

Poster Abstracts

On-site poster session 3
11th May 1:15-2:10 pm

Tools and Modelling

Emerging technologies

Poster session: Tools and modelling

Electrochemical impedance spectroscopy probing for novel anti-bacterial agents in waste

Emil Rosqvist¹, Paola San-Martin-Galindo², Ellinoora Holmström¹, Jari Yli-Kauhaluoma³, Jouko Peltonen¹

¹*Physical Chemistry, Laboratory of Molecular Science and Engineering, Åbo Akademi University, Porthansgatan 3–5, FI-20500 Åbo, Finland*

²*Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5E, FI-00014 Helsinki, Finland*

³*Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, Viikinkaari 5E, FI-00014 Helsinki, Finland*

Within the multi-institute project ABC Health (Anti-Bacterial Channelling from waste to human health, Jane and Aatos Erkko Foundation) we develop new tools for detecting novel antimicrobials from waste samples — aimed at fighting antimicrobial multi-resistance. An electrochemical impedance spectroscopy (EIS) sensor system is deployed for monitoring biofilm growth and its disruption. The system will be applied for analysing environmental samples, specifically, to detect antimicrobial-rich waste fractions. Waste samples that show a promising biological activity will be further investigated within the project. Subsequently, the underlying response mechanisms will be looked into.

To detect the response of biofilms to antibiotics (AB), EIS was chosen as a robust analysis method. Electrodes for the EIS sensor can be printed on almost any material, thus enabling flexible, low-cost and sustainable solutions for large-scale screening. Validation of the approach was done with *S. aureus* cultures being treated with varying concentrations of AB scrutinising for differences between treated and untreated biofilms. Parallel cell-surface interaction studies were done with quartz crystal microbalance (QCM) and surface plasmon resonance (SPR). Both QCM and SPR measures adsorption of matter onto a surface and were used to describe the responses of biofilms to AB treatments, when used in conjunction with EIS studies.

Poster session: Tools and modelling

Influence of spatially varying shear stress on biofilm formation in a millifluidic flow chamber

Cornelius Wittig, Sedef Ilk, Si Suo, Thomas Crouzier, Wouter Metsola van der Wijngaart, Shervin Bagheri

KTH Royal Institute of Technology, Sweden

In the biomedical field, the formation of biofilm under shear stress is of great interest, specifically in the context of infectious diseases. Endovascular stents experience wall shear stress in the range of 0.22 - 6.72 Pa [1], while the flow in a typical artery has a Reynolds number of 500 [2]. Drinking water distribution systems and food processing plants often encounter similar shear stresses [1].

Experimental investigations, however, are typically conducted in one of two scenarios. In microfluidic environments, low flow rates are combined with minuscule Reynolds numbers ($Re < 1$) and comparably high wall shear stress. On the other hand, macrofluidic assemblies typically experience turbulent flow with high Reynolds numbers. In this study, a millifluidic device with a cross section of 20 mm × 1 mm is developed to bridge this gap and investigate the dependence of biofilm development on flow rate and wall shear stress in the range of $Re \approx 100$ and $\tau_w \approx 1$ Pa. Several channel geometries are used to evaluate the influence of spatial shear gradients on the development of biofilms.

A *Pseudomonas fluorescens* (PCL1701) biofilm is grown under constant flow to encourage adjustment to the local flow regime. The development of the biofilm is characterised using several features, such as substratum coverage, biofilm volume, surface roughness, and porosity. These parameters are measured using optical coherence tomography (OCT) and epifluorescent microscopy. OCT enables in-situ measurement during the growth of the biofilm. The growth in several regions of interest (i.e. behind a backward-facing step or at several positions in a contraction/expansion) is monitored for several days. A range of Reynolds numbers is investigated ($Re_{Dh} = 100, 200, 400$), corresponding to shear stresses from 0.2 Pa to 1 Pa in an unmodified rectangular channel.

[1] Gomes, L.C. and Mergulhão, F.J.M. (2021) 'A Selection of Platforms to Evaluate Surface Adhesion and Biofilm Formation in Controlled Hydrodynamic Conditions', *Microorganisms*, 9(9), p. 1993.

[2] Miller, G.E. (2012) 'Biomedical Transport Processes', in *Introduction to Biomedical Engineering*: Elsevier, pp. 937–993.

Poster session: Tools and modelling

Galleria mellonella larvae as a model for studying implant-associated biofilms

Kamran A. Mirza, Lara Theime, Oliwia Makarewicz, Mathias W. Pletz

Institute for Infectious Diseases and Infection Control, University Hospital Jena, Am Klinikum 1, 07747 Jena, Germany

Background: Biofilms can develop on almost all medical implants such as catheters and cardiovascular implants. Due to the biofilm-mediated antimicrobial tolerance, implant-associated infections are extremely difficult to treat. Invertebrate models such as the *Galleria mellonella* (Greater wax moth) larvae infection model are increasingly being explored to learn more about the pathogenesis of bacterial infections due to less ethical constraints compared to mammalian models. However, biofilm-associated infections have hardly been analysed in the *G. mellonella* model. The study aimed to evaluate different methods of forming biofilms on medical implants in *G. mellonella* larvae to mimic implant-associated biofilms.

Methods: Biofilm formation on the implant inside the larvae (in vivo) and pre-formed biofilm on the implant (in vitro) implanted in the larvae were the two approaches compared. Three clinical isolates of each *Staphylococcus aureus* and *Enterococcus faecalis* with a standardized dose of 10⁵ CFU/larvae were used. In the first approach (in vivo biofilm), the implant was implanted one hour before the larvae were infected. In the second approach, implant was pre-incubated with bacterial suspension of 0.5 McFarland for 24 h at 37 °C in a 96-well microtiter plate and implanted into the larvae through the proleg. Survival of the larvae was monitored as well as bacterial attachment by colony-forming unit (CFU) determination and scanning electron microscopy (SEM).

Results: Compared to planktonic infection, biofilm development approaches resulted in lower survival rates for larvae e.g., 100% survival of larvae was observed with planktonic infection compared to biofilm, which was 60%. Despite this, both methods resulted in a 10⁶ CFU of bacterial adhesion to the implant. The insertion of pre-infected implants in larvae resulted in a higher survival rate compared to in vivo biofilm method, which involved bacteria inoculation into hemolymph resulting in sepsis and lower survival rate in the larvae.

Conclusion: Overall, the *G. mellonella* model was able to produce biofilms on implants and showed the capability of assessment of qualitative (biofilm visualization) and quantitative (CFU count) parameters.

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Dynamics of mono- and dual- species biofilms grown on nanostructured surfaces

Paola San-Martin-Galindo¹, Emil Rosqvist², Stiina Tolvanen², Kirsi Savijoki¹, Päivi Tammela¹, Jari Yli-Kauhaluoma³, Adyary Fallarero¹, Jouko Peltonen²

¹*Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5E, FI-00014 Helsinki, Finland*

²*Physical Chemistry, Laboratory of Molecular Science and Engineering, Åbo Akademi University, Henriksgatan 2, FI-20500 Åbo, Finland*

³*Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, Viikinkaari 5E, FI-00014 Helsinki, Finland*

Biofilms facilitate the protection of bacteria as response to external environment conditions. As the biofilm formation is a complex and dynamic process that involves the bacteria response to surfaces, it demands the use of orthogonal approaches embracing multiple bacterial assays as well as versatile parameterization of the surface when investigated. In this study, factors influencing *Staphylococcus aureus* grown on nanostructured polymer-coated surfaces, in a mono-species biofilm model, were explored in detail, in order to connect several physico-chemical parameters with the biological response. To this end, the *S. aureus* biofilm growth (cell viability, poly- β -1,6-N-acetyl-D-glucosamine [PNAG] fraction and surface-associated proteins) and the polymer-coated surfaces (surface chemistry and topography) were thoroughly characterized. The influence of two different biofilm-forming assays (agar plate- and well plate-based assays) on the *S. aureus* growth was also analyzed. The outcomes showed that certain roughness parameters of the polymer-coated surface drove the biological response differently depending on the biofilm assay used. The bacterial viability and PNAG fraction responded to surface properties, with surface polarity, fine structure and valley and peak properties being particularly influential. Furthermore, abundance profile of several virulence proteins (e.g. surface proteins related to pathogenic activity and host invasion) was observed to strongly correlate to at least one roughness parameter. Additionally, this effect was also evaluated in dual-species *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms model by quantifying the viable cells number. It was shown that the polymer-coated surface showed a significant increase survival of *S. aureus* cells when cultured with *P. aeruginosa* when compared to a reference bare surface. Overall, these results highlight the importance of evaluating and combining several different surface parameters, comprehensively accounting for both physical and chemical properties of the surfaces as well as the impact of the choice of investigation when studying bacterial response, and during the functionalization of material surfaces.

Poster session: Emerging technologies

Self-regulating degradation of antimicrobial functionalized hydrogels as a novel treatment against biofilm-forming microorganisms

E. Uhlmann^{1,2}, C. Hein¹, A. Brehmer¹

¹*Fraunhofer Institute for Production Systems and Design Technology IPK, Germany*

²*Institute for Machine Tools and Factory Management IWF, Technische Universität Berlin, Germany*

Bacterial resistance to conventional antibiotics combined with the increasing awareness of the essential role of biofilms in nosocomial infections caused by medical devices has led to a growing interest in new antimicrobial strategies. Since the formation of bacterial resistances represents a permanent risk in the drug treatment of biofilms, optimization of drug release to prevent microbial adherence prior to the formation of a mature biofilm continues to gain attention. In particular, prophylactic release of antibiotics often increases the risk of resistance development. Flexible and self-regulated drug release based on the presence of microorganisms is desirable. For this purpose, different biodegradable hydrogel matrices like gelatin or poly(lactic-co-glycolic acid) (PLGA) were modified for a stable application in the body. Clinically relevant microorganisms were tested for their ability to degrade these biopolymers. By utilizing enzymatic metabolic processes of certain microorganisms, the degradation of the hydrogels could be induced and influenced. The hydrolysis ability of certain microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* was then linked to an antimicrobial therapy option. For this purpose, model antibiotics were incorporated into the hydrogels. The gel solutions were then dip-coated onto conventional catheter materials, cured, and tested for antimicrobial efficacy and drug release profile in a model of catheter-associated urinary tract infections. *Staphylococcus aureus* in particular showed sensitive behavior to antimicrobial treatment by autonomous hydrolysis of the modified hydrogels. Even at low cell counts, the active ingredient was released in a sufficient quantity. Biofilm formation was thus avoided over a period t of $t = 10$ days. Using this approach, an on-demand antimicrobial treatment for emerging nosocomial urinary infections was achieved. The results offer great potential for reducing biofilm formation on medical devices, such as catheters or antimicrobial wound dressings. However, the commercial implementation of this technology on a large scale requires further investigation.

Poster session: Emerging technologies

Engineering biofilm hydraulic resistance on the microscale

Samuel Charlton¹, Dorothee Kurz¹, Steffen Geisel², Eleonora Secchi¹

¹*BioMatter microfluidics, Institute of Environmental engineering, ETH Zurich, Switzerland*

²*Soft materials laboratory, Institute of material science, ETH Zurich, Switzerland*

Bacterial biofilms are amongst the most successful modes of life in the terrestrial environment and ubiquitous within porous systems, such as soils and membrane interfaces. The bacteria within a biofilm are bound together by self-secreted extracellular polymeric substances (EPS), yielding a natural gel-like structure. EPS provides a protective shield and structural architecture to constituent cells of the biofilm, which shapes internal mass transport within the biofilm and flow field of the colonized porous media. The interplay between biofilm formation and flow leads to the formation of preferential flow pathways and significant increases in membrane hydraulic resistance, as investigated in macroscale experimental systems. However, the complex multiphase interaction between biofilm proliferation and flow within irregular porous media make the deduction of scalable physical relationships challenging. Consequently, a mechanistic understanding linking EPS composition, biofilm morphogenesis and porosity with hydraulic resistance is still missing.

In this work, we investigate the time evolution of biofilm morphology and hydraulic resistance as a function of flow conditions and EPS composition. We grow the model bacterium *Bacillus subtilis* in two types of microfluidic channel and use a library of EPS mutants devoid of polysaccharide (EPS-O), protein (TasA) or hydrophobin (BslA) to modulate EPS composition. Firstly, we grow and characterize the biofilm during its morphogenesis in a novel microfluidic platform where the biofilm is grown on a cellulose nanofibril (CNF) membrane barrier. The CNF platform allowed precise measurement of single colony biofilm hydraulic resistance as a function of pressure-driven flow and EPS composition, whilst retaining full optical access. To validate the scalability of our findings, we use an unconfined microfluidic geometry, a model 2D porous media, which increases the biofilm volume by approximately two orders of magnitude. We measure hydraulic resistance and apply advanced optical visualization techniques to quantify biofilm morphogenesis and internal transport. This study allowed us to quantify how flow and EPS composition shape the hydraulic resistance of biofilm at the micro and mesoscale. The relationships derived from these studies enhance our understanding of how we can engineer biofilms to tune their hydraulic resistance.

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Microalgae Microbiomes – A Natural Source for the Prevention and Treatment of Diseases in Aquaculture

Ines Krohn, Lutgardis Bergmann, Yuchen Han, Wolfgang Streit

University of Hamburg, Germany

Aquaculture is one of the fastest growing food sectors in the world with land-based aquaculture gaining increasing interest. Diseases caused by bacteria like *Pseudomonas* sp., *Flavobacterium* sp., *Aeromonas* sp. or *Edwardsiella* sp. are an obvious challenge to the aquaculture industry exacerbated by many pathogen's biofilm building abilities. Antibiotics are still in use in many regions of the world, contributing to increased antimicrobial resistance. Our work builds on the hypothesis that a novel approach to disease treatment in aquaculture can be achieved by exploiting the healthy properties of microalgae and their associated microbiomes.

To advance this field, we performed both functional and sequence-based screening of microalgae and microbial consortia for anti-biofilm activities. Five promising candidates of microalgae which showed significant anti-biofilm traits were chosen for further investigation. Ongoing metagenomes-, metaproteomes- and metatranscription analyses gave first insights into the main enzymes involved in these antibiofilm effects and potential candidates for industrial applications.

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Reinforced CS-based coatings and self-standing films to control biofilm formation by release of active species

E. A. Kukushkina^{1,2}, M. C. Sportelli¹, N. Ditaranto^{1,2}, R. A. Picca^{1,2}, N. Cioffi^{1,2}

¹*Dipartimento di Chimica and*

²*CSGI (Center for Colloid and Surface Science) Bari Unit, Università degli Studi di Bari Aldo Moro, Bari, Italy*

Due to the spread of resistance-related health and economic problems, development of new materials to control the growth of biofilms is needed as much as the creation of the alternatives to antibiotics. Taking into account a positive trend towards sustainability, new active materials potentially have to demonstrate “green” nature and low negative impact on the environment.

Chitosan (CS) is a well-known natural polymer, which has a variety of attractive properties: it is biodegradable, non-toxic, relatively cheap and it has intrinsic antimicrobial action. By all means, it has the potential to work as a “green” alternative to petroleum-based packaging materials with additional property to prevent food spoilage caused by pathogenic microorganisms. The main disadvantage for CS-based materials is related to their poor performance in humid environment, which could limit their real life-application. There are several ways to overcome this, one of them is addition of crosslinking agent(s).

Tannic acid has been proved to be a compatible and effective crosslinker in combination with CS [1]. Apart from a better compatibility with aqueous environment, it potentially brings additional antimicrobial and antibiofilm effect, thus leading to synergistic performance of the CS/TA composite. To assure more robust antibiofilm action and additional effect against resistant pathogens, inorganic nanophases of known antimicrobial action could be incorporated or in situ synthesized and immobilized in the polymer matrix [2]. Addition of metal nanoparticles (MeNPs) beneficially increases the performance of synergistic composite due to the generation of reactive oxygen species (ROS) and ion release .

The liquid starting material for creation of solid forms, mother solution (MS), contains all the above mentioned components: CS as a base, TA as crosslinker and metal nanoparticles as inorganic additive. It was further used to create thin coatings on various substrates (ex. stainless steel) or to produce self-standing films by solution casting method. Both MS and the final materials were studied and characterized by various techniques and will be subjected to biofilm growth control tests and antimicrobial activity tests to evaluate the potential for food packaging application.

[1] A.-K. Koopmann et al., *Molecules*, 2020, 25, 4910.

[2] E.A. Kukushkina et al., *Nanomaterials*, 2021, 11(7), 1687.

Poster Abstracts

Online poster session 3
11th May 1:15-2:10 pm

Tools and Modelling

Emerging technologies

Online

Poster session: Tools and modelling

Staphylococcus aureus biofilm metabolism and susceptibility to ciprofloxacin on thiol-ene polymer surfaces

Jéssica Amorim², Cristina D. Cruz¹, Markus Haapala², Tiina M. Sikanen², Päivi Tammela¹

¹*University of Helsinki, Faculty of Pharmacy, Drug Research Program, Division of Pharmaceutical Biosciences, Finland*

²*University of Helsinki, Faculty of Pharmacy, Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Finland*

Microfluidic devices are a promising tool for the study of bacterial biofilms and their susceptibility to antibacterial treatments. However, before applying new microfabrication materials to biofilm research, it is necessary to characterize the material-cell interactions to understand their possible impacts on biofilm growth and maturation. Thiol-enes are one of the emergent manufacturing materials in the field of biological microfluidics, composed of two monomers, one with thiol groups (here, PETMP) and another with allyl groups (here, TATATO). In the present study, we examined the metabolic activity and the growth of *Staphylococcus (S.) aureus* ATCC 29213 biofilms on thiol-ene polymers prepared using these monomers in different stoichiometric ratios (25% molar excess of thiols, 25% molar excess of allyls or stoichiometric). *S. aureus* biofilms were grown on uncoated (polystyrene, control) and thiol-ene coated 96-well plate in Tryptic Soy Broth + 1% glucose without antibiotic or in Muller Hinton Broth for 24h with ciprofloxacin (1–0,25µg/mL). For metabolic assessment, the biofilms were washed and incubated for 20 minutes with a solution of 4µg/mL of resazurin, followed by fluorescence measurement (n=5 technical and n=3 biological replicates). For growth assessment, the biofilms were washed, diluted serially by 10-fold, drop-plated in Tryptic Soy Agar and incubated at 37°C overnight (n=3 technical and biological replicates). Without the presence of antibiotics, cells grown on thiol-ene surfaces had a significantly higher metabolic state compared with those grown on polystyrene, while the number of bacteria was similar on each surface. When challenged with ciprofloxacin, cells grown on thiol- and allyl-rich surfaces showed a Minimum Biofilm Inhibitory Concentration of 0,67µg/mL i.e., 45% higher than on the polystyrene surface according to the resazurin data. In contrast, the stoichiometric surface was not different from polystyrene. Based on these results, the monomer ratio has an influence on the biofilm susceptibility to ciprofloxacin. On one hand, the stoichiometric composition appears as the most feasible for microfluidic antibiofilm screening (biologically equivalent to the control, polystyrene). On the other hand, the higher concentration of ciprofloxacin required on thiol- and allyl-rich surfaces warrants further research to reveal its underlying mechanistic basis, which could provide new insights into the development of antibiofilm materials/surfaces.

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Facile and rapid in situ synthesis of synergistic AgCl/BAC into Montmorillonite against harmful bacteria

Syed Imdadul Hossain^{1,2}, Maria Chiara Sportelli¹, Rosaria Anna Picca^{1,2}, Luigi Gentile^{1,2}, Gerardo Palazzo^{1,2}, Nicoletta Ditaranto^{1,2}, Nicola Cioffi^{1,2}

¹*Dipartimento di Chimica and*

²*CSGI (Center for Colloid and Surface Science) Bari Unit, Università degli Studi di Bari "Aldo Moro", Bari, Italy*

Green synthesis and characterization of synergistic hybrid Nanoantimicrobials (NAMs) have attracted extensive attention in food, medicines, agriculture, and cosmetic sectors. NAMs allow enhancing the desired qualities in food production, storage, nutrient stability, while inhibiting or eradicating communities of bacteria. If antimicrobial drug-resistant are not tackled now, 20 person could die every 1 minute by 2050 worldwide.¹ Bacteria have propensity to colonize on biotic and abiotic surfaces forming so called biofilms, and obtain peculiar resistance mechanism. One of the potential route in racing against antimicrobial resistance and post antibiotic era, and eradicate harmful biofilms is no wait but continuous development of potential synergistic hybrid NAMs.

In this regards, AgXs is considered as potential antimicrobial which is effective in killing both airborne and waterborne bacteria by providing a tailored concentration of biocidal Ag⁺ ions into environment ². However, one of the challenges still remain is green, facile, and rapid synthesis of AgCl into bioactive substrate. Synthesis of AgCl NPs into montmorillonite (MMT) could play extremely important role due to the possibilities of creating synergistic antibacterial hybrid nanocomposite with slow release of Ag species in order to obtain a prolonged antibacterial activity and overcome toxicity. Herein, in-situ synthesis of AgCl/BAC NPs is carried out in the presence of refined MMT while AgCl NPs get entrapped into/onto MMT substrate provided that electrostatic interaction between AgCl NPs and MMT lead to formation of synergistic hybrid NAMs. Characterization studies on the nanocomposite were done by TEM, XPS, UV-Vis and Infrared spectroscopies. The nanocomposite is expected to be additive for food packaging, aiming at either bactericidal or long lasting bacteriostatic effects, based on the active species loading. Further study on impregnation of colloidal AgCl/BAC into Cu intercalated MMT are planned to be carried out to acquire synergistic effect of nanocomposite against harmful biofilms.

References

1. Jack, A. & Campbell, C. The threat of antibiotic resistance — in charts. <https://www.ft.com/content/d806dcf5-23f8-4714-ad04-ca11a66061e2> (2020).
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Online

Poster session: Emerging technologies

Expression of recombinant Green Fluorescent Protein (GFP) using *Escherichia coli* biofilms: effects of culture media and surface properties

Fábio Carvalho¹, Ana Azevedo^{1,2}, Luciana C. Gomes^{1,2}, Gabriel A. Monteiro³, Filipe J. Mergulhão^{1,2}

¹*LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

²*ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*

³*iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, 1049-001 Lisboa, Portugal*

The production of engineered proteins using numerous expression systems has gained increasing attention in the last decades. Despite the many advantages of biofilm systems compared to suspension growth cultures, they have been scarcely investigated for the production of recombinant proteins. Previous studies have shown that high specific concentrations of enhanced Green Fluorescent Protein (eGFP) could be obtained from *E. coli* biofilms, even with no optimization of the cultivation conditions. This work intends to assess the effect of surface material and culture media in *E. coli* biofilm formation and eGFP production to find the optimal combination that maximises eGFP yield.

E. coli JM109(DE3) cells transformed with the pFM23 plasmid carrying the eGFP gene were used and assays were performed in 12-well plates for 9 days using surface materials with distinct morphological and physicochemical properties (stainless steel (SS), polyvinyl chloride (PVC), and silicone rubber (SIL)). Their individual properties were analyzed by Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), and contact angle measurements. The surfaces were combined with culture media containing different sources of carbon and nitrogen (Lysogeny broth (LB), Terrific broth (TB), and M9ZB broth). Colony-forming units (CFU), fluorescence microscopy, fluorimetry, and High-Performance Liquid Chromatography (HPLC) were used to monitor bacterial growth, eGFP production, and plasmid copy number (PCN).

The surface image analysis by SEM revealed that SIL has protuberances, which contributes to the higher surface roughness parameters determined by AFM when compared to SST and PVC. Contact angle determination indicated that SIL was more hydrophobic ($-75 \text{ mJ}\cdot\text{m}^{-2}$) than SST and PVC (-10.9 and $-12.3 \text{ mJ}\cdot\text{m}^{-2}$, respectively).

Results showed that biofilm formation was higher using TB medium for all surfaces studied, where PVC presented the highest biofilm amount with increasing biofilm thickness over the days, ranging from $191.0 \pm 54.1 \mu\text{m}$ at day 1 until $1357.8 \pm 319.8 \mu\text{m}$ at day 9. Regarding specific eGFP production, cells grown in TB medium synthesized more protein than cells grown in LB medium or M9ZB medium, wherein PVC presented the highest amount of protein with $206.8 \pm 40.9 \text{ fg}\cdot\text{cell}^{-1}$. Hence, using PVC and TB medium seems to be the most advantageous conditions to obtain the highest specific eGFP production in *E. coli* biofilms.

Online

Poster session: Emerging technologies

Surface proteomics of mycobacterial biofilms to develop biofilm-binding synthetic nanobodies

Milka Marjut Hammaren^{1,3}, *Hanna Luukinen¹, Alina Sillanpää¹, Kim Remans³, Karine Lapouge³, Tânia Custodio⁴, Christian Löw⁴, Henna Myllymäki¹, Ihalainen Teemu¹, Toni Montonen¹, Markus Seeger⁵, Robertson Joseph⁶, A. Nyman Tuula⁶, *Kirsi Savijoki^{1,2}, *Parikka Matalena¹

*Equal contribution

¹Faculty of Medicine and Health Technology, FI-33014, Tampere University, Finland

²Faculty of Agriculture and Forestry, FI-00014, University of Helsinki, Finland

³European Molecular Biology Laboratory, DE-69117 Heidelberg, Germany

⁴European Molecular Biology Laboratory, DE-22607 Hamburg, Germany

⁵Institute for Medical Microbiology, University of Zurich, 8006 Zurich, Switzerland

⁶Institute of Clinical Medicine, Department of Immunology, University of Oslo, Oslo, Norway

Antibiotic tolerant biofilms were recently shown to exist in tuberculous mycobacterial infection in vivo. As tuberculosis continues to be the deadliest bacterial infection and is difficult to cure with antibiotics, investigating the biofilm forms for more efficient and targeted treatment is crucial. In this study, using biotinylation tagging of intact *Mycobacterium marinum* biofilms and total biofilm lysates, we identified the most abundant surface-exposed proteins in the extracellular matrix, surface-enriched proteins as well as proteins enriched inside the biofilm-embedded cells. The dataset gives an overview of composition of mycobacterial biofilms that can be utilized in the development of biofilm-targeting treatments. We are especially interested in the surface-structures of mycobacterial biofilms to develop in-house binding reagents for future applications. GroEL1 and GroEL2 proteins were identified among the top 10 most abundant surface proteins in cultured biofilms as well as in vivo in infected tissues extracted from *M. marinum* infected adult zebrafish. Synthetic nanobodies, so-called sybodies, originally developed in the Seeger lab, can be produced to bind any surface-exposed target protein with high affinity. We purified the two mycobacterial recombinant GroEL proteins using *E. coli*, and used the sybody library to identify binders against GroEL1&2. We characterized the best binders and showed that we could find clones that bind intact mycobacterial biofilms of *M. marinum*.

Online

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Deep RNA seq analyses in multispecies microbial communities involved in wound and lung infections

Raphael Moll¹, W.R. Streit¹, I. Alio¹, T. Hoffmann¹, H. Rhode², U. Mamat³, U. Schaible³, Kai Papenfort⁴

¹*Department of Microbiology and Biotechnology, University of Hamburg, Hamburg, Germany*

²*Institute for medical microbiology, Virology and Hygiene, Universitätsklinikum Eppendorf, Hamburg, Germany*

³*Cellular Microbiology, Priority Area Infections, Research Center Borstel, Borstel, Germany*

⁴*Institute for microbiology, Friedrich-Schiller-Universität Jena, Germany*

Until recently microbiology and biofilm research has primarily centered on single species microorganisms as the fundamental drivers of infections. However today with a better understanding on the human microbiome it has become clear that in lungs and wounds complex microbial consortia are drivers of infections. These consortia contain different microbial species originating from all lineages of the tree of life. To further develop our comprehension on the infection process with relation to the complex interaction of the various microorganisms, we are establishing artificial communities that serve as model consortia and help us to address scientific questions connected with infection, host defense and drug target delivery.

For this, we are working with fluorescence labelled strains of the pathogens *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and the emerging pathogen *Stenotrophomonas maltophilia*. Furthermore, we have incorporated *Candida albicans* into these multispecies consortia and have successfully established mixed species biofilms under flow and static conditions. Utilizing LSM imaging, a particular dissemination and arrangement of every species in these biofilms was noticed.

Dual species and triple species RNA seq analysis of these multispecies biofilms and the use of promotor fusion constructs has shown explicit and different expression patterns for every species as compared to the control single species biofilms, proposing that the various species recognize and react to the presence of others in these complex communities. By applying our multispecies model consortia, we will be able to address research questions regarding complex inter species interactions as well as interaction with the host and drug target delivery.

Poster session: Emerging technologies

Synthetic mimics of antimicrobial peptides based on itaconic acid as antimicrobial polymeric coatings

Sibylle Rau¹, Diana Lorena Guevara Solarte¹, Lea Sollka², Karen Lienkamp^{2,3}, Elmar Hellwig¹, Ali Al-Ahmad¹

¹*Department of Operative Dentistry and Periodontology, Medical Center of the University of Freiburg, Faculty of Medicine, University of Freiburg, Hugstetter Strasse 55, 79106 Freiburg, Germany.*

²*Bioactive Polymer Synthesis and Surface Engineering Group, Department of Microsystems Engineering (IMTEK) and Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany*

³*Institut für Materialwissenschaft und Werkstoffkunde, Universität des Saarlandes, Campus, 66123 Saarbrücken, Germany.*

Aim of the study: Due to the increase of antibiotic resistance worldwide, there is a demand for alternative treatment of infections. The aim of this study was to evaluate the antimicrobial effects and toxicity towards eukaryotic cells of itaconic acid (IA)-based synthetic mimics of antimicrobial peptides (SMAMPs) (IA-SMAMPs).

Material and Methods: A copolymer of Itaconic Acid-4-N-Boc-2'-Aminoethylamide-co-N,N-Dimethylacrylamide was synthesized and evaluated regarding the antimicrobial activity against planktonic bacteria (*Escherichia coli* and *Staphylococcus aureus*), effects on biofilm formation of *Proteus mirabilis*, antibiotic resistance development of *E. coli* in comparison to ciprofloxacin and their suitability for preserving pharmaceutical ointments. Additionally, the antimicrobial activity of substrates coated with IA-SMAMPs was tested to simulate the effects on initially adhered bacteria. The biocompatibility of IA-SMAMPs was also evaluated by the hemolysis assay with red blood cells. Cell compatibility of the coated surfaces was tested using human gingival mucosal keratinocyte cells and the alamar blue assay.

Results: Inhibitory effects (60-100%) were shown for concentrations of IA-SMAMPs in the range of 50-400 µg/ml against *E. coli* and *S. aureus*, respectively. The biofilm formation of *P. mirabilis* was highly reduced at lower concentrations (≤ 100 µg/ml). Over an exposition time of 20 days the inhibitory effects of IA-SMAMPs on *E. coli* remained constant, whereas the inhibitory effects of ciprofloxacin decreased from 90 to 30% revealing the development of antibiotic resistance. In six different pharmaceutical ointments, IA-SMAMPs eradicated *E. coli* and *S. aureus* at a killing rate of up to 100%. The IA-SMAMPs-coated silicon surfaces showed a high antimicrobial activity and killed adherent bacteria at arrange of 2.8-4.4 Log₁₀. The hemolysis assay revealed a high biocompatibility level of IA-SMAMPs towards red blood cells at antimicrobial concentrations. The alamar blue assay as well as live/dead-staining and microscopic analysis showed also toxicity of the surfaces coated with IA-SMAMPs to eukaryotic cells.

Conclusions: IA-SMAMPs are promising polymers for coating of medical devices such as urine catheter and other biomaterials to prevent biomaterial-associated infections.