Probing microplatform for the study of biological adhesion forces

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A tool for the study of biological adhesion forces with the atomic force microscope (AFM) is introduced. The tool, a "microplatform," can be functionalized with variety of specimens such as bacterial cells and used to study adhesion between the specimen and a surface. This tool is easily created using commercially available silicon AFM tips and an AFM, and can be customized in size to fit specific applications. Two custom fabricated microplatforms, ~1 and ~2.5 μm were tested. The method of microplatform fabrication, as well as adhesion force data between E. coli bacteria and a nanofiltration membrane is presented. © 2003 American Institute of Physics.

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I. INTRODUCTION

Bacterial adhesion is important in a variety of fields including bioremediation, biomaterial development, and marine biofouling. The process of cell adhesion to a conditioned substratum is a key event in biofilm development, and is the subject of considerable research efforts. Although macroscopic properties of bacteria such as hydrophobicity and surface charge have been shown to generally correlate with the extent of bacterial adhesion to a surface, mechanisms of initial attachment are not fully understood.

The invention of the atomic force microscope (AFM) in 1986 provided an important tool for the study of biological macromolecules and cell surfaces. Unlike the electron microscope, which must operate in a vacuum, the AFM can be operated in any environment including liquid mediums that duplicate natural physiological conditions. The AFM thus serves as an ideal tool for the investigation of cell attachment/detachment processes since it can be used to measure interaction forces between bacteria and a sample surface. Several methods have been used to study adhesion interactions between bacteria and substrates. The simplest method is to use an uncoated silicon or silicon nitride AFM tip to probe bacteria attached to a surface. This approach is limited however, due to the small number of materials used for the manufacture of AFM tips and to variations in tip radius. In order for quantitative force data to be extracted from force-versus-distance curves, an accurate determination of tip radius (r) is required. This can be problematic since manufacturers typically quote only a maximum limit for this parameter, i.e., <20 nm, and methods of determining the actual tip radius (scanning electron microscope (SEM), DMT theory) are often time consuming and cumbersome. In addition, manipulation force microscopy has been used to measure the force necessary to displace cells adhering to surfaces. The main limitation to this method of obtaining cell adhesion force measurements is that the cantilever base is small compared to the cell cross section. Hence, high local stresses can damage the cell. Alternately, bacteria have been attached directly to a cantilever tip, and to the end of a tipless cantilever in the form of cell probes. Unfortunately, attaching cells directly to an AFM tip is often difficult and time consuming. One reason for this difficulty is the relatively large size of microbes in comparison to an AFM tip. The radius of a sharp tip, as previously mentioned, is on the order of 20 nm while many microbial cells are much larger. E. coli bacteria, for example, are approximately 0.5–1 μm wide and 1–2 μm long. Lastly, microspheres with a known diameter (usually 5 μm) have been coated with bacteria and then attached to the end of an AFM cantilever (often, a tipless cantilever). Although microspheres provide more surface area for bacterial attachment, the tip preparation process requires not only coating the microsphere with bacteria, but attaching it to the cantilever as well. Furthermore, this method requires additional equipment such as a high-powered microscope and micromanipulator.

This article introduces a tool, the probing microplatform, which, in one method of production, is produced using only the AFM via simple modifications to a standard AFM tip. The microplatform enables the attachment of bacterial cells, or other specimens, to a flat probing region with less difficulty and using fewer instruments than previously reported. These probes can then be used for monitoring sample interactions with surfaces.
II. MATERIALS AND METHODS

A Dimension 3000 AFM (NS III Controller, Digital Instruments, Santa Barbara, CA), operated in contact mode, was used to grind silicon AFM probe tips (Multi75, Nanodevices, Santa Barbara, CA) by setting the scanner to exert a large force between an aluminum oxide sample and the silicon tip. This was accomplished by engaging the tip on the aluminum oxide surface, then manually lowering the tip another 10–15 \( \mu \text{m} \) to increase the grinding force on the tip. The scan size and rate were increased to the maximum settings \( 100 \mu \text{m} \) and 61 Hz, respectively; and the AFM was allowed to run in this manner for 15–30 min, depending on the desired tip size. Varying the grinding time created a variety of microplatform sizes ranging from 1 to 7 \( \mu \text{m} \) in width. SEM images of two of the microplatforms (designed with widths of 1 and 2.5 \( \mu \text{m} \)) are shown in Fig. 1.

Contact mode AFM images (512 \( \times \) 512 pixel) of a calibration standard (200 nm deep, 10 \( \mu \text{m} \) pitch) were taken in air before and after each cantilever was ground. Cross sectional data extracted from these images was studied in conjunction with images obtained with a SEM (47S-4700, Hitachi, Tokyo, Japan). It was determined that the change in pitch dimension approximately equaled the new tip width. Figure 2 depicts how changes in tip width affect the apparent diameter of the pits in the standard: (a) a sharp tip can image the entire 5 \( \mu \text{m} \) width of the pit, while (b) the ground tip can only image a portion.

The 1 and 2.5 \( \mu \text{m} \) microplatforms were tested for ease of bacteria attachment. The microplatforms were first soaked for 15 min in a 4% solution of poly(ethyleneimmine) (PEI) (MW = 1200 Daltons), prepared by diluting 100% stock solution (Polysciences Inc., Warrington, PA) in ultrapure water (Milli-Q, Millipore Corporation). PEI is a polymeric compound with a net positive charge that served to enhance the adhesion of cells to the silicon nitride microplatform surface. E. coli cells (strain JM109, obtained from the Biology Department, University of Nevada, Reno) were then attached to the PEI-coated microplatform. This was accomplished by using the piezotube of the AFM to bring the microplatform into contact with cells, suspended in a droplet of aqueous solution, which were incubated for approximately 30 min. Force-versus-distance curves were obtained with the bacteria-coated tips on a clean nanofiltration (NF) membrane (HL, Osmonics, Minnetonka, MN). Forty force curves were taken on the membrane with the bacteria-coated microplatform.

![Fig. 1. SEM images of silicon AFM tips modified to make microplatforms: (a) ~1.0 \( \mu \text{m} \) tip and (b) ~2.5 \( \mu \text{m} \) tip. The platform surfaces are outlined in black. Both scale bars are 2.0 \( \mu \text{m} \).](image1)

![Fig. 2. Inset in (a) and (b) is cross sectional data from AFM images of a calibration standard (200 nm deep, 10 \( \mu \text{m} \) pitch) taken with the same tip (a) before and (b) after grinding. Below the cross-sectional data are sketches depicting how changes in tip width affect the apparent diameter of the pits in the standard: (a) a sharp tip can image the entire 5 \( \mu \text{m} \) width of the pit, while (b) the ground tip can only image a portion.](image2)
Following the force curve measurements, SEM images were taken to verify the presence of bacteria on the microplatform surface. For comparison, 40 force curves were also taken on the same membrane with clean microplatforms of similar size.

III. RESULTS AND DISCUSSION

The microplatform, a useful tool for biological adhesion measurements was produced and tested. Bacteria on the microplatform surface greatly affected the measured adhesion force between the microplatform and the membrane surface. It was shown that ~2.5 μm microplatforms are a reasonable size for the attachment of bacteria since the success rate of attaching bacteria to the flat region of the microplatform was higher for the ~2.5 μm microplatform than for the ~1 μm microplatform (data not shown). The average adhesion force between a clean ~1 μm microplatform and a clean membrane was 9.97 nN, and the average adhesion between a bacteria-coated ~1 μm microplatform and a clean membrane was 8.17 nN. The relatively equal average values of adhesion for both the clean microplatform—membrane interaction and the bacteria coated microplatform—membrane interaction indicate the absence of bacteria on the microplatform. SEM images confirmed this. They indicated the presence of several bacteria on the sides of the microplatform, but none directly on the microplatform surface. On the other hand, the average value of adhesion between the clean membrane and the clean ~2.5 μm microplatform was 12.46 nN, and the average value of adhesion between the bacteria coated ~2.5 μm microplatform and the clean membrane was 147.15 nN. Thus, the average adhesion force obtained when using the bacteria-coated tip was more than 1 order of magnitude higher than the adhesion force observed using a clean microplatform to probe the membrane surface. SEM images provided further evidence for the conclusion that bacteria were present on the microplatform surface. Figure 3 shows adhesion force histograms obtained from each set of interactions as well as SEM images of the ~2.5 μm microplatforms used. Figure 3(a) shows an uncoated microplatform. Figure 3(b) shows a bacteria coated microplatform, as evidenced by the rounded protrusions on the tip sidewalls and microplatform surface. The adhesion force values for the bacteria-membrane interaction that we report seem reasonable based on comparison with literature-reported values: they are similar to the 80–240 nN range of adhesion forces reported by Bowen et al. for interactions between a yeast cell and a cleaved mica surface.

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20 S. K. Lower, C. J. Tadanier, and M. F. Hochella, Jr., Geochim. Cosmo-
23 This adhesion range was observed with several 2.5 μm platforms includ-
ing the one that was later coated with bacteria and presented in this article.