Cell wall deformation and Staphylococcus aureus surface sensing
Harapanahalli, Akshay

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Chapter 6

General Discussion
General discussion

Bacterial communication with their environment takes place in two different systems: 1) Quorum Sensing (QS) and 2) mechanosensing (1, 2). The mechanism of QS is described in literature (1) for many bacteria, including *Staphylococcus aureus*. However, very little is known about the mechanism of mechanosensing which contributes to sensing of the environment when it is in contact with surfaces. Therefore, in this thesis we have studied the adhesion of *S. aureus* to biomaterials in terms of 1) Physical characteristics to the bacterial cell wall that contribute to surface sensing and 2) Gene specific responses of bacteria adhering to different biomaterials. To study physical characteristics (adhesion forces and cell wall deformation), we have used advanced state of the art techniques, like Atomic Force Microscopy (AFM) and Surface Enhanced Fluorescence (SEF). Molecular changes at the gene level due to external stress were studied using quantitative real time polymerase chain reactions.

**Cell wall deformation determined by atomic force microscopy and surface enhanced fluorescence**

In the field of microbiology, AFM is widely used to measure nanomechanical properties of the living cell. Properties such as visco-elasticity, single protein functionality, cell wall deformation and adhesion forces can be measured to understand nano-scale organization, dynamics of cell membranes and cell walls (1-3). Adhesion forces and cell wall deformations between the cell and the substratum can be quantified in the range of piconewtons (~ 10\(^{-12}\) N) and nanometers, respectively (3). In chapter 2 we have directly determined the cell wall deformation by measuring the polar radii (height images) of two *S. aureus* strains and their isogenic \(\Delta{pbp4}\) mutants (strains with a softer cell wall) to demonstrate that, \(\Delta{pbp4}\) mutants are 40% more deformable than their parent strains. In chapter 4 we have measured adhesion forces of the same wild-type and mutant strains on three different biomaterials (stainless steel, poly-methyl methacrylate and polyethylene) and have shown that, adhesion forces are substratum specific and stronger for the wild-type *S. aureus* than the \(\Delta{pbp}\) mutant strains. Although, the wild-type *S. aureus* strains have more rigid and less deformable cell walls, we can anticipate that differences in adhesion forces between the wild-type cells and the biomaterials can induce, adhesion force dependent substratum specific deformation.

Adhesion forces and cell-wall deformations measured using AFM give considerable understanding about cell–substratum interactions, but there are a few critical drawbacks.
using AFM. Firstly, AFM requires an external load, which is required to image the cell surface, which is used to calculate the cell wall deformation and its rigidity. Secondly, bacteria are immobilized (using α-poly-L-lysine or dopamine) to the AFM cantilever or the surface through chemical treatment (4). Application of an external load and chemical treatment can potentially introduce artifacts during studying bacterial adhesion forces when compared to the bacterial adhesion force to surfaces under natural conditions as in a flow chamber. For instance, cell wall deformations obtained using AFM imaging for S. aureus Δpbp4 mutants attached to α-poly-L-lysine coated surface, showed deformations between 49 – 82 nm (chapter 2) that were more or less similar than deformations measured by SEF (20 – 25 nm) (chapter 3). With SEF deformation of bacterial cell walls can be determined under natural conditions and at a macroscopic level (3 x 10^8 cells cm^{-3}), while the AFM can only be applied to a single bacterial cell and requires many experiments in order to get similar statistics as SEF. Therefore, application of new methods like SEF are a more appropriate and accurate method to evaluate cell–substratum interactions than AFM.

SEF as applied in this thesis is a very reliable and powerful method to measure the cell wall deformation. However, SEF also has a few drawbacks. Firstly, SEF (also known as metal enhanced fluorescence) can only be applied on metal surfaces due to the nature of the method. Secondly, we assumed that distribution of the fluorophores in the bacterial cell is homogeneous. To validate this assumption, other microscopical methods, like the super-resolution microscopy can be applied (5). With this method single molecule localization within a spatial resolution of 1 nm can be determined. Therefore, in order to determine cell-wall deformation SEF is the preferred technique but has the limitation that it can only be used on metal surfaces, therefore the AFM method is a good alternative. Moreover, the best way to overcome these limitations would be to apply both methods wherever possible to compliment the findings of one another.
General discussion

**Gene specific responses to biomaterials and mechanosensing in bacteria**

Sensing environmental stresses is an important part of bacterial survival. For mechanosensing, some bacteria have developed extracellular appendages like flagella, pili or curli to respond to adhesion or physical stress (6). Interestingly, *S. aureus* does not possess any extracellular appendages, yet it can respond to physical contact, suggesting that a generalized surface sensing approach must exist for all microorganisms and cells to respond to localized surface stresses upon adhesion. Eukaryotic cells have several points of contact between the cell and the surface, these points of contact are called focal adhesion points, which connect the extracellular membrane to the transmembrane linkers through an actin-myosin networks (7). In *Pseudomonas aeruginosa*, transmembrane links are established through MreB cytoskeleton, which has a similar function as the actin-myosin network and also regulate the type IV pili of *P. aeruginosa* to sense adhesion forces to surfaces and transmit regulatory signals for biofilm formation (8). The surface sensing of *S. aureus* possibly takes places through adhesion force induced cell wall deformation. In chapter 4, we show that cell wall deformation of *S. aureus* strains is due to adhesion forces caused by different biomaterials. Although, this is not a direct quantification of the cell wall deformation, we determined molecular changes that took place upon adhesion to three different biomaterials, stainless steel, poly-methyl-methacrylate and polyethylene. Matrix associated poly-N-acetylglucosamine and its corresponding icaA gene expression showed a remarkable correlation with adhesion forces measured on the three surfaces, confirming the effects of adhesion force induced cell wall deformation. Studies also show that icaA expression in *Staphylococcus epidermidis* is substratum specific in presence of gentamicin (9).

Bacterial cell wall deformation in its natural surface adhesive state can be quantified using SEF. The molecular effects of nanoscale cell wall deformations can be linked to adhesion forces arising from the substratum surfaces. Furthermore, investigating molecular links between mechanosensitive channels and cell surface proteins can reveal more insights into mechano-transduction, bacterial sensing of substratum surfaces and adaptability.
**Future perspectives**

Bacteria can sense and interact with the substratum and gain resistance. In this thesis, we have shown that membrane proteins can detect and respond to adhesion forces experienced by the cell wall to up-regulate antibacterial resistance. Mechanisms such as two-component systems serve as key models in understanding surface sensing and regulation of antibacterial resistance. However, bacteria regulate antibacterial resistance through more than one mechanism, and at an alarming rate. Infection causing strains detected in 2013 were resistant to four classes of antibiotics, which were still effective treatments in 2009 (10). Therefore, identifying alternative targets and mechanisms are very important in order to keep pace with growing antibacterial resistance. Mechanosensitive channels are ideal candidates for surface sensing and perhaps in regulating antibacterial resistance upon surface adhesion via opening and closing of the channels. Therefore, it would be worthwhile to investigate the impact of adhesion forces on channel opening and regulation of antibacterial susceptibility in the wild-type strains and compare it with mutants lacking mechanosensitive channels in biomaterial associated infections.
General discussion

REFERENCES


