Detection of surface brush on biological cells *in vitro* with atomic force microscopy

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Observation of a brush on the cell surface with the atomic force microscopy (AFM) *in vitro* is reported. The number of methods to study brushes that coat living cells is limited despite their biological importance. Moreover, it is important to take into account the brush layer when studying cell mechanics. Here the authors present an AFM method to detect the length and grafting density of the brush on viable cells with resolution that considerably surpasses any existing method. The authors demonstrate this method using cultured human cervical epithelial cells, but it can be applied to any type of cell. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2757104]

Molecular brushes on living cells, composed of the glycocalyx layer and the pericellular molecular coating,[1,2] are known to be responsible for cell-cell interaction, cell migration, differentiation, and proliferation.[3,4] The brush can also define the degree of invasiveness of cancer cells.[1,2,5] The cellular coat can be imaged with a conventional (dry) and environmental electron microscopy.[6] Three-dimensional visualization of the molecular coat around the cell can be done with particles (or fixed red blood cells), exclusion assays.[6,7] Finally, fluorescent labeling, in particular, various lectins and specific binding proteins, can also be used. Electron microscopy requires complicated procedures to provide sufficient contrast and to avoid artifacts. The optical techniques are limited in resolution by the diffraction limit. Finally, none of these techniques can provide quantitative information about the grafting density of the brush.

Atomic force microscopy (AFM) has been used to study cell mechanics.[8–10] Despite the potential of single molecule sensitivity,[11] detection of a brush layer on eukaryotic cells has not yet been reported with the AFM technique. This is presumably due to the two problems: (1) a typically sharp AFM probe can easily penetrate in between the brush without detectable resistance, and (2) the probe also deforms the cell body which is soft itself, hiding the mechanical response of the brush.

In the method presented we use a 5 μm silica particle as the AFM probe to overcome problem 1. A sufficient amount of the brush will touch such a probe, providing enough force to deflect the AFM cantilever. Problem 2 will be resolved by means of a method that decouples squeezing brush from the deformation of the cell body. The decoupling method is as follows. The probe of radius R deforms both the brush and cell body (Fig. 1). Simple geometrical reason gives $h=Z-Z_0+i+d$. Because we are dealing with a quasistatic situation when doing the measurements, the forces pushing both layers are the same, and are equal to the load force $F=kd$ (where k is the spring constant defined by the resonance method, using built-in option of the AFM software). In these notations the sought force of the probe-brush interaction [$F(h)$, the force if the brush were attached to an absolutely rigid substrate] is found if we get d as a function of h. Parameters Z and d in the above equation are directly measured while collecting the force curves. To derive $d(h)$, the other two parameters (i, Z_0) have to be found by the fitting of the collected force curves $d(Z)$. Young's modulus E of the cell body can be found by using the Hertz-Sneddon model[12] $i=(9dk/16E)^{2/3}R^{-1/3}$. After that the sought $d(h)$ is unambiguously defined.

The raw force curves (the displacement of the cantilever versus vertical position of the scanner, d vs Z, not shown) were collected over 16 viable cells in Hank's balanced salt solution buffer using force-volume mode. To process the force curves, they were averaged over 30–50 individual curve measurements collected over relatively flat regions of each cell. As a result of the processing, we found that the average Young's modulus of the cell body was 2.1 ± 0.5 kPa. The extracted force curves for the brush-AFM probe interaction are shown in Fig. 2(a). One can see clearly the exponential dependence, which is an indication of a brush.

To describe the brush force quantitatively, we use the steric interaction equation derived in Ref. 13 for polymeric brushes. Using the Derjaguin approximation, one can get the

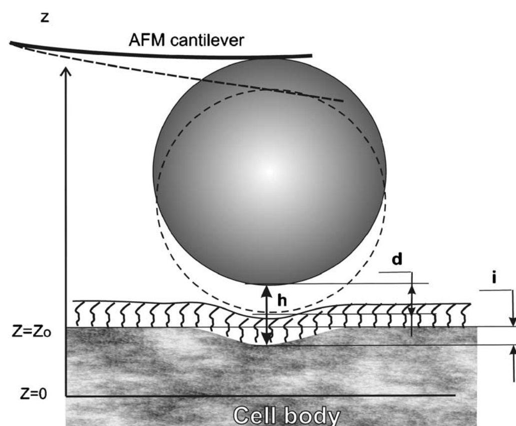

FIG. 1. Interaction of a spherical AFM probe with two-layer cell structure. Z is the relative scanner position of cantilever, d is the cantilever deflection, Z_0 is the nondeformed position of the inner layer of sample, i is the deformation of sample, $Z=0$ is for the maximum deflection (assigned by the AFM user), and h is the separation between the cell body and the AFM probe.

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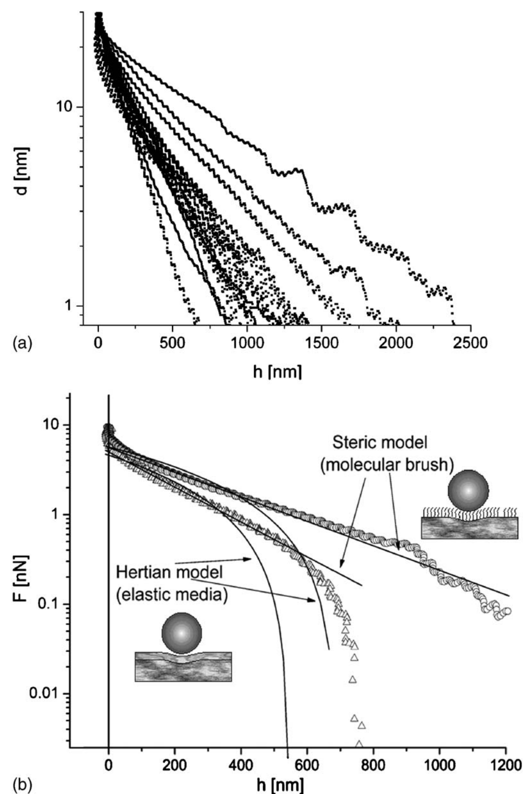

FIG. 2. (a) Forces of interaction between the AFM probe and the brush layer obtained by processing raw force data. (b) Fitting Eq. (1) for an example of two representative force curves of (a). The best fits are also shown for these curves if they were due to the elastic response on the medium of the first layer described by the Hertzian model.

force of interaction between the spherical AFM probe and flat surface,

$$F_{\text{brush}} \approx 50k_BTRN^{3/2}\exp\left(-\frac{2\pi}{L}h\right)L. \tag{1}$$

Here L is the equilibrium thickness of the brush, N is an effective surface density of the brush molecules (grafting density), R is the radius of the AFM probe, and T is the temperature. We consider grafting density effective because the brush can include complex "molecules," such as microvilli (see below). Equation (1) is a valid description of a brush for $0.2 < h/L < 0.9$. Processing data shown in Fig. 2(a), we can get the distribution of parameters N and L.

Figure 2(b) shows fittings for an example of two representative force curves. The best fits are shown for both possibilities: Eq. (1) (the top layer as a brush) and if the top layer were the elastic medium (Herztian model, see, e.g., Ref. 14). One can see that the fit of the brush model is obviously better than the elastic medium model. The fitted brush parameters are shown in histograms of Fig. 3. Cells have a brush length averaged at $L = 2360 \pm 970$ nm. The grafting density $N = 290 \pm 160$ brush molecules/μm^2. Relatively large standard deviations come from variability between dif-

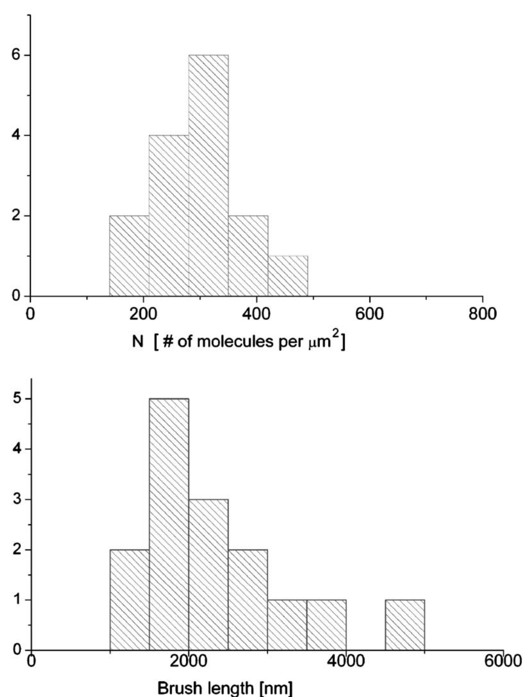

FIG. 3. Distribution of the brush parameters obtained for human cervical epithelial cells.

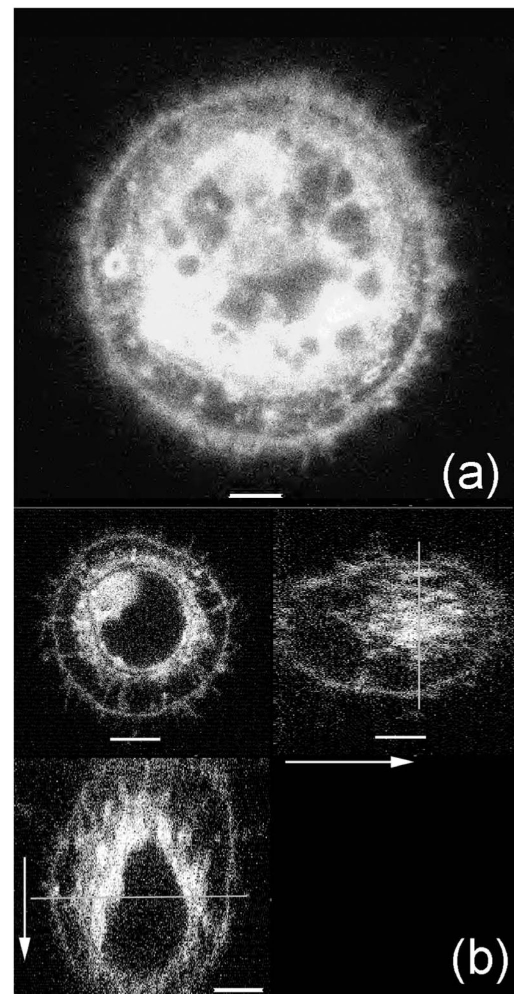

FIG. 4. (a) Representative confocal image of a cell showing the brush. To distinguish the cell surface brush and filopodia developed on the Petri dish surface, the images were taken at the middle of cell height. (b) We also show three dimensional cross sections of cells. The arrows indicate vertical direction pointing out of the Petri dish. Scale bars are 5 μm.

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ferent cells. The accuracy of the fitting (using Levenberg-Marquardt and simplex algorithms) is typically single percents within single cell.

It should be noted that the chondrocyte glycocalyx or pericellular coat[15] is a known example of large molecular brush on the cell surface, which is comprised of molecules of lengths[16] comparable to those found here. It is plausible to expect that we are dealing with a similar type of molecular brush. Staining with alcian blue dye indicated the glycoconjugate composition of the brush (not shown). However, high-resolution confocal images of cells stained with nile red (a dye that shows lipid layers) (Fig. 4) also show cell brushes comparable in size with those derived from the AFM data. This means that the brush detected by AFM has a complex nature, a mix of membrane corrugation, presumably microvilli and microridges, as well as glycocalyx. The biological nature of the observed brush still has to be investigated.

The method described here can be applied to virtually any kind of cell. In contrast to the existing techniques, the AFM method described can provide precise quantitative data on both lengths and effective grafting densities of the brush by collecting force curves on viable cells.

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