Bacterial transmission

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Summary

Bacterial adhesion is a main problem in many biomedical, domestic and industrial environments and forms the onset of the formation of a biofilm, in which adhering bacteria have grown into a multi-layered film, embedding themselves in a matrix of extracellular polymeric substances. Transmission is a common pathway of bacterial contamination of surfaces in diverse environments. Bacterial transmission is an event of adhesion on the receiving surfaces and subsequent detachment of bacteria from donor surfaces. Since bacteria prefer to grow in a biofilm, findings on the mechanism of bacterial and biofilm transmission and how to prevent them are urgently needed.

In **Chapter 1**, we first have described basic mechanisms of bacterial transmission and an overview of the importance of bacterial transmission is given. In order to increase the understanding of bacterial transmission the aim of this thesis was to study the effect of various environmental and intrinsic factors on bacterial transmission from a donor surface covered with a multi-layered bacterial biofilm. The main environmental factors studied were the surface (nano-) structure of receiving surfaces and the pressure and shear forces applied during transmission. The main intrinsic factors were the bacterial species and in particular the impact of the visco-elastic properties of the extracellular polymeric substances (EPS) matrix on biofilm transmission.

Biofilm transmission of an EPS producing and non-EPS producing *Staphylococcus epidermidis* strain between two stainless steel surfaces under high and low contact pressures were evaluated in **Chapter 2**. Donor biofilm thicknesses before and after transmission as well as biofilm thickness on receiver surfaces after transmission were measured using optical coherence tomography. After optical coherence tomography, biofilms were dispersed in buffer and numbers of bacteria in donor and

receiver biofilms enumerated in a Bürker-Türk counting chamber. Biofilms were further visualized through confocal laser scanning microscopy and two photon laser scanning microscopy. After transmission, donor surfaces remained fully covered with biofilm, albeit thinner than before transmission indicating transmission through cohesive failure in the biofilm. Donor and receiver biofilm thicknesses after transmission did not add up to the pre-transmission donor biofilm thickness, as did numbers of biofilm bacteria, suggesting more compact biofilms after transmission, especially for non-EPS producing staphylococci. Combination of thickness and number of bacteria in biofilms per unit substratum area yielded an increase in staphylococcal density per unit biofilm volume from 0.20 μm^{-3} to 0.52 μm^{-3} for transmission of the non-EPS producing strain under high contact pressure. The EPS producing strain had similar densities before and after transmission of around 0.17 μm^{-3}. This suggests three different phases in biofilm transmission: 1) compression yielding compaction of the biofilm, 2) separation and 3) biofilm relaxation during which the viscoelasticity of possible EPS produced restores biofilm structure to its pre-transmission density.

Two types of stress may have impact on bacterial transmission: compressive and shear stress. Bacterial transmission often occurs under compressive stress as described in chapter 2. However, bacterial transmission under shear is at least equally, if not more common, as for instance during slicing of meat, intra-venous catheter insertion through the skin or urinary catheter insertion attracting peri-urethral bacteria to the catheter surface. Therefore, in **Chapter 3***,* a new device had been described to study shear-induced bacterial transmission from a (stainless steel) pipe to a (silicone rubber) tube and compared transmission of EPS-

producing and non-EPS producing staphylococci. Transmission of an entire biofilm from the donor to the receiver tube did not occur, indicative of cohesive failure in the biofilm rather than of adhesive failure at the donor-biofilm interface. Biofilm was gradually transmitted over an increasing length of receiver tube, occurring mostly to the first 50 cm of the receiver tube. Under high shearing velocity, transmission of non-EPS producing bacteria to the second half decreased non-linearly, likely due to rapid thinning of the lowly lubricious biofilm. Oppositely, transmission of EPS-producing strains to the second tube half was not affected by higher shearing velocity due to the high lubricity and stress relaxation of the EPS-rich biofilms, ensuring continued contact with the receiver. The non-linear decrease of ongoing bacterial transmission under high shearing velocity is new and of relevance in for instance, high-speed food slicers and food packaging.

Recently there is a rising interest in various forms of engineered surfaces for the use in hospitals and nursing homes where the risk of nosocomial infections and epidemic spreads are high. Therefore, in **Chapter 4**, we had focused on bacterial transmission from biofilms of an EPS producing and a non-EPS producing staphylococcal strain from smooth silicon (Si) donor surfaces to smooth and nanopillared Si receiver surfaces. Staphylococcal biofilms were fully covering the donor surface before transmission. However, after transmission biofilms only partly covered donor and receiver surfaces regardless of nanopillaring, indicating bacterial transmission through adhesive failure at the interface between biofilms and donor surfaces as well as through cohesive failure in the biofilms. The number of bacteria per unit volume in EPS producing staphylococcal biofilms before transmission was two-fold smaller than that of the non-EPS producing strain. This difference increased after

transmission in biofilm-left-behind on the donor surfaces, due to an increased bacterial density for the non-EPS producing strain. This suggests that biofilms of the non-EPS producing strain remained compressed after transmission, while biofilms of the EPS producing strain were induced to produce more EPS during transmission and relaxed towards their initial state after transmission due to the viscoelasticity conferred to the biofilm by its EPS.

Bacterial adhesion is often described in terms of surface thermodynamics and by adhesion force analysis. Bacterial transmission, however, is mechanistically different from adhesion, as it involves bacterial detachment from a donor surface followed by adhesion to a receiver one. In the general discussion **(Chapter 5)** surface thermodynamics were opposed to adhesion force analyses, such as Atomic Force Microscopy measurements and transmission probabilities based Weibull analyses of adhesion forces as applied in the current literature towards bacterial adhesion, with their appropriate extensions towards transmission. Opposition of surface thermodynamics and adhesion force analyses will distinguish between transmission of bacteria from a donor covered with a (sub)monolayer of adhering bacteria or a multi-layered biofilm. Opposing adhesion and transmission not only yields a better understanding of bacterial transmission, but may stimulate researchers to more carefully consider whether an adhesion or transmission model is most appropriate in the specific area of application aimed for, rather than routinely relying on adhesion models.