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Determination of Staphylococcus epidermidis fibrinogen binding protein Fbe adhesion forces using atomic force microscopy

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Title (English)

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Title (Swedish)

Abstract

The surface morphology and fibrinogen binding capacity of two *S. epidermidis* strains, a wild type expressing the fibrinogen binding protein (Fbe) and its isogenic mutant, were compared. Adhesion forces between fibrinogen and Fbe were investigated by using atomic force microscopy. The results showed a significant lower number of adhesion events in the Fbe mutant.

Keywords

Biofilm, catheter infection, *Staphylococcus epidermidis*, fibrinogen, atomic force microscopy, force measurements

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Determination of *Staphylococcus epidermidis* fibrinogen binding protein Fbe adhesion forces using atomic force microscopy

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Sammanfattning

Staphylococcus epidermidis är en vanligt förekommande och normalt sett ofarlig bakterie som utgör en del av vår hudflora. Om bakterien däremot kommer in i kroppen, exempelvis vid introduktionen av ett medicinskt implantat, kan den påbörja bildandet av en biofilm vilket kan ge upphov till svårbehandlade infektioner.

I det här examensarbetet har ett högupplösande atomkraftsmikroskop använts för att studera två *S. epidermidis* stammars ytstruktur och bindningsförmåga till plasmaproteinet fibrinogen. Stammarna var en vildtyp som på cellytan uttrycker det fibrinogenbindande proteinet Fbe och en mutant som saknar proteinet. Vidare har mekaniska egenskaper och interaktioner mellan fibrinogen och Fbe undersökts. Detta är möjligt då atomkraftsmikroskopet, vilket kan jämföras med en gammeldags grammofonspelare vars spets rispar sig fram över ytan, konstruerar en bild över den nativa ytan samtidigt som atomkrafter mellan spets och yta registreras.

När de två *S. epidermidis* stammarna fick binda in till fibrinogentäckta mica ytor bildade mutanten betydligt färre kolonier jämfört med vildtypen. Mätningar av adhesionskrafter mellan fibrinogen och den Fbe negativa mutanten visade på en signifikant minskning av antalet adhesioner jämfört med vildtypen. Resultaten bekräftar att Fbe proteinet spelar en viktig roll vid den initiala adhesionen av bakterien till medicinska implantat.

Examensarbete 20 p

Civilingenjörsprogrammet i molekylär bioteknik

Stockholms Biovetenskapliga Forskarskola maj 2007

1. Introduction

The introduction of medical devices, such as catheters, frequently results in infections. Catheter related infections are believed to result from a two-step process (1). The implant is initially covered in host factors facilitating the adherence of bacteria to the surface. Next, the bacteria accumulate and a biofilm is formed. *Staphylococcus epidermidis*, a common member of the skin flora, an opportunistic pathogen and known biofilm producer, is the foremost cause of medical device-related infections (2, 3). In the USA catheter-associated urinary tract infections constitute 40% of all infections resulting from medical care and *S. epidermidis* infections alone cost 1 billion $ per year and contribute to the already extensive use of antibiotics (2, 4).

The interactions between bacteria and the implant surface are mediated by a number of factors (3). One of the molecular mechanisms for *S. epidermidis* adhesion and biofilm formation is its binding to fibrinogen, a soluble plasma protein involved in coagulation (5). In 1998 Nilsson and co-workers identified the *fbe* gene in *S. epidermidis* and found the Fbe-protein to be responsible for binding to fibrinogen (5). The same group created an *fbe* knock-out which bound less well to fibrinogen compared to the wild type strain (6).

The first aim of this study was to set up a model system to study the ability of *S. epidermidis* wild type and its derivate lacking the *fbe* gene to bind to fibrinogen coated surfaces and the properties of these two bindings. The binding of the two strains was studied on air dried samples by Light Microscopy (LM) and was further characterised by Atomic Force Microscopy (AFM), a technique increasingly used for studying morphological characteristics of microbial surfaces (7). The AFM technique, first developed by Binnig in 1986, is a high-resolution type of scanning probe microscopy providing three-dimensional images (7, 8, 9). The AFM technique offers a method for biological samples to be studied in their native environment and also enables quantitative measurements of forces exhibited between tip and sample surface. Drying the samples is however not optimal if the goal is to study bacteria in a physiological environment. Therefore, the second aim was to set up a system for imaging and measuring mechanical properties of hydrated bacteria. Single cells were immobilized in the pores of a polycarbonate filter membrane, a method of mechanically trapping the cells without changing the chemical composition of the bacterial surface. The immobilized bacteria were imaged in liquid and force measurements of tip-surface interactions with and without

fibrinogen functionalized tips were performed. The results showed that the Fbe positive wild type frequently exhibits stronger adhesion forces when probed with a fibrinogen-coated tip compared to the Fbe mutant.

2. Materials and methods

2.1 Bacterial strains

Staphylococcus epidermidis strains used in this study were obtained from Jan-Ingmar Flock, Karolinska Institutet, Sweden (Table 1.).

Table 1. Bacterial strains used in this study

Strain	Description	Reference
HB	Isolated from a human patient with osteomyelitis	Nilsson M., et al. (1998)
SE56	HB, *fbe*::Gm^R	Pei L. and Flock J. (2001)

2.2 Media and growth conditions

S. epidermidis were routinely grown on horse blood agar plates or in 20 ml cultures of Luria-Bertani broth medium (10 g Bacto tryptone, 5 g Bacto yeast extract, 10 g NaCl per liter) in 100 ml Erlenmeyer flasks at 37 ºC with shaking at 200 r.p.m.

2.3 Fibrinogen coating

AFM imaging of air-dried samples required a good fibrinogen binding substrate with a smooth surface suitable for AFM. A fibrinogen coating assay was set up to compare two types of surfaces: plastic Thermanox cover slips (Nunc, Denmark) known to bind fibrinogen and atomically flat mica surfaces Grade V-4 (SPI Supplies, USA). Freshly cleaved mica slides of approximately 1 cm^2 and Thermanox surfaces (0.5 cm Ø) were placed in 6 well Corning culture plates (4 cm diameter) and coated with concentrations ranging from 0.0007 to 40

μg/ml of human fibrinogen, obtained from Jan-Ingmar Flock, in phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.4 mM KH_2PO_4 [pH 7.4]) over night. Rapid washing of the surfaces was done with 2 ml PBS-Tween 20 (0.05% v/v) (Sigma) two times to prevent unspecific binding and blocking was done with 2 ml 2% (w/v) bovine serum albumin (BSA) (Sigma) in PBS at 37 ºC for 1 hour. After washing each surface five times 1.5 ml rabbit antibodies against human fibrinogen (DAKO, Denmark) were added (1:500 dilution) and incubated at 37 ºC for 1 hour without shaking. Surfaces were once again washed five times and antibody binding was detected with 1.5 ml (1:2000 dilution) HRP-labeled antibodies (DAKO) against rabbit IgG antibodies incubated at 37 ºC for 1 hour. The substrate solution was prepared from *o*-phenylenediamine dihydrochloride tablets (DAKO) according to the manufacturer's instructions. The colorimetric reaction was stopped with 200 μl 0.5 M H_2SO_4 after ten minutes and the product was measured spectrophotometrically at λ=490 nm. Fibrinogen coating was also carried out to functionalize AFM probes prior to force measurements on bacterial surfaces. The cantilever was submerged in a 10 μg/ml fibrinogen solution for one hour, subsequently washed three times in PBS and used immediately to prevent the fibrinogen from drying.

2.4 Sample preparation

Samples studied in a dry condition were prepared by growing diluted (1:100) overnight cultures to mid-exponential phase of A_{600}=0.5. The bacteria were washed three times in 10 ml PBS, dissolved in water with 0.05 % (v/v) Tween 20 to an A_{600} of 1.0. Fibrinogen coated mica slides of approximately 1 cm^2 were placed in 6 well Corning culture plates (4 cm diameter) with 2 ml bacterial suspension. Bacteria were allowed to adhere for 30 minutes at room temperature without shaking and subsequently surfaces were immersed in double distilled water five times. Surfaces were air-dried at room temperature in a dust free environment and glued onto glass microscope slides using nail polish. For AFM imaging in liquid, cultures were grown in LB with 0.05 % (v/v) Tween 20 to A_{600}=0.3. A syringe was used to gently push 10 ml cell suspension through the smooth side of an isopore polycarbonate membrane (Millipore, Sweden) with a filter diameter of 14 mm and a pore diameter of 1.2 μm. The filter was washed carefully in destilled water, mounted with double adhesive water-resistant tape on a glass microscope slide, covered with a drop of PBS and used immediately.

2.5 Light Microscopy

Dried samples were studied with an Eclipse E400 light microscope (Nikon instruments) with a 10 x objective and images were taken with a connected CCD camera (Nikon instruments).

2.6 Atomic Force Microscopy

AFM imaging was performed at room temperature using a Bioscope (Veeco Instruments Inc, USA) connected to an optical microscope. Imaging in air was done on dried samples and imaging in liquid was performed in a drop of 200 µl PBS forming a meniscus between the filter surface and the glass fluid tip holder. All images were obtained in contact mode by using V-shaped cantilevers with conical silicone nitride tips with a nominal force constant of 0.05 N/m and at a scan frequency of 1.47 Hz. Data were analysed with the software WSxM (Nanotech Electronics, Spain www.nanotech.es). In AFM contact mode, the cantilever is raster scanned in the X, Y direction in gentle contact with the sample surface. The deflection of the cantilever is detected by a photodetector sensing the reflection of a laser beam focused on the reflective side of the cantilever (Fig. 1).

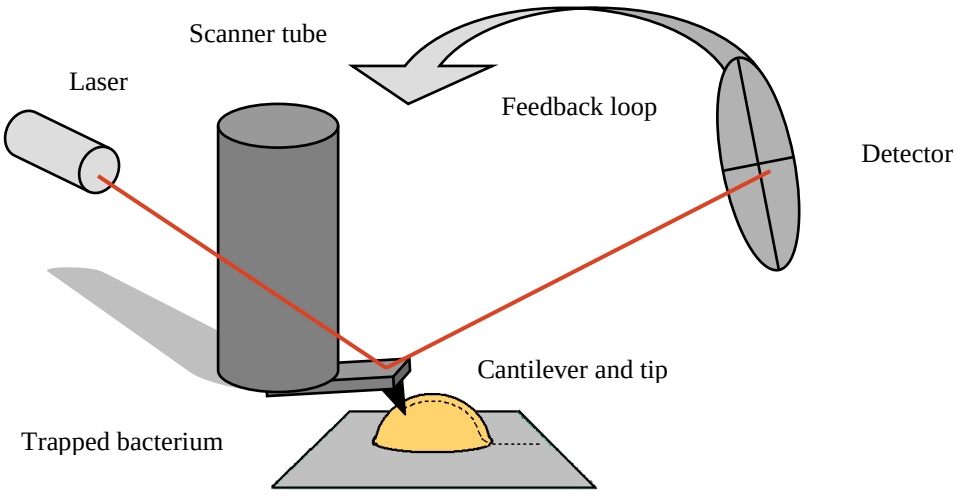


Figure 1. Schematic illustration of the AFM technique.

To minimize the force on the sample surface, a feedback loop attempts to keep the deflection of the cantilever constant which is accomplished by the vertical movement of the piezoelectric tube to which the cantilever is attached. Recordings of the tube motion enable construction of an image of the sample on a computer screen. (7, 9)

2.7 Force imaging

AFM can be used to determine mechanical properties by recording a force versus distance curve when measuring interactions between tip and sample surface. The force bending the cantilever is measured as the tip is brought close to, or in contact with, the sample surface and then pulled away (Fig. 2). Force curves hold information about the adhesion between tip and sample as well as about the local Young's modulus, a measure of a surface elasticity (10).

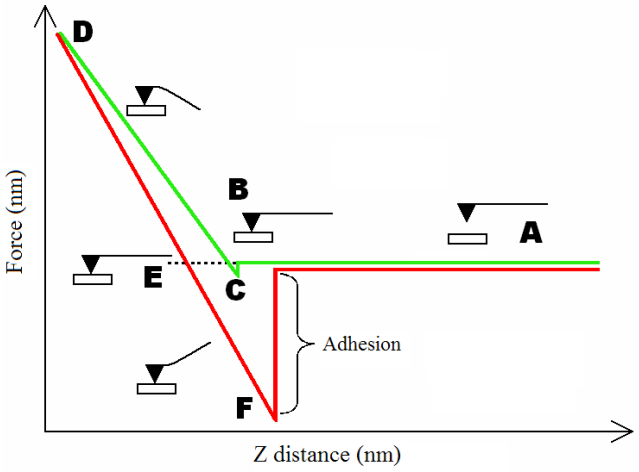


Figure 2. The anatomy of an idealised force versus distance curve. A) The tip approaches the surface. B) As the tip is brought close to the surface attractive forces will cause it to jump into contact with the surface. C) Tip in contact, cantilever deflection increases. D) Cantilever is withdrawn. E) Cantilever comes free from the surface.

Forces versus distance curves were recorded for both strains, with and without a fibrinogen coated tip. All experiments were performed using tips with or without fibrinogen coating on several individual bacteria from the two strains. Force measurements were performed by probing the tip on the cell and one to nine force curves were obtained from each individual cell. Of the overall 188 force curves 49 were recorded using fibrinogen coated tip on wild type HB, 51 from uncoated tip on wild type HB, 41 from fibrinogen coated tip on mutant SE56 and 47 from probing an uncoated tip on the mutant SE56. The force curve can be described according to Hook´s law, $F = k\Delta Z$. The restoring force F in Newton (N) exerted by the spring (cantilever) is obtained by multiplying the spring constant k (N/m) with the distance ΔZ (m) which the spring is elongated, or in this case the deflection sensitivity. To obtain accurate absolute values from force-distance measurements the spring constant and the deflection sensitivity of the system have to be determined which was not done in this study. However, force curves were not recorded to obtain absolute values of the forces, but rather to obtain a comparison between the wild type and mutant.

3. Results

3.1 Mica binds fibrinogen

Performing AFM imaging on air-dried samples requires strong immobilization of the cells to a substrate. In this study we wanted to coat substrates suitable for AFM with fibrinogen to compare the fibrinogen binding capacity of the fibrinogen binding wild type HB and its isogenic mutant SE56. Previous studies have used Thermanox or other plastic materials for fibrinogen coating (5). However, we wanted to use the atomically flat mica surface since it is more suitable for AFM imaging. The fibrinogen binding capacity of Thermanox and mica surfaces was examined in a coating assay. Saturation of fibrinogen binding to the two surfaces was reached at about 0.5 μg/ml and the results indicate equivalence in the fibrinogen binding properties of both surfaces (Fig. 3). Hence, mica was chosen as a substrate and the coating concentration was set to 10 μg/ml to ensure saturated binding to the surface.

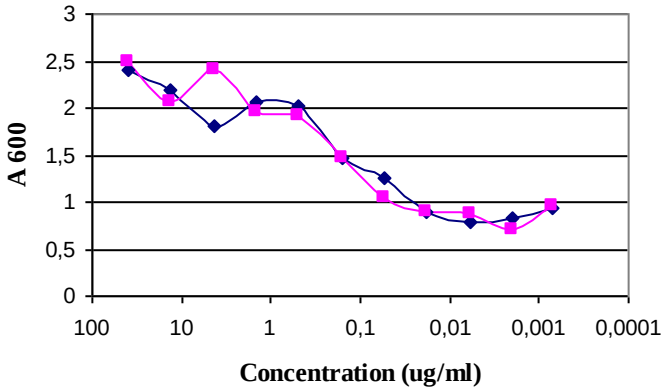


Figure 3. Fibrinogen coating assay. Surfaces were coated in a concentration range of 0.0007 to 40 μg/ml fibrinogen in PBS over night. Thermanox, Mica.

3.2 Binding of HB and SE56 to fibrinogen coated mica slides

Next, we used AFM to examine if the choice of solvent would affect the air-dried samples of wild type bacteria binding to fibrinogen. Bacteria were dissolved in either PBS or water before they were allowed to adhere to the fibrinogen-coated surfaces for 30 minutes. Contact mode AFM imaging of the two *S. epidermidis* strains bound to fibrinogen-coated mica surfaces was performed on representative dried samples. AFM imaging showed that the choice of solution was crucial for obtaining good quality AFM images. Bacteria dissolved in PBS left patterns from salt crystals while bacteria dissolved in water did not leave any traces from the solvent (Fig. 4). Therefore, water was chosen as solvent.

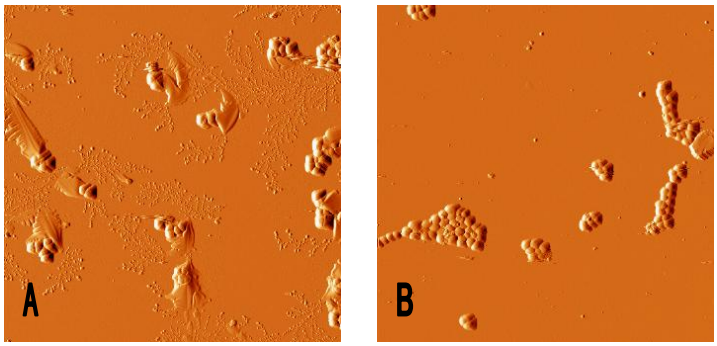


Figure 4. AFM deflection images, 40x40 µm, of strain HB dissolved in (A) PBS and (B) water allowed to adhere to fibrinogen coated surfaces for 30 minutes.

3.3 The effect of water, PBS and Tween on cell viability

Since water proved to be a more suitable solvent for the bacteria when performing AFM on the dried samples we wanted to confirm that solving bacteria in water did not affect cell viability. Bacteria were dissolved in either PBS or water for 30 minutes at room temperature and viable counts from the two conditions were carried out. The results were also compared to viable counts from bacteria dissolved in LB. There was no significant difference between the number of viable cells dissolved in H_2O, PBS or LB (Table 2). This suggests that the effect of PBS and water on the cells are equal under our conditions and that dissolving bacteria in water did not lyse the cells. Further, to facilitate AFM imaging we wished to prevent bacteria from aggregating before attaching to the coated surface. To reduce non-specific binding between bacteria and surfaces in the adherence stage 0.05 % of the detergent Tween was subsequently added to the solvent. Tween did not affect the growth rate of either *S. epidermidis* strain (Fig 5.) in liquid cultures and therefore 0.05 % Tween was added to all liquid cultures.

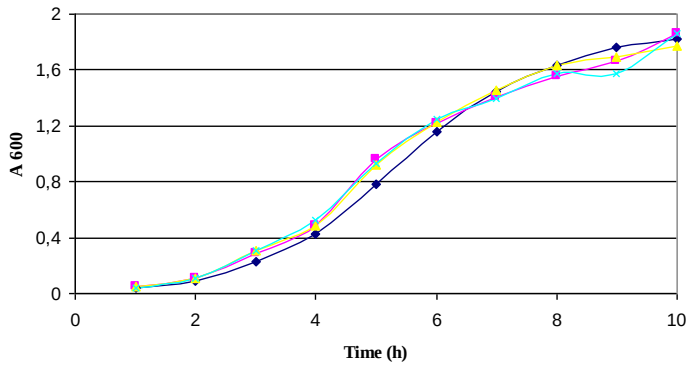


Figure 5. Growth rates of the two strains in LB and LB 0.05 % Tween. HB in LB, HB in LB, 0.05 % Tween, SE56 in LB, SE56 in LB 0.05 % Tween.

	HB	**SE56**
LB	$2.3x10^8$	$2.0x10^8$
PBS	$1.9x10^8$	$1.3x10^8$
H_2O	$1.2x10^8$	$9x10^7$

Table 2. Viable counts of the two strains from three solvents.

3.5 LM and AFM imaging of dried bacteria

Having established conditions that fulfilled the requirements of both *S. epidermidis* fibrinogen binding and the AFM technique, samples were prepared for both light microscopy and atomic force microscopy. Bacteria were allowed to adhere to fibrinogen coated mica surfaces for 30 min. The surfaces were washed by gentle immersion in double distilled water and air-dried before being glued onto microscope glass slides. The surfaces were first studied with the light microscope and images were taken at several different locations. Comparisons of the two strains collectively showed that the SE56 mutant formed significantly fewer colonies than the HB wild type (Fig. 6). This finding agrees with results obtained in a previous study (6).

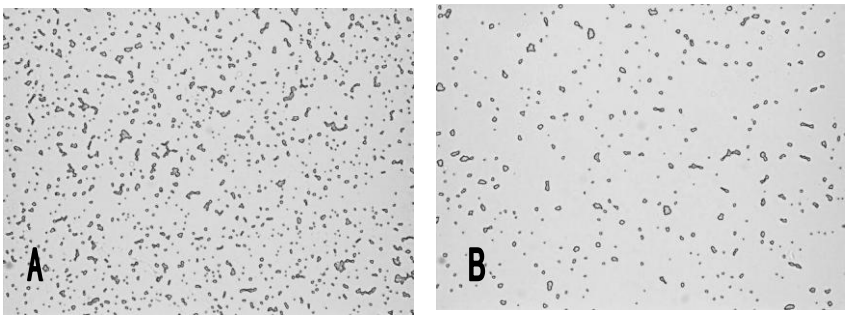


Figure 6. Representative light microscope images of (A) HB and (B) SE56 dissolved in water and allowed to adhere to the fibrinogen coated mica surfaces before air-dried.

High-resolution AFM deflection images providing high contrast morphological details were used to examine if an apparent difference of wild type and mutant binding to fibrinogen could be observed. Representative AFM deflection images of HB and SE56 are shown in Figure 7. AFM imaging of wild type HB and mutant SE56 dissolved in water and adhered to fibrinogen coated mica surfaces showed no apparent difference cell surface morphology. Both strains exhibited differently sized colonies of tightly packed cells attached to the fibrinogen-coated surface.

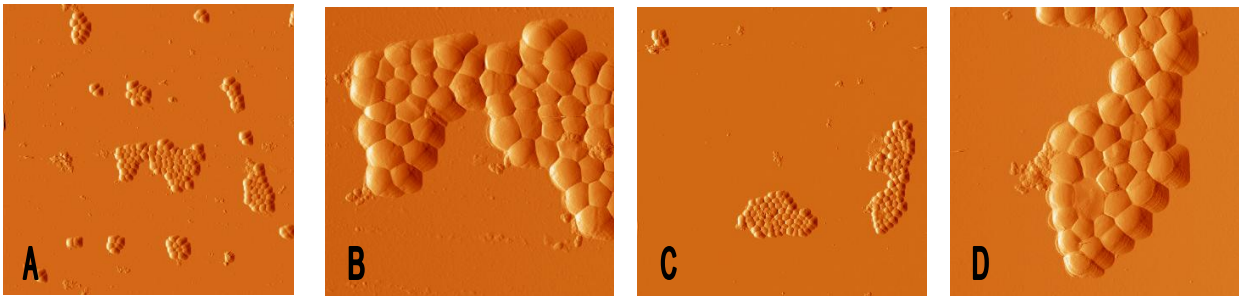


Figure 7. AFM deflection images (A) HB 40x40 μm, (B) HB 20x20 μm, (C) SE56 40x40 μm, (D) SE56 20x20 μm.

Several of the features seen in samples from both wild type and mutant are represented in Figure 8. Slime, defined by having properties of sticking to the AFM tip and creating smeary lines, was however more commonly observed in HB. A non-sticky substance, probably debris from collapsed cells, could be observed attached to the cells in both strains.

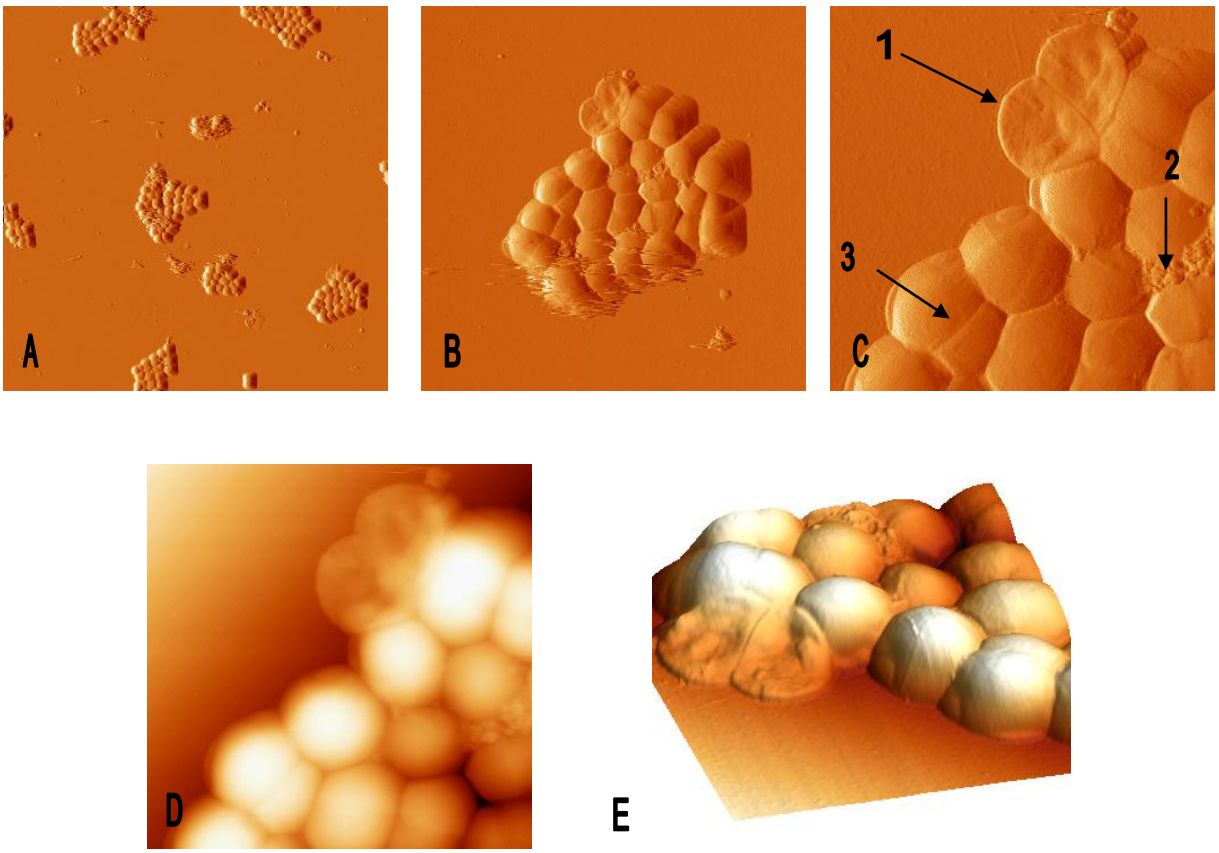


Figure 8. AFM deflection images of HB on fibrinogen coated surfaces shown in different scan sizes: (A) 40x40 µm, (B) 10x10 µm (C) 5x5 µm. Collapsed cells (arrow 1), cell debris (arrow 2) and scars from cell division (arrow 3) were observed. (D) Topography image, with light colours representing elevated regions, used to create a 3D image (E).

3.6 Polycarbonate membranes efficiently immobilize bacteria

AFM imaging in liquid offers the advantage of keeping the cells in their native environment and it also reduces sample damage since less force is required for tracking the tip over the sample surface. The next step was to set up a system for AFM imaging of bacteria in a liquid solution and performing force measurements. AFM imaging in liquid and the use of bacterial cells as force probes requires a strong immobilization of the cells to the substrate. In addition, force measurements on bacterial cell surfaces under physiological conditions demands an

immobilization method not affecting the chemical composition of the surface (11). Mechanically trapping in pores of a polycarbonate membrane with a pore diameter comparable to the cell diameter has been shown to be a successful method for immobilization of single cells (12, 13, 14). In this study single trapped cells, well anchored in pores, were identified in a topography image with 40x40 µm scan sizes. The zoom function was used to obtain scan sizes of 1.5-3 μm^2 of a single bacterium before force measurements were conducted (Fig. 9).

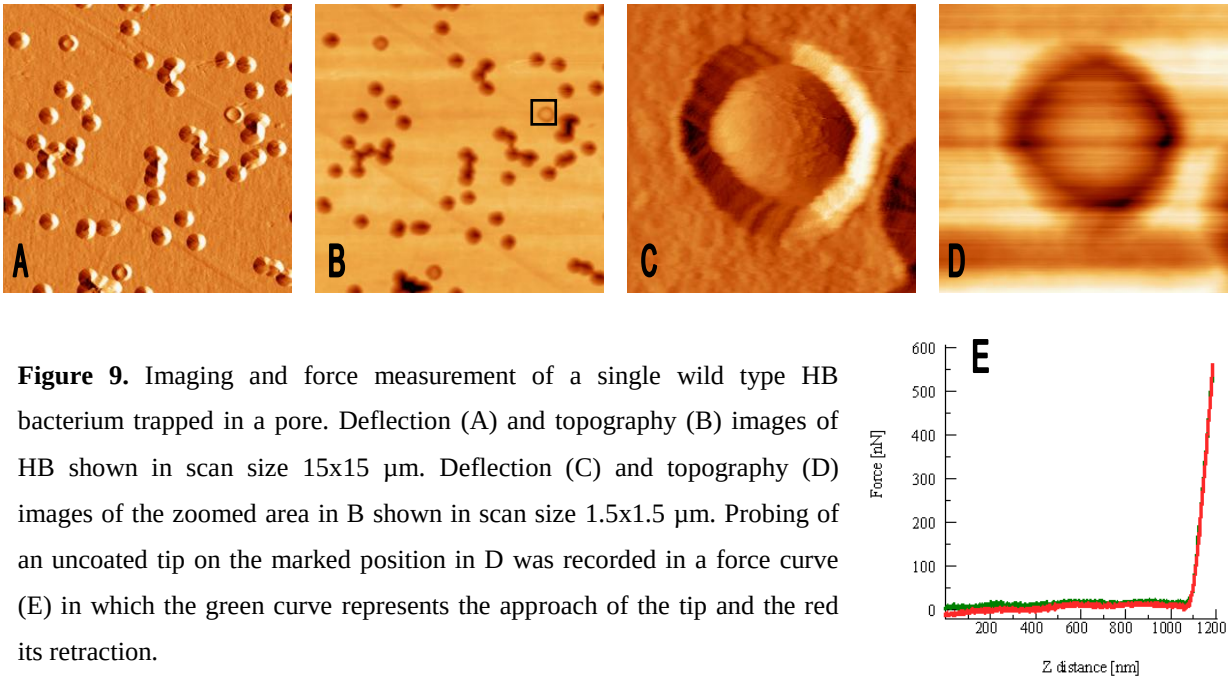


Figure 9. Imaging and force measurement of a single wild type HB bacterium trapped in a pore. Deflection (A) and topography (B) images of HB shown in scan size 15x15 µm. Deflection (C) and topography (D) images of the zoomed area in B shown in scan size 1.5x1.5 µm. Probing of an uncoated tip on the marked position in D was recorded in a force curve (E) in which the green curve represents the approach of the tip and the red its retraction.

3.7 Force versus distance curves

The AFM force measurements performed on hydrated bacteria resulted in a total of 188 force curves. The force curves were characterized for the incidence or absence of three different properties: adhesion peaks, curvature and oscillations (Fig. 10) which were used for comparisons of the wild type and mutant strains. Firstly, force curves exhibiting adhesion peaks in the retraction curve were examined. When measuring forces of biological cells these events are often referred to as unbinding events between the tip and components on the samples surface, for example proteins (15). Secondly, the shape of the curvature in the contact region was defined as significant if a contact or zero force point could not be determined. An interpretation of curvatures is that they are the result of long-range repulsive forces upon approach of the tip (16). The third property was initially observed in measurements of the

wild type HB with fibrinogen-coated tip. More or less severe oscillations enabled measurements to be carried out and oscillations were also observed in experiments with other tip-sample combinations.

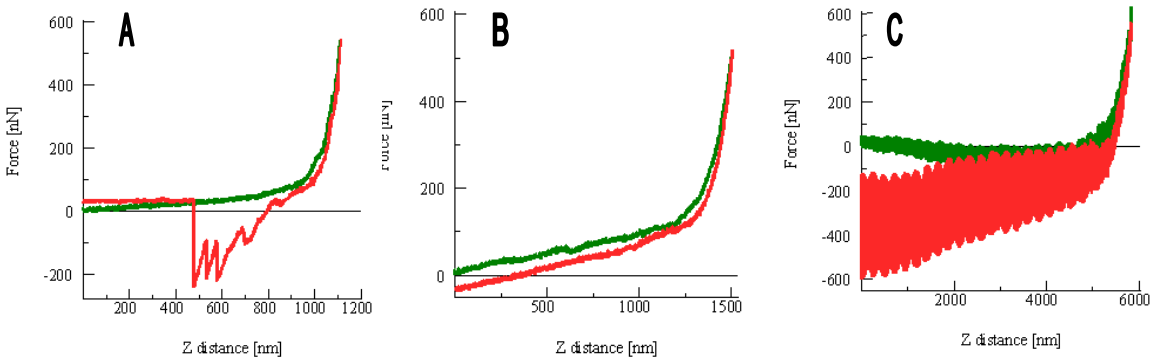
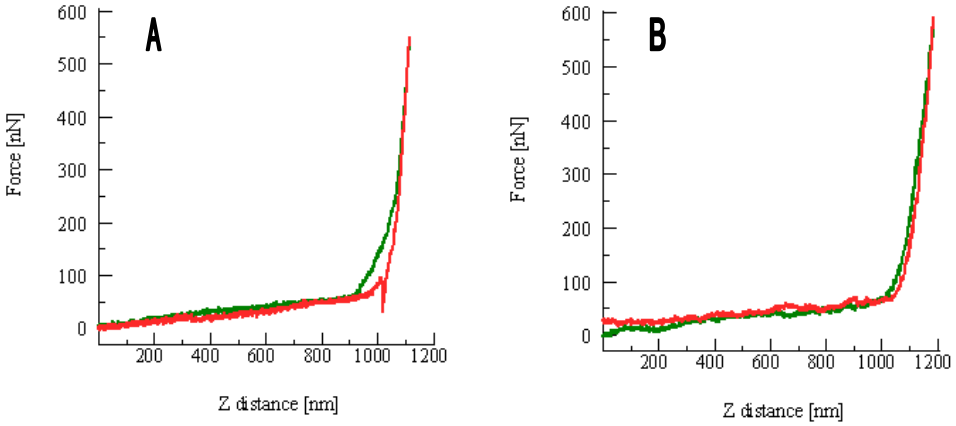


Figure 10. Examples of force curves recorded when probing wild type HB with a fibrinogen-coated tip exhibiting the three different features: adhesion peaks (multiple) in the red retraction curve (A), distinct curvatures (B) and heavy oscillations (C).

Representative force curves of the two strains probed with and without coating of the tip can be seen in Figure 11. A distinct adhesion peak is visible in the red retraction curve from probing HB with a fibrinogen-coated tip (Fig. 11 A). Neither wild type HB probed with uncoated tip (Fig. 11 B) nor mutant SE56 probed with or without fibrinogen coating (Fig. 11 C and D) showed any signs of adhesion peaks, curvature or oscillations in these force curves.



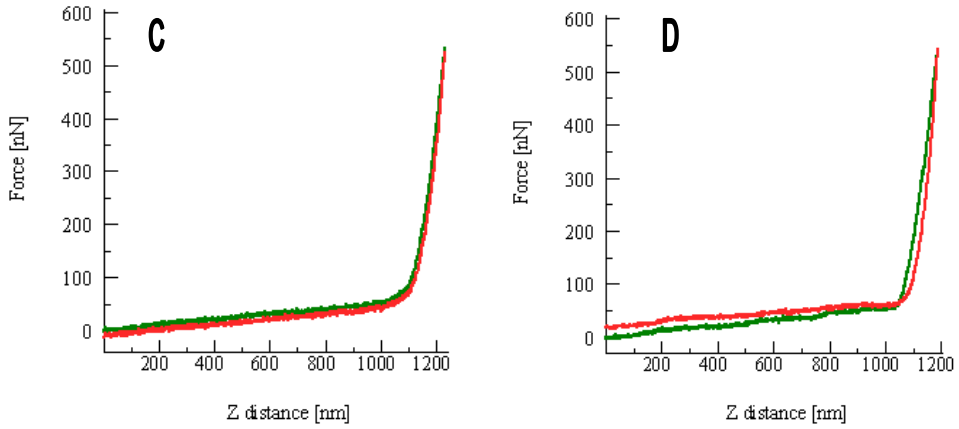


Figure 11. Representative examples of force curves recorded from each tip-sample combination: fibrinogen-coated tip on wild type HB (A), uncoated tip on wild type HB (B), fibrinogen-coated tip on mutant SE56 (C) and uncoated tip on mutant SE56 (D).

The distribution of adhesion peaks, curvature and oscillations among the total 188 obtained force curves can be seen in Table 3. Nearly 30 % of the force curves acquired from probing a fibrinogen-coated tip on the Fbe positive wild type HB showed significant adhesion peaks. In contrast, only about 7 % of the force curves showed adhesion peaks when probing with a fibrinogen coated tip on the Fbe negative mutant SE56. Further, less than 5 % of the force curves from probing mutant and wild type with an uncoated tip exhibited adhesion peaks. Hence, these interactions probably represent non-specific interactions.

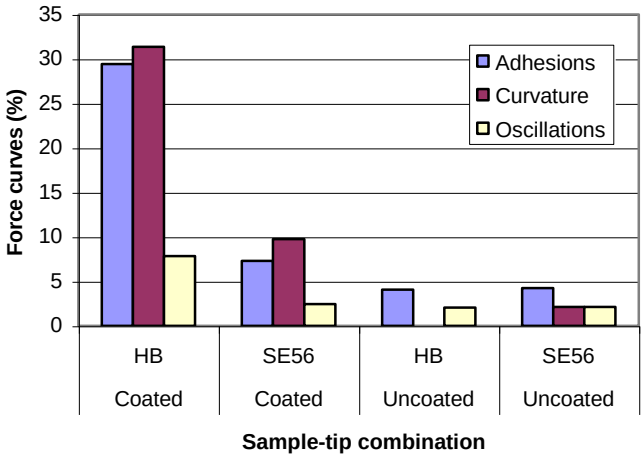


Table 3. Distribution of adhesion peaks, curvatures and oscillations among the total 188 obtained force curves.

The presence of curvature was seen in more than 30 % of the force curves probing wild type HB with a fibrinogen-coated tip and in about 10 % of force curves probing mutant SE56 with

a fibrinogen-coated tip. The strongest and most frequent oscillations, 8 % of the force curves, were observed when probing the Fbe positive wild type HB with a fibrinogen-coated tip. However, this number only represents the completed force curves and do not include the more than twice as many trials interrupted by heavy oscillations of the system.

4. Discussion

In this study AFM has been used both as a tool for imaging of *S. epidermidis* bound to fibrinogen coated mica surfaces and for measuring of adhesion forces between fibrinogen and fibrinogen binding protein, Fbe, on bacterial surfaces. No apparent difference in morphological details of the wild type and mutant strains was observed in high-resolution AFM imaging. However, a significant difference in fibrinogen binding of wild type HB and mutant SE56 could be seen with LM. AFM force measurements with fibrinogen functionalized probes showed that the Fbe positive wild type frequently exhibited stronger adhesion forces when probed with a fibrinogen-coated tip compared to the Fbe negative mutant.

When setting up a model system a range of parameters have to be analysed to attain stringent conditions. The conditions for the first aim of this study, imaging of S. epidermidis on fibrinogen coated mica surfaces, had to meet requirements of both *S. epidermidis* fibrinogen binding and the AFM technique. The sample preparation used for the air-dried samples was shown to be stringent enough to detect a difference in fibrinogen binding between *S. epidermidis* wild type and mutant in LM but not in AFM. However, AFM provided information about cell morphology, surface characteristics and debris from collapsed cells. Features like the smoothness of Gram-positive cell surfaces and ridges from cell division previously described by Umeda and co-workers were also observed in this study (17). The main challenge addressed in the sample preparation procedures for air-dried samples was to reduce substances interfering with the AFM imaging. In general two substances were observed: a non-sticky substance, most probably debris from collapsed cells, and a sticky slime which severely impaired AFM imaging. Sticky slime and dried media not removed by washing was more frequently seen in samples from the wild type HB compared to the mutant

SE56. If this slime is present by chance or associated with the expression of *fbe* is still to be investigated as is the influence of the washing procedures.

Setting up a system for AFM imaging in liquid allowed adhesion force measurements of bacteria under native conditions. The obtained force curves from wild type and mutant were characterized for the incidence or absence of adhesion peaks, curvature and oscillations. The adhesion peaks seen in force curves recorded with fibrinogen-coated tip on the wild type HB imply strong interactions between fibrinogen and Fbe surface proteins. However, similar adhesion peaks have previously been interpreted to reflect an event where the tip is picking up material from the cell wall during indentation (15). *S. epidermidis* is not known to have many adherence components for the extracellular matrix, however there is a possibility that the interaction is not specific (18). Background of non-specific interactions was seen when probing with a coated tip on the mutant as well as with an uncoated tip on both wild type and mutant. An example of a surface protein that could be capable of interacting with the tip is the slime substance called polysaccharide intercellular adhesin (PIA) seen in several *S. epidermidis* strains (18).

The interpretation of the force curves curvatures is more difficult than adhesion peaks. One theory is that they are the result of long-range repulsive forces upon approach of the tip (16). In this study all measurements were performed in PBS buffer of pH 7.0 causing both the coated and uncoated tip to have a negative charge (15). If a negative tip approaches a positive surface a snap-in effect can be seen in the curve of the approaching tip. No such snap-in was observed in the force curves and this indicates that the surface most likely is negatively charged. This would be in line with the general accepted concept of bacteria having an overall negatively charged surface. If this is the case and both surface and tip are negatively charged this would give rise to long-range repulsions. However, the large number of curvatures observed in contact regions in force curves of wild type HB probed with fibrinogen may have different interpretations. If a coated tip was to be more negatively charged than an uncoated tip this would result in more long-range repulsion in force curves with a coated tip. In this case curvatures would have been as frequent in force curves probing a coated tip on the mutant SE56 as on the wild type, however this is not supported by the data. Curvatures may also be due to deformation of the soft sample and since no high-resolution images were taken after force measurements it cannot be ruled out that sample damage has contributed to the observed long-range repulsion forces (16, 19).

Oscillations were first observed in force measurement attempts probing a fibrinogen coated tip on wild type HB. The strong oscillations of the system made it impossible to carry out measurements in more than half of the attempts to create force curves. These attempts are not included in the data. The observed oscillations could be due to strong specific interactions between a highly fibrinogen coated tip and the Fbe protein on the wild type bacterial surface. Another possible explanation to an oscillating system is that the fibrinogen coating affects the physical properties of the cantilever and thereby altering e.g. the spring constant, which in turn might thwart proper measurements. However, the observed oscillations when probing mutant SE56 with fibrinogen-coated tip were considerably fewer compared to wild type HB. This implies that the interaction between fibrinogen and Fbe protein most likely is connected to the formation of oscillations.

Taking these results in consideration from a cell-material interaction or biomaterial perspective we confirm that the Fbe protein plays an important role in the initial adherence to fibrinogen coated surfaces. Altogether, the many promising applications of AFM make it one of the most progressing and powerful tools for determining specific forces in for example microbial adhesion processes (16). The next step in this study would be to determine absolute values of the adhesion forces and compare these to values of other adhesins as well as to determine their specificity. We have done attempts to confirm the specificity of the fibrinogen binding capacity of Fbe by introducing soluble Fbe protein during force measurements with fibrinogen-coated tips but no difference in interaction forces was observed and additional work on recording conditions is required (20). AFM force measurements could also have promising applications for determining other receptor-protein interactions and finding drugs capable of blocking these interactions. However, functionalizing the tip is a critical step since the binding between molecule and tip has to be stronger than the interaction studied and the molecule must still be mobile enough to interact with the sample (20).

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