopy. Single-bacterial cell atomic force microscopy will be utilized to characterize the initial interactions between the Detroit 562 cell surface glycan structures, and planktonic GAS.

**Results:** No significant difference was seen in adherence of planktonic GAS to exoglycosidase pre-treated and untreated monolayers. Similarly, biofilm cell viability remained unchanged in biofilm formed on exoglycosidase pre-treated and untreated monolayers. However, removal of Detroit 562 pharyngeal cell surface glycans resulted in an increase in GAS biofilm biomass on cell monolayers. Biofilm biomass increase was both glycan, and strain dependent.

**Conclusion:** These data suggest that cell surface glycans may offer a protective advantage against GAS biofilm formation. Differences in biofilm formation following glycan removal cannot be attributed to differences in initial bacterial adherence or changes to GAS viability within the biofilm. Future assessment of EPS via staining and scanning electron microscopy may provide a greater understanding of the observed phenotype.
Introduction: In the food processing environment biofilms are thought to be an important source of contamination of food by spoilage and pathogenic bacteria, leading to economic losses and potential health threats of consumers.

Hypothesis and aims: The aim of this study was to identify biofilm hotspots in the food processing environment investigating food contacting (FCS) and non-food contacting sites (NFCS), which could potentially lead to contamination of the food product.

Methodology: In an Austrian meat processing facility 108 samples (47 FCS, 61 NFCS) were taken using a scraper-flocked swab method. The presence of microorganisms was confirmed by microbiological and DNA-based methods. Furthermore the presence of matrix components was determined using the phenol-sulfuric acid method for carbohydrates, SDS-PAGE for proteins and NanoDrop measurements for extracellular DNA (eDNA). Additionally bacteria were isolated, identified and their biofilm forming abilities were determined.

Results: In four samples (three from NFCS and one from FCS) we detected bacteria and all three matrix compounds (carbohydrates, proteins and eDNA), resulting in 3.7% biofilm-positive sites. Additionally, at five sites the presence of two of three components was confirmed; either eDNA or proteins were not detectable (4.6%). The identified biofilm hotspots were drains, water hoses and a screw conveyer. Brochothrix thermosphacta and Pseudomonas spp. were among the most prevalent isolated bacteria.

Conclusion: Only a few biofilm hotspots could be identified. In rooms where clean operation is essential for product safety (slicer room, packaging area) no biofilms could be detected. Water hoses as biofilm hotspots represent an under-investigated potential contamination site. Further investigation on water hoses in the food processing environment will be conducted. In the next step the microbiome of the identified hotspots will be analyzed.
**202: Covalent lectin inhibition and application in bacterial biofilm imaging - Wagner S**

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**Introduction:** Biofilm formation by pathogenic bacteria is a hallmark of chronic infections. In many cases, lectins play key roles in establishing biofilms. The pathogen Pseudomonas aeruginosa often exhibiting various drug resistances employs its lectins LecA and LecB as virulence factors and biofilm building blocks.

**Hypothesis and aims:** Inhibition of the function of these proteins is thought to have potential in developing "pathoblockers" preventing biofilm formation and virulence. Therefore, we designed the two diastereoisomeric galactose-derived epoxides 2 and 3 as potential covalent active site inhibitors of LecA.

**Methodology:** We have developed the first covalent inhibitor of carbohydrate binding sites by rational structure-based design. Both diastereomers of the epoxygalactoheptoside 2 and 3 were synthesized and biologically evaluated using a competitive binding assay, an MS-based sequencing using MALDI in source decay (ISD), crystallization of LecA in complex with 3 and staining of *P. aeruginosa* in vitro biofilms.

**Results:** LecA displayed a strong diastereoselectivity for the 6d epimer 3 over its 6l isomer 2. The binding site and its covalent nature at physiological pH was established using mass spectrometry-based sequencing and the non-covalent crystal structure of 3 in complex with LecA was solved at pH 4.6. Finally, we used the fluoresceine-derivative 17 for the LecA-specific staining of *P. aeruginosa* biofilms.

**Conclusion:** A covalent lectin inhibitor specific to a carbohydrate binding site is described for the first time. Its application in the LecA-specific *in vitro* imaging of biofilms formed by *P. aeruginosa* is also reported. Such conjugates may lead to the development of pathogen-specific imaging agents to localize bacterial biofilm-associated infections inside an infected host enabling pathogen- and tissue-directed therapy.
203: Validation of the EPA approved single tube method using a wide range of biocides - Westgate S

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Introduction: The single tube method was recently approved by the Environmental Protection Authority and is used to quantitatively assess biocides against bacterial biofilms. The method can be used to register biocides for biofilm removal claims, demonstrating a product ability to remove biofilm from hard, non-porous surfaces. Validation of the methodology is important to ensure reproducibility between laboratories and geographies.

Hypothesis and aims: The aim of the study was to validate the single tube method against a range of biocides.

Methodology: Staphylococcus aureus and Pseudomonas aeruginosa biofilms were grown in a CDC reactor for 48 hours. Following incubation, reactor coupons were rinsed with Phosphate Buffer Saline to remove planktonic organisms. The pre-formed biofilms were immersed in each biocide for 10 minutes. Following exposure, treatments were neutralised and remaining microorganisms were recovered using vortexing and sonication. Remaining total viable organisms were quantified. All assays were sampled with three biological replicates and two technical replicates to produce statistically testable data.

Results: An average of 8.75 ± 0.12 Log_{10} CFU mL^{-1} viable P. aeruginosa were recovered from untreated controls. An average of 5.44 ± 0.35, 5.67 ± 0.25, 7.93 ± 0.23, 8.64 ± 0.15, 8.48 ± 0.11 and 4.70 ± 0.16 Log_{10} CFU mL^{-1} viable P. aeruginosa were recovered from Biocide A, B, C, D, E and F treated coupons, respectively. An average of 7.62 ± 0.14 Log_{10} CFU mL^{-1} viable S. aureus were recovered from untreated controls. An average of 4.00 ± 2.98, 3.30 ± 0.54, 3.56 ± 0.50, 7.39 ± 0.24, 6.80 ± 0.14 and 3.06 ± 0.41 Log_{10} CFU mL^{-1} viable S. aureus were recovered from Biocide A, B, C, D, E and F treated coupons respectively.

Conclusion: The methodology differentiated between biocidal products following 10-minutes treatment. EPA-approved and validated biofilm methods ensure testing has been carried out to a high standard and is highly repeatable between tests, between scientists and between laboratories.
Introduction: Biofilm reactors are of major importance in a world with limited resources. Whereas they are applied in certain areas already, there is still a necessity for extending the fields of application and improving the biofilm formation process itself.

Hypothesis and aims: We aim at understanding the influence of shear forces on the early stages of biofilm formation by addressing different magnitudes of shear forces. First, we determine the minimal detachment forces of single bacteria due to lateral shear forces in the nN range. Second, we quantify the vertical adhesion forces under flow conditions. Further, we apply different microstructures on the substrate to create sheltered areas for the bacteria with the aim of improving the growth conditions for the biofilm.

Methodology: We apply a Quartz Crystal Microbalance to determine the influence of microstructures on the amount of attached and subsequently detached bacteria. Thereby, the microstructures are manufactured using direct laser writing and consist of titanium-coated polymer. For imaging the microstructured titanium-coated quartzes and the associated bacteria, we use Scanning Electron Microscopy. Further, we implement single bacteria studies by using Lateral Force Microscopy and Scanning Force Spectroscopy.

Results: Microstructures on an oscillating quartz promote the attachment of Staphylococcus sciuri. Further, they hinder the detachment and lead to a preferential alignment of the bacteria behind the hemispherical structures. By applying Lateral Force Microscopy on different materials, we found a correlation between the applied force in the nN range and the number of moved Paracoccus seriniphilus bacteria as well as between the number of detached bacteria and the surface energy of the substrate. Further, any structuring of the substrate as well as acidic conditions hinder the detachment.

Conclusion: Microstructures on a titanium surface promote the initial attachment of bacteria and at the same time hinder their detachment. The minimum detachment forces of single bacteria correlate with the substrate’s surface energy.
205: Effect of Faraday waves on the bacterial attachment and biofilm formation - Xia H

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Introduction: The mechanical effects of physical factors such as wave and vertical acceleration to the biofilm formation needs systematic studies. The laboratory studies of surface waves, often using the Faraday wave (wave generated on the surface of vertically shaking container), have shown a wide range of behaviors, from pattern formation to turbulence. Recent publication showed that small amplitude vibrations promote the biofilm formation. However, the range of the parameters in those experiments was very narrow.

Hypothesis and aims: We propose to apply a wide range of the vibrations (frequencies and accelerations) to study the bacteria attachment and the biofilm formation. A model experiment is set up in the physics lab under similar conditions using passive particles and advanced imaging techniques to investigate the mechanisms of the wave/wave-driven flow on the biofilm formation.

Methodology: The experimental facility includes the computer-controlled electrodynamic shaker, a temperature-controlled incubator and sample holders. The acceleration of the sample will be monitored using an accelerometer. Each cell of the 6 well culture plates containing 2ml of nutrient broth is inoculated with 20 ul of overnight bacteria culture. Several plates will are stacked and mounted on the shaker and shaking continually for different time periods (up to 48 hours) in the incubator. The control sample is incubated separately.

Results: Initial results show greatly increased bacteria attachment and biofilm formation in a range of experimental conditions. Various patterns are observed for different frequencies and accelerations. The patterns in the biofilm might be related to the wave patterns.

Conclusion: These results show that vibration and surface wave can be used to control biofilm formation and therefore presents a novel and potentially cost effective means to manipulate the development and yield of biofilms in a range of important industrial and medical processes.
206: Active layer fluctuations drive a critical pinning transition in biofilm surface roughness - Young E

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**Introduction:** Biofilm surface roughness, or variation in surface height, can play a crucial role in biofilm function. Surface roughness controls diverse characteristics such as the extent of pathogen adhesion, genetic mixing and hence potential for cooperation, antibiotic penetration and the chances of fixation of antibiotic resistant mutants. These characteristics, in turn, can feed back on the roughness of the biofilm.

**Hypothesis and aims:** Our aim is to create a phase diagram of biofilm surface roughness as a means to understanding the link between spatial structure and function.

**Methodology:** We have used the iDynoMicS agent-based biofilm modelling software to model *Pseudomonas aeruginosa* biofilms in a flow cell set up change with nutrient availability and bacteria maximal growth rate. We examine in detail the variations in the thickness of the layer of actively growing cells at the top of the biofilm, or 'active layer'.

**Results:** We observe that local gaps in the active layer thickness can cause the biofilm interface to become stationary, or 'pinned', relative to the moving front. We distinguish three phases based upon this behaviour: a smooth phase in which there are no pinning sites, a depinned phase in which pinning sites arise but close up again and a pinned phase in which pinning sites arise but cannot be overcome. We are able to quantify these phases and their corresponding roughness behaviors in terms of the normalised standard deviation of the active layer.

**Conclusion:** Our results indicate a key role for the dynamics of the active layer in biofilm spatial structure. As many cell functions are related to whether or not the cell is metabolically active, we believe that this is an important step towards linking the structure of the biofilm to its function.