Quantification of the lateral detachment force for bacterial cells using atomic force microscope and centrifugation

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A B S T R A C T

To determine the lateral detachment force for individual bacterial cells, a quantitative method using the contact mode of an atomic force microscope (AFM) was developed in this study. Three key factors for the proposed method, i.e. scan size, scan rate and cantilever choice, were evaluated and optimized. The scan size of 40 × 40 μm² was optimal for capturing sufficient number of adhered cells in a microscopic field and provide adequate information for cell identification and detachment force measurement. The scan rate affected the measurement results significantly, and was optimized at 40 μm/s considering both force measurement accuracy and experimental efficiency. The hardness of applied cantilevers also influenced force determination. The proposed protocol for cantilever selection is to use those with the lowest spring constant first and then step up to a harder cantilever until all cells are detached. The lateral detachment force of Escherichia coli cells on polished stainless steel and a glass-slide coated with poly-L-lysine were measured as 0.763 ± 0.167 and 0.639 ± 0.136 nN, respectively. The results showed that the established method had good repeatability and sensitivity to various bacteria/substrata combinations. The detachment force quantified by AFM (0.639 ± 0.136 nN) was comparable to that measured by the centrifugation method (1.12 nN).

1. Introduction

It is well documented that bacteria may attach to surfaces and subsequently form biofilms in both natural and artificial environments [1]. A biofilm can be defined as a structured community of bacterial cells being enclosed in a self-produced polymeric matrix and adhering to an inert or a living surface [2]. A widely accepted model [3] has been proposed to describe several general stages of biofilm formation and development, including (I) initial reversible attachment of suspended bacterial cells, (II) transition to irreversible attachment, (III) development of biofilm architecture, (IV) development into mature biofilms and (V) dispersion of cells. Since the attachment of a single cell on a substratum (Stages I and II) is the limiting step in the development of a biofilm, many studies have focused on these two stages in the attachment of bacterial cells to various surfaces.

There are several physical and chemical distinguishing features between the two stages of the single cell attachment process [4]. In the first stage, bacterial cells swim or are transported close enough to the surfaces and then initial attachment takes place because of van der Waals forces, electrostatic forces and hydrophobic interactions [1,5,6]. The adhered cell can be easily removed by fluid shear forces at this stage [7]. In the second stage, bacterial cells stick themselves on the surface by the production of exopolysaccharides and/or specific ligands [8]. In the transition from initial attachment to irreversible attachment, covalent and hydrogen bonds, and hydrophobic interactions are involved [4]. After irreversible attachment, much stronger forces (e.g. scraping, scrubbing or chemical cleaners) are required to detach bacteria from the surfaces [1].

To provide deeper insights into single cell adhesion/detachment processes, various methods have been applied to count adhered bacteria or determinate the adhesion strength between the cell and the substratum. The methods for counting bacteria include transmission light microscopy, epifluorescence microscopy, scanning electron microscopy, plate counting, spectrophotometry for stained cells, ATP measurement, as well as nucleic acid probes (reviewed in Ref. [9]). However, the counting methods are considered to be more useful to analyze Stage I of adhesion [10]. For adhesion Stage II, the cells–substratum adhesion strength has been estimated in previous studies using shear forces to detach the adhered cells and measure the lateral detachment force, e.g. flow chambers [11], spinning disks [12], centrifugation [13] as well as air bubbles [14]. These studies provide useful information related to Stage II of the bacterial adhesion process. However, most of these methods are limited by several disadvantages, including low sensitivity and precision, complicated and time-consuming preparation procedures, inability to apply both controlled and directed forces to a specific location, as well as difficulty in estimating the critical force value to detach all the adhered cells [10,15].

Atomic force microscopy (AFM) is a powerful technique with which one can scan samples at the nanometer or sub-nanometer
scale as well as probe interactions in adhesion processes with high force resolution in either dry or wet environments [16]. These advantages can provide large application potential for measuring the detachment forces between cell–cell and cell–substratum in situ [17–19]. Previous studies (reviewed in Refs. [20,21]) have developed a specific approach to measure cell–substratum interactions using an AFM tip coated with a single cell or a layer of bacterial cells. These research provided quantitative information on various physicochemical forces (van der Waals forces, electrostatic interactions, ionic strength, chemical bonds as well as hydrophobic interactions) between the cell and the substratum and gave deep insight into Stage I of the cell adhesion process. Research on Stage II are quite limited. Sénechal et al. [15] evaluated the detachment force between Enterococcus faecalis and polymers using the deflection set-point-applied force calibration curve via AFM and provided an accurate approach to evaluate the adhesion forces for Stage II. More recently, Deupree and Schoenfisch [10] developed a new methodology to determine the lateral detachment force of bacteria according to the total compression of the cantilever, probe geometry and cantilever orientation using the contact mode of AFM and the more accurately measured lateral detachment forces of cells with statistical significance. Unfortunately, this approach did not consider the influence of a cantilever bend in the determination of lateral detachment forces. To overcome the limits mentioned above, in this study, a quantitative method was developed and optimized to quantify the lateral detachment forces of bacterial cells considering the consequent influence of the cantilever bend. Moreover, the detachment force quantified by AFM was compared to that measured by the centrifugation method.

2. Materials and methods

2.1. Bacterial species and culture

Escherichia coli wild-type strain K-12 was obtained from the E. coli Genetic Stock Center (Department of Biology, Yale University)
and used in this study as the test bacterium. *E. coli* was cultivated at 37 °C and 150 rpm in a sterilized (121 °C for 20 min) Luria–Bertani (LB) medium, which contained 10 g of tryptone, 5.0 g of yeast extract, 5.9 g of NaCl (controlling the ionic strength of medium at 100 mM) and 1 L DI water, and pH was adjusted to 7.4. Then the cells were harvested in the log phase at a concentration equivalent to an OD600 nm value of about 0.1 (approximately 10⁷ cells/mL). This bacterial solution was then used for further experiments immediately.

2.2. Substrata preparation

The stainless steel was polished using a Micropolish alumina solution with particle size of 0.3 μm (No. 40-6352, Buehler, USA). After that the polished surfaces were washed by sonication for 10 min in DI water and in dehydrated ethanol for the second wash (Merck KGaA, Germany) to remove the residual alumina particles and organics. Then the polished stainless steel were dried under a nitrogen flow and glued on glass-slides using Crystalbond 209 (Ted Pella, USA). For poly-L-lysine coated glass-slides, the slides were firstly immersed in an ethanol/HCl (v/v — 70/1) solution overnight, washed thoroughly using sterilized DI water, then immersed in 0.01% poly-L-lysine solution (Sigma-Aldrich, USA) for 5 min and finally dried at 40 °C for 12 h. All the prepared substrata were stored in a desiccator at room temperature (23 ± 1 °C) before use.

2.3. Bacterial adhesion

First, 150 μL of the prepared bacterial solution was dripped onto the surfaces of prepared substrata and cultivated at room temperature for 1 h. After that the samples were carefully washed three times with sterilized 100 mM PBS solution (0.29 g KH₂PO₄, 1.19 g

![Image](https://via.placeholder.com/150)

Fig. 2. Three consecutive vertical deflection images of the same *E. coli* cell adhered on polished stainless steel detected at V<sub>setpoint</sub> Values of (A) 0.3 V, (B) 0.5 V and (C) 1.0 V. This cell was detected to be intact in (A). The detected cell partially appeared in (B) and totally disappeared in (C), indicating that the cell was detached exactly at the black line in (B). The value of V<sub>deflection</sub> can be determined according to the peak value (round cursor) of the cell detachment line (black line in B) in the vertical deflection image (D).
K$_2$HPO$_4$ and 4.93 g NaCl in 1 L of DI water, pH adjusted to 7.2 and sterilized at 121 °C for 20 min. Then the samples were immersed in the PBS solution mentioned above and placed in a humid chamber at room temperature for 12 h till AFM and centrifugation measurements were carried out.

2.4. Quantification of lateral detachment force

According to Deupree and Schoenfisch [10], the lateral detachment force may be calculated based on the total compression of the cantilever, probe geometry and cantilever orientation using contact mode AFM, as expressed by Eq. (1) (Fig. 1A)

$$F_{lat} = kSV_{total} \sin(\Phi + \theta) \cos \theta$$  \hspace{1cm} (1)

where $F_{lat}$ is the lateral detachment force (nN), and $k$ and $S$ are the spring constant (nN/nm) and sensitivity (nm/V) of the applied cantilever, respectively. Angles $\theta$ and $\Phi$ are parameters related to the probe geometry and cantilever orientation. $V_{total}$ is the total vertical deflection of the reflected laser beam on the photodiode detector and indicates total compression of the cantilever.

Actually, for contact mode, the cantilever will slightly bend due to the counterforce from the bacterial cell to the tip, and angles $\theta$ and $\Phi$ would change correspondingly (Fig. 1B). However, this bending was ignored in the study of Deupree and Schoenfisch [10] when considering the cantilever length versus deflection. In order to reflect the actual situation and calculate more accurately, a modified method that considered the influence of a cantilever bend was developed to quantify the lateral detachment force of bacteria in this study. Fig. 1C shows a schematic illustration of the force applied by a bending cantilever on an attached cell. As the probe geometry would not change, the increase in angle $\theta$ equals the decrease in angle $\Phi$. Therefore, Eq. (1) should be modified to Eq. (2)

$$F_{lat} = kSV_{total} \sin(\Phi + \theta) \cos \Phi$$  \hspace{1cm} (2)

The vertical deflection of cantilever, $H$, can be calculated from cantilever deflection $d$ and angle $\Phi$, or from the difference of OB and $CA$, which are related to length of the applied cantilever $L$, and angles $\Phi$ and $\Phi$ (to simplify the calculation, the length of $OA$ is assumed to be equal to its arc length $L$). Moreover, cantilever deflection $d$ can be determined by the product of $V_{total}$ and $S$. Therefore

$$V_{total} \cos \Phi = H = L(\sin \Phi - \sin \Phi)$$  \hspace{1cm} (3)

Thus, angle $\Phi'$ can be calculated using the double-angle formula (two solutions will be obtained from a quadratic equation and the abnormal value was stripped) as shown below

$$\Phi' = 2 \arctan \left[ \frac{L - \sqrt{(V_{total}S)^2 + (L \cos \Phi)^2}}{V_{total}S + L \sin \Phi} \right]$$  \hspace{1cm} (4)

Then $\theta$ can be determined as follows:

$$\theta = \Phi + \theta - 2 \arctan \left[ \frac{L - \sqrt{(V_{total}S)^2 + (L \cos \Phi)^2}}{V_{total}S + L \sin \Phi} \right]$$  \hspace{1cm} (5)

Finally, the lateral detachment force of bacterial cells can be calculated as follows:

$$F_{lat} = kSV_{total} \sin(\Phi + \theta) \times \cos \left\{ \Phi + \theta - 2 \arctan \left[ \frac{L - \sqrt{(V_{total}S)^2 + (L \cos \Phi)^2}}{V_{total}S + L \sin \Phi} \right] \right\}$$  \hspace{1cm} (6)

In Eq. (6), $k$ and $S$ can be calibrated by resonance frequency of the cantilever and the slope of the force spectrum between tip and substratum using software provided by the manufacturer, respectively. The two angles, $\theta$ and $\Phi$, and length of cantilever $L$ are fixed for a given AFM system. For the AFM system used in this study, $\theta = 10$° and $L = 300–350$ μm. $V_{total}$ can be determined by the sum of $V_{setpoint}$ and $V_{deflection}$. $V_{setpoint}$ is the user-defined cantilever compression. $V_{deflection}$ shows an additional compression of the cantilever when bacterial detachment occurs at a detachment line (Fig. 2B and D). In this study, only the deflection record of the trace scan (movement direction from the tip to the cantilever end along the cantilever axis) was used to obtain the $V_{deflection}$ values, as buckling of the cantilever may occur when it moves in the re-trace direction [10].

Strictly, the lateral detachment force determined by Eq. (6) was neither a measure of a detachment force nor an adhesion force, but a characterization of bacterial adhesion strength in phase II to substrata, since other parameters, including scanning time of the applied force on the specific cell and the static friction between the cell and the substratum, could also influence the detachment event. In the present study, these influences were neglected and only the total deflection of the AFM cantilever was applied to calculate the lateral detachment force and characterize the adhesion strength between bacterial cells and the substrata.

![Fig. 3](image_url) Horizontal (A) and vertical (B) schematic figures of slide arrangement in centrifugation method. (C) Lateral centrifugal force imposed on adhered cells, which was similar to that in AFM method, by this fixing pattern.
2.5. Optimization of AFM scanning

AFM images were obtained in the contact mode using a JPK NanoWizard AFM (JPK Instruments, Germany). To optimize AFM scanning operation for detachment force measurement, three crucial factors of AFM application were selected and evaluated separately, including scan size (80 × 80, 40 × 40 and 20 × 20 μm²), scan rate (24, 40 and 80 μm/s), as well as hardness of the applied cantilevers. Silicon cantilevers (CSC37 and CSC38, Mikromasch, Estonia) with a series of spring constants of 0.03, 0.05, 0.08, 0.3, 0.35 and 0.65 nN/nm were applied to acquire images and calculate the force. To ensure parallel experiments, the probe was adjusted to the same orientation after changing the cantilever. Before each measurement, the sensitivity and spring constant of the applied cantilever were calibrated on a clean glass-slide using software provided by the manufacturer. Three to five fields were selected randomly to obtain sufficient cells for statistical analysis. All the experiments were conducted independently (using independent cultures and substrate preparations) for each parameter and lateral detachment forces were measured in the PBS solution mentioned above.

2.6. Measurement of lateral detachment force by centrifugation method

In this study, the centrifugation method was modified according to the previous studies [13] to measure lateral detachment of the adhered bacteria. After cultivation, the glass-slides coated with poly-L-lysine were immersed into a 100 mM PBS solution and fixed in 50 mL centrifugal tubes. Then the centrifugal tubes were placed in a specific position, as shown in Fig. 3. In this situation, the adhered cells would be imposed on a shear centrifugal force, which was similar to that in the AFM methodology. Then each glass-slide was spun in a centrifuge (Jouan KR22i, USA) for 5 min at a speed in the range 1875–15000 g at room temperature. After centrifugation, the orientation of glass-slides was checked again and the remained cells were stained with acridine orange and counted (8–10 fields) using a 100 × objective under a fluorescence microscope (Nikon Eclipse E600, Japan) [22]. Averages of remained cells were calculated for each centrifugal speed and compared with controls (without centrifuging) to estimate the percentage of detached cells under the specific shearing force. The centrifugal force \( F_c \) applied to each bacterial cell can be determined as follows:

\[
F_c = \left( \rho_c - \rho_m \right) V_c \cdot RCF \quad (7)
\]

where \( \rho_c \) and \( \rho_m \) are the densities of the cell and medium, respectively, \( V_c \) is the volume of the cell and RCF the relative centrifugal force. The experiment was repeated 3 times. According to the study of Godin et al. [23], \( E. coli \) density was estimated to be 1.16 g/cm³ and the density of PBS was 1.006 g/cm³. The volume of \( E. coli \) used in this study was calculated to be 1.75 μm³ (estimating the shape of \( E. coli \) to be cylindrical) by measuring the cell length of 2.14 ± 0.16 μm and width of 1.02 ± 0.17 μm using tapping mode AFM (totally 8 cells).

3. Results and discussion

3.1. Scan size

For the present method, it is important to obtain sufficient pixel information in order to distinguish the bacterial cell morphology
from that of the substratum surface as well as acquire accurate force data. Thus, three different scan sizes (80 × 80, 40 × 40 and 20 × 20 μm²) were evaluated to find the optimum scan size. The rod-shaped bacterium E. coli K-12 was measured to have a length of 2.14 ± 0.16 μm and width of 1.02 ± 0.17 μm using tapping mode AFM. Due to the fact that the direction of the rod-shaped cells dispersed randomly, the cells that are perpendicular to the fast scan direction were chosen and used to predict the critical pixel numbers of bacterial cells and the influence of scan size. The critical pixel number of adhered cell occupied can be calculated by the quotient of cell width and side length of one pixel. In this study, the scan size was optimized at 40 × 40 μm². According to Deupree and Schoenfisch [10], the resolution of AFM images was maintained at 512 × 512 pixels. As a result, the pixel length of the 80 μm scan size was 0.156 μm, whereas the pixel length of the 40 and 20 μm scan sizes were 0.078 and 0.039 μm, respectively. Accordingly, the critical pixel numbers of the E. coli cell were 6, 13 and 26 in the three scan sizes (Fig. 4). The images with smaller scan size could provide more information for cell identification and force measurement. However, the number of cells in a field will decrease with smaller scan size. According to Deupree and Schoenfisch [10], the resolution with approximately 10 pixels could provide sufficient information for identifying cells and determining forces. Thus, in the present study, the scan size was optimized at 40 × 40 μm² for measuring the lateral detachment force of E. coli.

3.2. Scan rate

Another key factor in AFM scanning is the scan rate, which represents the rate of tip movement and may affect the result of detachment force measurement significantly. In this study, three different scan rates (80, 40 and 24 μm/s) were chosen to evaluate their effects on detachment force measurement. Fig. 5 showed that the detachment forces were 0.880 ± 0.222 nN (39 cells), 0.747 ± 0.150 nN (31 cells) and 0.624 ± 0.105 nN (27 cells) at scan rates of 80, 40 and 24 μm/s, respectively, indicating that scan rates significantly (P_{80/40}=0.002 and P_{40/24}=0.008) affected the measurement of lateral detachment forces. This could probably be caused by the increase in linear momentum of the moving tip at the higher scan rate, which magnified the counterforce from the bacterial cell to the scanning tip. Moreover, the relative standard deviation (RSD), which presented the precision of the measurements, also increased from 17% to 25% along with the scan rate, possibly due to the increased noise range of the AFM signal at the higher scan rates.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Values of cantilevers’ parameters and their detection limits in the AFM system used in this study (θ=10° and Φ=10°).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantilever</td>
<td>k (nN/nm)</td>
</tr>
<tr>
<td>A</td>
<td>0.0268</td>
</tr>
<tr>
<td>B</td>
<td>0.0515</td>
</tr>
<tr>
<td>C</td>
<td>0.0785</td>
</tr>
<tr>
<td>D</td>
<td>0.134</td>
</tr>
<tr>
<td>E</td>
<td>0.239</td>
</tr>
</tbody>
</table>

Fig. 5. Lateral detachment forces of E. coli on polished stainless steel at three different scan rates. The markers show measured values for individual cells and lines (solid line for 80 μm/s; dashed line for 40 μm/s; and dotted line for 24 μm/s) show the average values.

Fig. 6. Lateral detachment forces of E. coli on polished stainless steel using five different cantilevers. The markers show measured values of individual cells and lines (— — — for cantilever A; — — — for cantilever B; — — — for cantilever C; — — for cantilever D; and — — — — — for cantilever E) show the average values.

Fig. 7. Replicated measurements of lateral detachment forces of E. coli on polished stainless steel (A) and poly-L-lysine coated glass-slide (B). The markers show measured values of individual cells and lines show average values of each replicate.
higher scan rate. Thus lower scan rates should be applied to
decrease the tip linear momentum and reduce the noise range of
signals. However, the scan time had to be correspondingly
increased if the scan rate decreased, i.e. 8.5, 17 and 28 min per
image at the scan rates of 80, 40 and 24 μm/s, respectively.
Therefore, to balance the accuracy of the measured force and
efficiency of the measurements, the scan rate was selected to be
40 μm/s in this study.

3.3. Hardness of cantilever

To evaluate the influence of cantilever hardness on measure-
ments, five cantilevers with different hardnesses (spring constant
from 0.02 to 0.2 nN/nm) were selected in this study (Table 1). Fig. 6
showed that the measured forces increased with the cantilever’s
spring constant. Similar lateral detachment forces were obtained
when using cantilevers A–C (cantilever A—0.749 ± 0.140 nN,
26 cells; cantilever B—0.732 ± 0.216 nN, 17 cells; cantilever
C—0.758 ± 0.083 nN, 16 cells). For harder cantilevers, much larger
forces were measured, such as 1.35 ± 0.318 nN (28 cells) and
1.83 ± 0.178 nN (17 cells) for cantilevers D and E, respectively.
The variation in the measured values using cantilevers of different
hardnesses was mainly caused by the detection limit of the applied
cantilevers. The detection limit, as shown in Table 1, was calculated
theoretically from $V_{\text{setpoint}}$, which is as low as possible but high
enough to maintain good image quality (for the system used in this
study, the minimum $V_{\text{setpoint}}$ was about 0.3 V) and $V_{\text{deflection}}$ of zero
according to Eq. (6). For cantilevers A–C, the measured forces were
much larger than their detection limits and the measured values
were not significantly affected by the detection limits. However, for
the two harder cantilevers, the detection limits already exceeded
detachment forces of the samples and the measurement results may
be overestimated. Thus, the proper protocol for choosing cantilevers
is to use those with the lowest spring constant (softest) first and then
change to a harder cantilever until all the cells are detached.

Various cantilevers with a wide range of spring constants (from
0.06 to 0.32 nN/nm) have been applied to detach the bacteria cells via
contact mode AFM in previous studies [10,15,18]. This study also
showed that softer cantilevers (spring constant of 0.0268 nN/nm)
could be used to measure the lateral detachment force. Such a wide spring constant range (0.02–0.32 nN/nm) could provide large scope of lateral detachment forces from 0.2 nN (detection limit) to 60 nN (assuming $V_{\text{total}}$ to be up to 10 V, i.e. the upper detection limit of the photodiode detector in the vertical direction) if $L$ and $\theta$ are the same as those used in this study. This wide range implies a large application potential to quantify the lateral detachment force of various cell–substratum combinations using the established methodology.

### 3.4. Repeatability

In this study, the lateral detachment forces of *E. coli* K-12 on the polished stainless steel and glass-slide coated with poly-\(\varepsilon\)-lysine were repeated 4 and 3 times, respectively, at different days to evaluate the reproducibility (Fig. 7). The measured forces were $0.747 \pm 0.150$, $0.749 \pm 0.140$, $0.758 \pm 0.166$ and $0.761 \pm 0.157$ nN for the four repeated tests using stainless steel as the substratum (the spring constants of used cantilevers varied from 0.0268 to 0.0396 nN/nm), and were $0.655 \pm 0.152$, $0.621 \pm 0.123$ and $0.642 \pm 0.135$ nN for the three repeated tests using a glass-slide coated with poly-\(\varepsilon\)-lysine as the substratum (spring constants of the used cantilevers varied from 0.0277 to 0.0378 nN/nm). No significant difference was found between each of the two replicates ($P_{1/2}=0.953$, $P_{1/4}=0.771$, $P_{2/1}=0.718$, $P_{2/3}=0.822$, $P_{2/4}=0.775$ and $P_{3/4}=0.941$ for stainless steel; $P_{1/2}=0.335$, $P_{1/3}=0.696$ and $P_{2/3}=0.531$ for poly-\(\varepsilon\)-lysine), indicating that the differences between the replicates were very small and the repeatability of the established method was acceptable.

By Eq. (5) and (6), the maximum error that was introduced for angles $\Phi$ and $\theta$ was about 4% for the cantilever with a length of 100 \(\mu\)m, leading to a 0.1% improvement for force measurement at maximum bending of cantilever ($V_{\text{total}}=10$ V) by considering that the cantilever was bending during measurement. Although this refinement cannot significantly improve the acquired forces in this study, the relative standard deviations were relatively larger (18–23%). The refinement was still considered to be necessary since it indeed reflected the actual situation and was expected to improve the accuracy of force measurements under a certain case (i.e., shorter cantilever with large sensitivity and measurement under a large set-point).

Several studies have been conducted to measure the lateral detachment forces of various cell–substratum combinations using contact mode AFM. Sénéchal et al. [15] determined the lateral detachment forces of *Enterococcus faecalis* on three different polymers. The detachment forces varied significantly from 0.7 to 19 nN depending on the applied substratum. Moreover, Deupree and Schoenfisch [10] found that the detachment forces needed to remove *Staphylococcus aureus* from the xerogel film to be doubled (from 4 to 8 nN) in 10 h. These findings indicated that the detachment forces were strongly dependent on the bacterial species, the substratum as well as cultivation time. In this study, the detachment forces of *E. coli* on different substrata were $0.755 \pm 0.153$ and $0.639 \pm 0.136$ nN, as shown in Fig. 7, which also presented significant difference ($P=0.001$). All of these results indicated that the established methodology was sensitive to different cell–substratum systems.

### 3.5. Comparison with centrifugation method

To evaluate the accuracy of the established AFM methodology, another independent method should be taken and compared with the results obtained from the AFM method. In this study, a method to measure the bacterial lateral detachment force using centrifugation [13] was selected and compared with the AFM method. Fig. 8 showed that the percentages of detached bacteria increased with the lateral centrifugal force. The removal percentages of the adhered cell number fitted well ($R^2=0.990$) with the dose–response curve of the Origin software (version 8.0) under a range of centrifugal forces. Calculated from the established curve, 50% of cells were detached at a centrifugal force of 1.12 nN. This value was considered to be representative and comparable with the average force obtained by AFM, since the adhered cells were detached by the lateral applied force in manners similar to the two methods. Considering unpredictable random errors caused by limited sample size and uncontrollable systematic errors caused by instruments and the experimenter, neither approaches could present the true values of the lateral detachment forces between the bacterial cells and the substrata. However, the narrow difference between the two independent methods (0.639 nN by AFM and 1.12 nN by centrifugation) increased the probability of measured values approaching the true values and the results obtained by AFM method in this study were reliable.

The AFM method, compared with centrifugation, can monitor the lateral detachment force of each observed cell. The distribution of measured detachment forces can be analyzed accordingly. Fig. 9 showed that the lateral detachment forces of *E. coli* adhered on the polished stainless steel (A) and poly-\(\varepsilon\)-lysine coated glass-slide (B). The solid lines represent normal distributions.

![Fig. 9. Data distribution of lateral detachment forces of *E. coli* on polished stainless steel (A) and poly-\(\varepsilon\)-lysine coated glass-slide (B). The solid lines represent normal distributions.](image-url)
stainless steel and poly-lysine coated glass-slides were fitted well with the normal distribution. The correlation coefficients \( R^2 \) were 0.889 (stainless steel) and 0.887 (poly-lysine coated glass-slide). Examination of the histograms in Fig. 9 strongly suggested that there may be a unimodal distribution in bacteria/substrata interactions, rather than a bimodal distribution indicated by the previous studies [24,25]. The centrifugation method cannot provide varied information and give deep insight into the internal relationships of the acquired data. Moreover, the AFM method had several other advantages, including high sensitivity and precision, obtaining the morphology and force information synchronously, ability of applying both controlled and directed forces on a specific location as well as estimate the critical force value to detach all the adhered cells [10,15].

4. Conclusion

(1) To determine the lateral detachment force of the adhered bacterial cell, a quantitative method using the contact mode of AFM was developed and optimized.

(2) The scan size of 40 \( \times \) 40 \( \mu \)m\(^2\) could capture sufficient number of adhered cells in a microscopic field and provide adequate information for cell identification and detachment force measurement.

(3) The scan rate of 40 \( \mu \)m/s had better force measurement accuracy and experimental efficiency compared with 24 and 80 \( \mu \)m/s.

(4) The proposed protocol for cantilever selection is to use those with the lowest spring constant first and then step up to a harder cantilever until all cells are detached.

(5) The established method had good repeatability and sensitivity to various bacteria/substrata combinations.

(6) The lateral detachment force quantified by AFM was comparable to that measured by the centrifugation method, which implied that the results obtained by the AFM method were reliable.

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