Determination of the shear force at the balance between bacterial attachment and detachment in weak-adherence systems, using a flow displacement chamber

Abstract

We introduce a procedure to determine shear forces at the balance between bacterial attachment and detachment under flow, that can be applied to determine adhesion forces in weakly adhering systems, such as polymer brush-coatings, which are currently in the center of attention for the control of bacterial adhesion and biofilm formation.
Introduction

Flow displacement systems, like the parallel plate flow chamber (PPFC), provide a powerful tool to study adhesion of colloidal particles, including bacteria, to surfaces under different hydrodynamic conditions [1]. Experimental observables, i.e. the number of adhering bacteria and their distribution on the surface, are used to derive attachment and detachment characteristics. A usual way to obtain qualitative information on the strength of the bacterium-surface bond in the PPFC is to simply pass an air-bubble through the chamber and analyze the number of bacteria remaining on the surface: the force exerted by the air-bubble on an adhering micron-sized particle is around $10^{-7}$ N [1]. Therefore, this method is too insensitive to be used in systems with weak bacterium-surface interaction forces, such as polymer brush-coatings.

One of the big advantages of PPFC is the adjustability of the shear rate and shear stress at the surface. These quantities are related through the equation: $\tau = F/A = \eta \sigma$ where $\tau$ is the shear stress, $F$ the force, $A$ the area on which the force is exerted, $\eta$ the absolute viscosity and $\sigma$ the shear rate. The wall shear rate is related to the flow rate $Q$ [2] according to $\sigma = 3Q/2b^2w$ with $b$, the half depth, and $w$, the width of the chamber. The force on a single adhering bacterium can then be approximated as the product of wall shear stress times the bacterial surface area exposed to the shear.

Sufficiently high shear stresses cause adhering bacteria to slide and roll over a surface, which may lead to detachment. In order to characterize attachment and detachment of bacteria with respect to wall shear, notions as “shear to prevent adhesion” and “shear to remove adhered bacteria” have been used [1,3-7]. Shear stresses in the range of 12 to 54 Pa have been reported for the removal of different bacterial strains from regular surfaces and usually a lower shear stress is required to prevent adhesion [8]. These characteristic shear stresses however, suffer from some ambiguity because the strength of adhesion can depend on the history of contact between a bacterium and a substratum surface, i.e. its residence time and the shear stress applied during adhesion.
[9,10]. Moreover, the shear to detach adhered bacteria cannot be obtained for a wide range of adhesion forces within the laminar flow regime.

Bacterial adhesion is the first step in the development of a biofilm and represents the onset of biomaterials implant-related infection, microbially induced corrosion, and fouling of membranes and heat exchanger surfaces in food processing systems [11]. Much attention has been directed towards the development of antifouling surfaces [12]. Polymer brush-coatings are currently considered as the most promising non-fouling coatings, as they weaken the attractive interaction forces between adhering bacteria and the underlying substratum [13,14]. Here, for the first time, we present a method that yields quantitative data on bacteria-surface affinity over a wide range of interaction forces, including the weak interaction forces as existing on non-fouling surfaces.

Materials and Methods

*Staphylococcus epidermidis* HBH276, *Staphylococcus aureus* ATCC12600 and *Pseudomonas aeruginosa* #3 were pre-cultured in 10-ml tryptone soya broth at 37°C for 24 h from blood agar plates. These pre-cultures were used to inoculate second cultures of 200-ml for 16 h. Subsequently, bacteria were harvested and washed twice with demineralized water. To break up bacterial aggregates, bacteria were sonicated for 3 times 10 s at 30 W. Finally, bacteria were suspended in phosphate-buffered saline solution (PBS: 10 mM potassium phosphate, 150 mM NaCl, pH 6.8) to a concentration of $3 \times 10^8$ per ml for all experiments.

Implant grade silicone rubber sheets (Medin, Groningen, The Netherlands) were rinsed with ethanol and demineralized water, sonicated in 2% RBS35 detergent and rinsed thoroughly with demineralized water, washed in methanol and rinsed with demineralized water again to remove oil contaminations and fingerprints. The silicone rubber was fixed in the bottom plate of the PPFC and exposed to a solution of 0.5 g l$^{-1}$
Pluronic F-127 (PEO$_{99}$PPO$_{65}$PEO$_{99}$, Sigma-Aldrich, USA) in PBS for 20 min. Non-attached polymer chains were removed from the surface by washing with PBS. This has been proven to result in a brush layer of PEO-chains on hydrophobic substrata [15-17].

Our flow chamber and image analysis system have been previously described [1]. The setup gives the possibility of having a steady flow as well as being able to quickly switch from low to high flow rates. We used a fluctuating flow protocol to study the attachment and detachment of different bacterial strains to pristine and PEO brush-coated silicone rubber. The protocol begins with flowing a bacterial suspension for 30 min at a wall shear stress of 0.005 Pa. Thereafter, the shear stress is instantly adjusted to either 0.005, 0.3, 0.7, 1.5 or 4.7 Pa, which is maintained for another 30 min. After each separate increase, the shear stress is reset to 0.005 Pa again. This cycle is repeated twice (Fig. 1a).

**Results and Discussion**

During the first 30 min at a shear stress of 0.005 Pa, bacteria attach to the surface. Increasing the flow rate implies a higher supply rate of bacteria to the surface, but also an increased shear stress acting on adhering bacteria. A relatively small increase leads to additional attachment of bacteria. Increasing the shear above a certain threshold will result in a net decrease in the number of adhering bacteria due to a dominant contribution of shear-induced detachment. Here, we refer to the shear stress at which additional attachment and detachment balance each other as the “critical shear stress”.

Attachment and detachment of *S. epidermidis* HBH276 on pristine and PEO-coated silicone rubber under fluctuating shear are presented in Fig. 1. The deposition rate (slope of the curve in a time frame) of *S. epidermidis* HBH276 on pristine silicone rubber is suppressed by increasing the shear stress $\tau$ due to the high wall shear
Fig. 1. Flow protocol to determine the critical shear stress at the balance between bacterial attachment and detachment. (a) Shear stress fluctuation between two values. The first shear stress $\tau_1$ is always 0.005 Pa, while the second shear stress $\tau_2$ is either 0.005, 0.3, 0.7, 1.5 or 4.7 Pa. (b and c) Adhesion profiles of *S. epidermidis* HBH276 on pristine silicone rubber and PEO-coated silicone rubber, respectively. These profiles are obtained from shear fluctuations of 0.005- X- 0.005- X Pa (0.005 ≤ X ≤ 4.7). Note the different scales along the y-axis.
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preventing newly arriving bacteria from attaching to the surface. Attachment and detachment events on PEO-coated surfaces are very different and the deposition rate at low shear is one to two orders of magnitude smaller than for pristine silicone rubber.

Fig. 2. The percentage change in number of adhering bacteria after application of the second shear stress as a function of the second shear stress $\tau_2$ ($\Delta = 100 \times (N_2 - N_1)/N_1$, where $N$ is the number of adhering bacteria within a cycle of shear fluctuation). The line represents a hyperbolic decay, from which the upper limit for shear-induced detachment (horizontal asymptote) and critical shear stress are derived (shear stress for zero change). Each data point represents the average ± range of two experiments with separately grown bacteria on separately prepared surfaces. ■, pristine silicone rubber; and ▲, PEO-coated silicone rubber.

Moreover, a noticeable portion of bacteria detach after application of the higher shear. The first and second cycles of shear fluctuation essentially yield the same detachment, indicating that the surfaces, particularly the PEO-coatings, have not been affected by
the high shear. At this stage, it is important to realize that all experiments are carried out in the initial phase of adhesion, i.e. where the numbers of adhering bacteria increase linearly with time (see also Fig. 1). Therewith all changes in attachment and subsequent detachment are due to fluctuations in shear and not to saturation of the surface. From Fig. 1 it can be seen that *S. epidermidis* HBH276 is much more loosely bound to the PEO-coating than to pristine silicone rubber surface.

In order to determine the above defined critical shear stress, we first calculate the net effect of increasing the shear and present the percentage change in number of adhering bacteria after application of the second shear stress as a function of the second shear stress (Fig. 2). This allows us to determine the critical shear stress and an upper-limit for shear-induced detachment. This change in the number of adhering bacteria upon increasing the shear shows a hyperbolic decay, as can be seen in Fig. 2. Zero change in the number of adhering bacteria, defining the critical shear stress, occurs at $2.7 \pm 1.1$ Pa for *S. epidermidis* HBH276 on pristine and at only $0.2 \pm 0.1$ Pa on PEO-coated silicone rubber, corresponding with critical forces of $2.1 \pm 0.9$ pN and $0.1 \pm 0.1$ pN, respectively on a single bacterium (assuming a staphylococcal radius of $0.5 \, \mu$m and of $0.6 \, \mu$m for *P. aeruginosa*, which is the radius of a sphere with equal volume as this rod-shaped organism). The upper limit of shear-induced detachment amounts 10% for *S. epidermidis* HBH276 on pristine silicone rubber, while over 90% of all adhering staphylococci can be detached from PEO brush-coated silicone rubber.

Table 1 compares the critical shear stress and upper limit of shear-induced detachment for the three different bacterial strains. Clearly, different bacterial strains adhere to surfaces with different strength. The adhesion strength of staphylococci is greatly decreased by the presence of a PEO-coating, while the adhesion strength of *P. aeruginosa* #3 is hardly affected. This is in line with other findings, showing that PEO brush-coatings are not effective against hydrophobic *P. aeruginosa* strains [13].
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Table 1. Critical shear stresses and forces at the balance between bacterial attachment and detachment and upper limits for shear-induced detachment of three bacterial strains on pristine silicone rubber (SR) and PEO-coated silicone rubber (PEO). ± indicates the standard error calculated from a series of five data points, each representing two experiments with separately grown bacteria on separately prepared surfaces.

<table>
<thead>
<tr>
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<th>Critical shear stress (Pa)</th>
<th>Critical shear force on a single bacterium (pN)</th>
<th>Upper limit of shear-induced detachment (%)</th>
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<tr>
<td></td>
<td>SR</td>
<td>PEO</td>
<td>SR</td>
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<td><em>S. epidermidis</em></td>
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<tr>
<td>HBH276</td>
<td>2.7 ± 1.1</td>
<td>0.2 ± 0.1</td>
<td>2.1 ± 0.9</td>
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<td><em>S. aureus</em></td>
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<tr>
<td>ATCC12600</td>
<td>1.0 ± 0.2</td>
<td>0.1 ± 0.0</td>
<td>0.8 ± 0.1</td>
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<td><em>P. aeruginosa</em></td>
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<tr>
<td>#3</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.3</td>
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</table>

AFM studies report adhesion forces mostly in the nN range for regular surfaces [10,18] while the largest critical shear force in this study is 2.1 pN for *S. epidermidis* on pristine silicone rubber. However, it has to be realized that AFM measures the forces acting perpendicular to a surface, whereas tangential forces are measured under flow. Moreover, in flow displacement systems, attachment and detachment take place spontaneously, whereas in AFM detachment is forced upon the system by retracting the tip. Adhesion forces have often been found absent or too weak to be measured by AFM upon separating bacterial cell surfaces from polymer brush-coatings [19], which constitutes a major advantage of the method proposed here, based on the shear force balance between bacterial attachment and detachment under flow.

Concluding, it can be stated that the method proposed provides better understanding of weak bacterium-surface interactions under controlled hydrodynamic conditions as it yields quantitative bond strength data, not attainable with conventionally developed methods.
References


