

## Oral multispecies biofilm model - a reproducible *in vitro* test system

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Dental implants play an important role in maintaining oral function after tooth loss. Despite medical advances, 20% of the patients suffer from a severe infection 5-10 years after implantation. This biofilm-associated infection called peri-implantitis causes inflammation of the implant surrounding tissue and bone degradation, which consequently can lead to implant loss. The current aim of ongoing research is therefore the development of new implant materials or surface coatings with characteristics that reduce or even prevent the formation of bacterial biofilms on the implant surface. For the evaluation of novel implant materials, appropriate test systems which mimic the *in vivo* situation are required. For this purpose, we developed a multispecies biofilm model, containing the four relevant oral bacterial species *Streptococcus oralis*, *Actinomyces naeslundii*, *Veillonella dispar* and *Porphyromonas gingivalis*. The biofilms were cultivated in standardized growth medium (a) in 96-well plates as well as (b) in a flow chamber system under anaerobic conditions. Live/dead staining and subsequent confocal laser scanning microscopy revealed a reproducible biofilm formation regarding biostructure and -volume. Application of quantitative real time PCR (qRT-PCR) and urea-NaCl fluorescence *in situ* hybridization (urea-NaCl-FISH) provided precise time-resolved information about the bacterial composition and the spatial species distribution within the biofilms. Our newly developed oral multispecies biofilm model reflects the *in vivo* situation of early native plaque, is robust and easy to handle and therefore well suited as an *in vitro* test system for both, high content screening applications in 96-well-plates and the evaluation of novel implant materials under physiological flow conditions.

## How does nano-silver get inside bacteria ? –Mechanistic studies using AgAu alloy nanoparticles

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Silver nanoparticles are nowadays frequently used to fight infections derived from multiresistant bacteria, however the toxicity to cells and hence the limited therapeutic window still restricts their application. In addition, the mechanism of silver-derived toxic and antimicrobial is to date not fully understood and furthermore, studies on the uptake of Ag nanoparticles by bacteria are rare.

In this work, we used gold-silver alloy nanoparticles as alternative antimicrobial agents. The particles were fabricated by pulsed laser ablation in liquids, a technique which does not require any artificial organic stabilizers and hence may avoid potential toxic cross-effects from ligands. [1] We could show that the composition of the AgAu particles could be controlled by the composition of the ablated targets and the generated particles exhibited a fully homogeneous elemental distribution (probed by EDX line scans) [2] and a crystal structure identical to the used targets (determined by XRD) [3]. In biological assays with oocytes, bacteria and human fibroblasts we found a non linear decrease of the antimicrobial activity with increasing gold molar fraction [4] [5], probably attributed to an inhibition of silver dissolution from the nanoparticles by the more noble metal gold. Consecutively, we studied the cellular uptake of AgAu alloy nanoparticles by the model bacterium *S. aureus*. Interestingly, we found only small particles inside the bacteria, however, these were significantly more silver rich than those originally exposed to the cell. Based on these findings we could conclude that these intracellular silver-rich particles were probably formed by dissolution and intracellular reduction of the silver ions. These findings may shed light on the mechanism of silver nanoparticle induced toxicity.

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## Innovative antimicrobial surfaces for orthopaedic applications

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Implants have changed our medicine, improving the lives of many patients. But there are still substantial problems with bacterial infections and inflammatory processes caused by impurities and defensive reactions in the body.

In general, the risk of infection for primary prostheses is relatively low (0.5 to 2%). Beyond that, register data indicates that the infection rate after implant revision is significantly higher (~ 15%). This is more and more relevant due to the demographic development. Various techniques have been established to generate antimicrobial implant surfaces. They range from drug release coatings to contact killing systems. However, many challenges have not yet been solved.

To prove the antimicrobial activity of our antimicrobial surfaces *in vitro* and *in vivo* we used an *in vitro* test model that is able to mimic the *in vivo* situation and gives a good indication about the antimicrobial activity under realistic conditions. Furthermore, we successfully established an osteomyelitis model to verify the antimicrobial activity *in vivo*.

The other aspect is the toxicity of microbes and their molecular components. If they are killed by antibacterial coatings, they are in direct contact with healthy tissue. This can lead to serious infections and inflammatory reactions.

A possible solution to this problem could be the development of a multifunctional antibacterial coating. This coating consists of a hydrogel which may trap and destroy pathogens.

To achieve this goal, interdisciplinary approaches that require highly specialized knowledge in the fields of physics, biology, chemistry and materials science are necessary. A joint cooperation of industry (Aesculap AG) and research institutes (DWI - Leibniz Institute for Interactive Materials at RWTH Aachen eV, Reutlingen University (HR) and the MPI for Medical Research (MPI)) works on this field.

## Liquid-infused titanium as biofilm-repellent implant surface

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Dental implants are frequently used in modern medicine. However, despite continuous biomaterial improvement, dental implants still suffer from high rates of implant-associated infections. This inflammatory disease, which is referred to as peri-implantitis, is caused by bacterial biofilms: firmly attached multispecies microbial communities, embedded into a self-produced matrix of extracellular polymeric substances, which show an altered phenotype compared to those in planktonic bacteria. As bacterial biofilms show inherent resistance to host immune response and antibiotic treatment, their retrospective treatment remains difficult. Therefore, ongoing research focusses on new implant materials, which aim at inhibiting biofilm formation. We recently developed laser-structured liquid-infused titanium surfaces, following the principle of “slippery liquid-infused porous surfaces” (SLIPS). This approach is based on superhydrophobic structures, which are infused with hydrophobic lubricants that are physicochemically entrapped as a thin film. Accordingly, a new liquid-liquid interface is created on the functionalized surface. Screening of four different structures generated by ultra-short pulse laser-ablation and five different lubricants yielded a lotus leaf inspired spike structure and a viscous perfluoropolyether as the most promising combination. The liquid-infused titanium showed an almost complete reduction of oral initial colonizer *Streptococcus oralis* monospecies biofilms either grown statically or in a flow chamber system. The same effect was observed for *in vitro* formed multispecies biofilms. As the surface’s components do not exhibit bactericidal characteristics, the liquid-infused titanium most probably physically inhibits bacterial attachment. Indeed, we could detect a reduced amount of initially adhering bacterial cells in a multi-well plate assay. Furthermore, single bacterial cell adhesion force spectroscopy revealed reduced unspecific and specific adhesion forces on liquid-infused titanium surfaces. In conclusion, we developed a liquid-infused titanium surface, which showed strong repellency of oral biofilms and additionally strengthened the hypothesis of bacterial repellency mediated through solely physical cell-surface interactions.

## **Monitoring of Infection Development by Multi Capillary Column - Ion Mobility Spectrometry (MCC/IMS)**

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Artificial joint replacement allows patients to become again mobile and is associated with a considerable increase in life comfort for the affected patients. However, in revision knee arthroplasties the risk of a periprosthetic joint infection (PJI) is as high as 10-15% and involves the high burden for the patient of a two-stage procedure with an implant free interval of at least six weeks where patients remain hospitalized. High PJI rates reflect the abundant use of antibiotics in different dosage forms (tablets, bone cements, spacers, infusions) which led over decades to the formation of multi-resistant bacteria which lack efficient antibiotic treatment. Polymerase chain reaction (PCR) or diagnostic kits, like the Synovasure® test, may prove an infection at a definitive time point when the treating surgeon has a suspicion of it. The development and course of the infection remain unclear. New efforts will be necessary to not only detect but also to monitor the course of an infection. Multi capillary column - ion mobility spectrometry (MCC/IMS) as a sensitive tool to find metabolites in the exhaled breath seems to be a method to identify and follow up the course of an infection development before it manifests to the surgeon by common blood parameters. Timely detection of an acute sepsis is of paramount importance to save the patient's life. In a first clinical trial exhaled breath from sixty patients at the Virchow Klinikum was collected. Patients were assigned either to an aseptic or a septic group. A differentiation between 10 mL exhaled air of patients with and without an infect and room air (inhaled air) was shown (accuracy 97 %, class precision between 86 % and 99 %). Finally, five peaks referring to five different analytes were identified as potential marker showing a significance level 99,9 % (including a Bonferroni-correction).

## Investigation of Peri-implantitis in Rats

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The extensive functional and esthetic reconstitution in partially and edentulous patients is part of the success story of dental implants with more than 1,000,000 replacements per year in Germany alone. Beside all material and surgical improvements over the last decades, biofilm-related inflammation of the peri-implant soft and hard tissue remains the major downside, being responsible for implant failure with a prevalence of 22% after five years.

For the development of innovative implant materials, surfaces or structures addressing this problem, experiments in animals are necessary, because of the complex interplay of tissue, bacteria and implant, which can be simulated only partially *in vitro*, and ethical considerations. Because of numerous similarities with humans regarding the periodontal anatomy and histopathology, rodents are widely used in periodontal research already. Despite low expenses, easy handling and the commercial availability of different genetic backgrounds, studies in rodents regarding peri-implantitis are less frequent than those performed in dogs or minipigs, mainly because of implant size and accessibility of the oral cavity. Furthermore, some of these models are limited to investigate antibacterial surface modifications of dental implants or the pathological mechanisms of a developing human peri-implantitis, since they use pre-infected implants or ligatures.

Together with our industrial partners we developed a miniaturized threepart implant system for rats, which enables us to mimic the procedure in patients and to modify the different compartments separately. This innovative model is used for basic peri-implantitis studies as well as preclinical studies regarding new implant and abutment surfaces.