Atomic Force Microscopy Investigation of Morphological and Nanomechanical Properties of Pseudomonas aeruginosa Cells

Atomic Force Microscopy (AFM) is unique in the aspect of studying living biological sample under physiological conditions. AFM was invented in 1986 by Binnig and Gerber and began in the early 1990’s to be implemented in life science. AFM can give a detailed three dimensional image of an intact cell, but also be used to examine the nanomechanical properties on single cell level. These qualities make AFM a powerful tool in biology and can be used to examine both morphological and nanomechanical response to various liquids environments, such as osmotic pressure, but also the effects of e.g. antibiotic treatment. Pseudomonas aeruginosa is a major opportunist human pathogen accounting for hospital-acquired infections, infections of ulcers and burn wounds, and is the predominant cause of chronic lung infections in Cystic Fibrosis patients. Regarding the treatment and control of P. aeruginosa infection multi-resistance clinical strains have become a rising problem. The last two decades a combination of the antibiotic ciprofloxacin and the antimicrobial peptide colistin, have proven efficient in controlling the infections. However, the exact mechanisms of action for colistin are not known. Advances of AFM are that sample preparation does not demand fixation, staining or coating and the sample it not examined under high vacuum. It is not surprising that mounting plantonic bacteria on a substrate and dehydration will lead to some extent of alteration. Here a flattening of both intact bacterial cells and nanostructures such as pili and flagella was obvious. The morphological effect of colistin and ciprofloxacin treatment was examined for dehydrated bacteria, and noticeable effects were observed. It is however, not possible to distinguish between primary effects of the antibiotic treatment and secondary effects caused by the dehydration. When visualizing bacteria in liquid the image resolution is reduced, but the bacteria are kept in the natural environment and therefore not subject to the same degree of artifact formation as observed for dehydrated bacteria. However, when imaging rode-shape Gram-negative bacteria such as P. aeruginosa the immobilization of the bacteria is a major challenge. Here gelatin coated mica proved to be an efficient and stable immobilization. AFM was used to examine the morphological and nanomechanical properties of P. aeruginosa after colistin treatment. AFM revealed significant changes in the fraction of individual bacteria and bacteria undergoing proliferation, and decrease of cell length of mother and daughter cells. The results indicated that colistin arrested the bacterial growth just after septum formation. Furthermore did the morphology change from a smooth bacterial surface to a more irregular and wrinkled phenotype, indicated loss of lipopolysaccharides and perhaps other surface proteins. Also the nanomechanical properties change significantly indication a stiffness of the bacterial cell wall and an increase turgor pressure. Microcontact printing of specific antibodies was used to selectively extract Escherichia coli out of both pure and mixed cultures. The technique was very efficient for E. coli both laboratory and wild-type strains, but was unsuccessful for P. aeruginosa and Salmonella typhimurium. Therefore the technique was not suitable for the broad spectrum diagnostic tool originally visualized. Low antibody-antigen affinity and inefficient exposure of the antibody recognition sites could be an explanation for the lack of success. Work presented in this thesis proves what powerful tool AFM is in bacteriology. AFM of bacteria in liquid can be used to examine the effect of changes in the surrounding environment and of antimicrobial peptides on nanoscale, both with regard to morphology and nanomechanical properties.

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