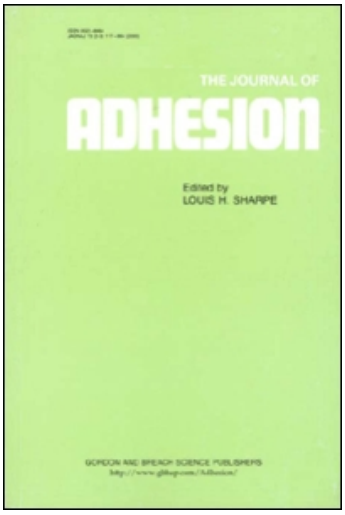


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# A Reliable Method to Measure the Adhesive Force with a Tiny Amount of Adhesive Material

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*A new method is proposed and developed to measure adhesive forces by use of the force-distance curve of a micro cantilever with an extremely small amount of testing material such as adhesive proteins. The contact area should be well-controlled at a reasonable value. Even though the area is desired to be as small as possible, a contact region of several micrometers by several micrometers is adopted in order to avoid obtaining meaningless measured values and uncertainty in the contact areas. An AFM cantilever is used after having been modified with a micro glass bead to enlarge the contact area for adhesion. A glass plate with micro-scale circular patterns is fabricated from a glass wafer by micro-machining processes in order to control precisely the contact area in adhesion tests. In the proposed method the adhesive materials are directly applied to the bead attached at the AFM cantilever before it is applied on the top area of the truncated cone on the fabricated glass plate. The developed method is applied to measure the adhesive forces of Cell-Tak® (which is a commercial extracted mussel adhesive) and recombinant Mgfp-5 (which is a recombinant mussel adhesive protein) and the statistical credibility of the measured adhesive force data is enormously improved as a result.*



**Keywords:** Adhesive force measurement; Adhesive protein; AFM; Cantilever modification; contact area; Force-distance curve; Substrate patterning

## 1. INTRODUCTION

In recent years, attention has been paid to the various characteristics of biomaterials by research scientists and engineers for the purpose of studies on life science and their applications. The adhesive property is one of the important characteristics of a material since some biomaterials are considered as excellent bio-compatible adhesives.

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The mussel adhesive protein is one of the promising adhesive bio-materials for practical applications in fields such as medical surgeries. It has higher tensile fracture strength than any other common epoxy resin but it is more ductile. It adheres tightly and permanently to the surfaces of various materials such as plastic, glass, metal, Teflon® and most biomaterials, even in water as well as in the air. Moreover, it is nontoxic to the human body and does not impose any immunogenic side effects [1,2]. Therefore, its chemical and physical properties have been studied to understand how it has such characteristics and functions [3–5] and to develop better adhesive materials for practical uses [6].

Unfortunately, a very small amount of such useful bio-materials as the mussel adhesive protein can be obtained or produced even today. It is known that more than 10,000 mussels are required to obtain 1 g of the mussel adhesive protein. Therefore, a quantitative measurement of adhesive force must be done with a tiny amount for very expensive adhesive materials such as the mussel adhesive protein.



The adhesive property can be obtained by directly measuring the adhesive force of a material of interest on a certain surface. In order to measure the adhesive force, the tensiometer can be used if the testing material can be obtained in a reasonable volume at low cost. Since the bio-compatible adhesive materials such as the mussel adhesive protein are available in a very small amount at high cost, AFM may be considered to be more adequate to measure their adhesive forces. The mussel adhesive protein is adopted as a testing sample here, to evaluate the developed method to measure the adhesive forces with a tiny amount of a testing material using the AFM. Even for the mussel adhesive protein, the tensiometer has been used in the past to evaluate its adhesive properties [7]. Recently, it was reported that AFM was used for successfully measuring the adhesive forces between one kind of mussel adhesive protein and silica [4]. Fant *et al.* claim that the adhesive properties may be evaluated by use of a Quartz Crystal Microbalance (QCM) [8]. However, since QCM can detect the amount of mass attached at its specific surface, it cannot measure the adhesive force directly. Rather, they did measure something that is affected by the adhesive force between the mussel adhesive protein and a gold electrode surface on quartz.

In this article, a method to measure the representative adhesive force is proposed. First, we propose the procedures adequate to reliably measure the adhesive force repeatedly, with only a tiny amount of the specimen. Then, we adopt the adhesive force per unit area as the representative 'adhesive force', Strictly speaking, in the reported measuring procedures using AFM by the reported techniques, the

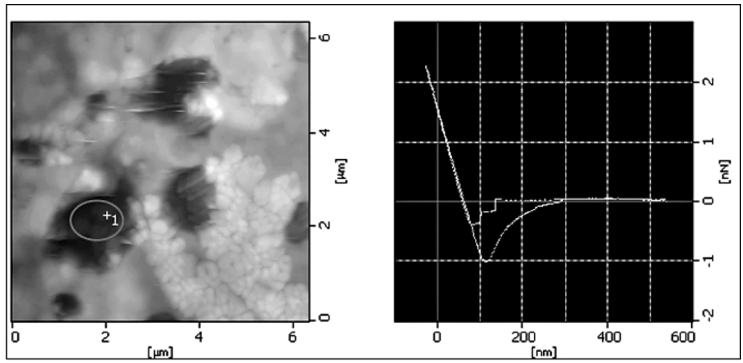
raw data of force-deformation curves must be distinguished from the adhesive force per unit area because the adhering area may be different during repeated tests and cannot be evaluated or measured. Therefore, we tried to control the adhering area. A modified AFM cantilever with micro-glass bead was adopted as used by Ducker *et al.* [9] so that the sufficiently-large adhering area could be guaranteed. And, the testing adhesive is applied first on the micro-glass bead directly in order to control the adhering area reliably. A substrate with micro-scale circular patterns fabricated by micro-machining process is also adopted to precisely control the adhering area at the known value. The adhesive force measured by the proposed method may provide very accurate, reliable data because the force-distance curves in the AFM can be used to measure such tiny forces as the receptor-ligand interaction [10], unfolding of a protein [11], antigen–antibody interaction [12,13], etc.



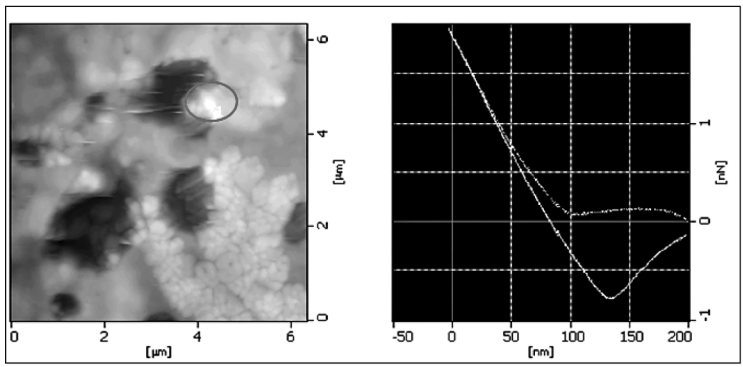
## 2. TECHNIQUES TO MEASURE THE ADHESIVE FORCE WITH AFM

### 2.1. Conventional Techniques

In the first stage, the surface adhesive property of a cured sample is pre-surveyed with a bare AFM cantilever using the commercial AFM equipment (SPA400, Seiko Instrument Inc., Tokyo, Japan). The silicon nitride AFM cantilevers used in the experiments are OTR35 (Olympus, Tokyo, Japan) for the contact mode. The nominal stiffness of the micro-cantilever is known as $k = 0.57\,\mathrm{N/m}$ provided by the manufacturer. Using the contact mode AFM, the topography of the glass surface is obtained where a sample such as Cell-Tak® (BD Biosciences, Bedford, MA, USA), a commercialized natural mussel adhesive, is put and dried in the air for 1 hour at room temperature. Then, after approaching the AFM cantilever until the interaction force is repulsive, the adhesive force is measured by use of the force-distance curve in Figure 1 obtained during pulling up the cantilever. The adhesive forces at several spots obtained by the procedures are presented in Figure 1. Since the topography is flat before the adhesive is applied, it is believed that the higher region contains more adhesive. Therefore, we expected that the adhesive force measured at the higher spot might be larger than that at the lower one. As can be seen in Figure 1, however, the expected trends are not found in the results. For example, the adhesive forces are measured to be higher in the area of lower altitude. Moreover, this trend is not consistent. In some cases, the force measured to be higher at the brighter (therefore, high




(a)



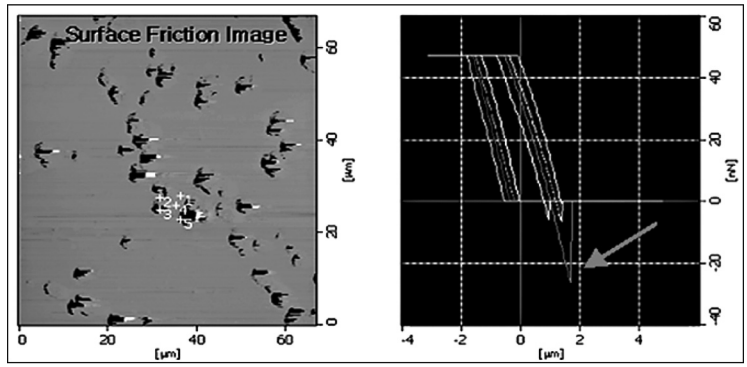

(b)

**FIGURE 1** AFM image of cured Cell-Tak (surface topography with contact mode and force-distance curve at the marked spots). F-D curve at (a) lower area and (b) higher area.

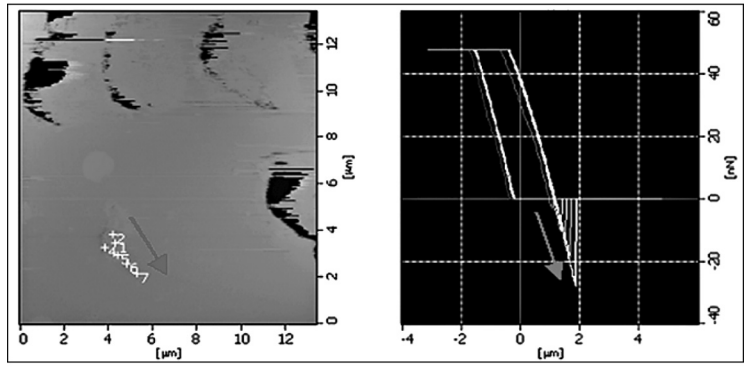
altitude) region. It can be explained by the following reasons. First, the tip of micro-cantilever might be contaminated during the processes of obtaining the topography of surface by the contact mode AFM method. Second, the contact area between the cantilever tip and the adhesive sample varies from one trial to another, because the tip is so small and the surface of the adhesive sample is too irregular to guarantee the contact area to be constant at each approaching process. The last reason may be that the amount of the sample is large enough to form irregular exposure patterns of the adhesive functional groups by the inner cross-linking known as so-called coating effects [14].

Figure 2 presents the Scanning Lateral Force Microscopy (LFM) images and the adhesive forces at several spots by use of the force-distance curves. The samples used in these experiments are of Bovine Serum Albumin (BSA) (Sigma Co., St. Louis, MO, USA). BSA is a protein material widely used as a control [15]. In the experiments, a BSA solution of concentration 0.144 mg/ml is used to make a sample layer. First, the solution is applied on the surface of glass. Then, it is cured in the air at room temperature. Since the lateral force is dependent on the friction force and since the friction force is affected by the adhesive force of the testing surface, a strong relationship had been expected. However, no consistent relationship can be found from these measurement data.






(a)




(b)

**FIGURE 2** AFM image of cured BSA (surface friction force with FFM and force-distance curves at the spots indicated by arrows). F-D curve at (a) around the frictional area and (b) around the frictionless area.

As mentioned before, the results obtained do not show any consistent relationship between the adhesive force and the information on friction forces from the LFM images. No consistent correlation can be found even after treating the LFM images to extract the information on the friction force only, by subtracting the topographical image information.

Therefore, we conclude that the measurement of the adhesive force using the bare conventional cantilever with a sharp tip for AFM may not be a useful technique to evaluate the adhesive property of a material, because the effects of indentation and contact condition of the tip to the surface of a testing adhesive can hardly be controlled. Therefore, we propose a new measurement scheme which makes it easier to obtain useful adhesive data using an AFM with a tiny amount of a sample. In this scheme, the testing procedures are changed and a modified micro-cantilever is used. In addition, a patterned substrate is introduced for the base of a sample to precisely control the contact area.

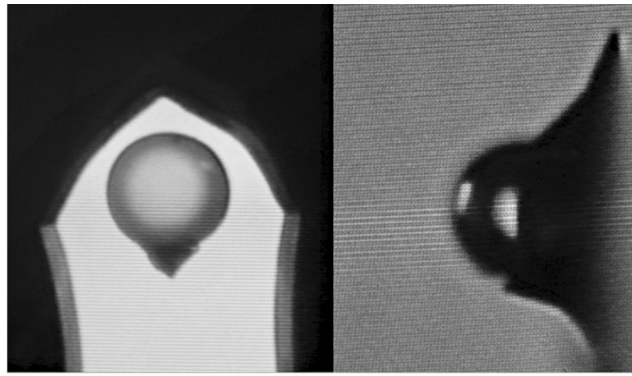


## 2.2. A New Scheme for Reliable Measurement

### *2.2.1. A Micro-Bead-Attached AFM Cantilever*

The typical size of a protein is known to be less than 10 nm. In this study, the adhesive (or bonding) force between one protein molecule and the glass surface is not of interest. However, the adhesive force between them over a macro-scale area is more useful for evaluation of materials such as an adhesive. Therefore, the contact area should be large enough that it contains a sufficiently large number of molecules and defects inside. In order to guarantee the contact area is sufficiently large, a micro-glass bead with a nominal diameter of 20 μm is selected instead of the sharp AFM tip with the nominal radius of curvature less than 20 nm. The micro-bead is attached at the end of an AFM micro-cantilever as shown in Figure 3. This kind of modified probe has been used earlier for measuring the adhesive force or controlling the contact status [9].

The micro-glass bead (borosilicate glass, microsphere 9020, Duke Scientific Co., Palo Alto, CA, USA) has a nominal radius of 20 μm and is attached at the end of an AFM micro-cantilever after an epoxy resin (Araldite, Vantico Inc., Los Angeles, CA, USA) is applied around its tip. After curing for more than 24 hours at room temperature, the modified AFM micro-cantilever is used for measurements. As can be seen in Figure 3, the bead is combined with the micro-cantilever at the right location in a clean state. Since the diameter of the micro-bead is about 20 μm, the contact area is expected to be larger than the area



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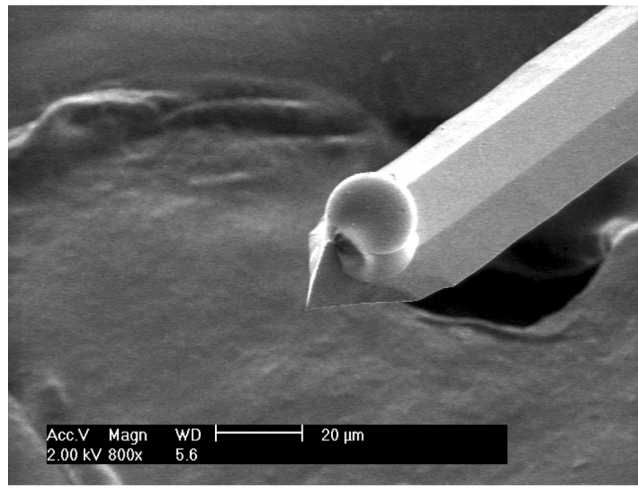



(b)

**FIGURE 3** Modified AFM cantilever with a glass bead: (a) an optical microscope image and (b) a SEM image.

of a circle with radius 1 µm, which is believed to be large enough to provide the average adhesive force in a macro-scale area.

### *2.2.2. New Measuring Procedures*

As mentioned in Section 2.1, since the surface of a sample deposited on a glass slide is too irregular to repeatedly control the contact area, we propose new procedures to solve the problem. The main idea is to apply the sample on the probe instead of the glass slide or substrate. If the sample is applied on the micro-bead carefully, the variation in

the contact area can be considerably reduced. The following are the experimental steps for measuring the adhesive force reliably:

(a) Prepare the modified AFM micro-cantilever by attaching a micro-glass bead at its end.
(b) Deposit an adhesive sample at a proper location on a glass slide.
(c) Apply the adhesive sample carefully at the end of the micro-bead by use of the magnified images from the CCD integrated in the AFM.
(d) Cure the adhesive sample in the air at room temperature.
(e) Move the probe to a clean surface of the slide and approach the probe to the surface until the measured contact force becomes repulsive.
(f) Pull up the probe until the probe is believed to be completely separated from the slide surface while recording the force-distance curve from the AFM.
(g) Repeat Procedures (e) and (f) until a sufficient number of data sets on the force-deformation curve are obtained.



Figure 4 shows a conceptual diagram of the proposed measuring process. The surface of the glass slide is assumed to be sufficiently flat and smooth. By adopting these procedures the variations in the measured adhesive force data can be considerably reduced. However, the absolute value of the contact area cannot be estimated with

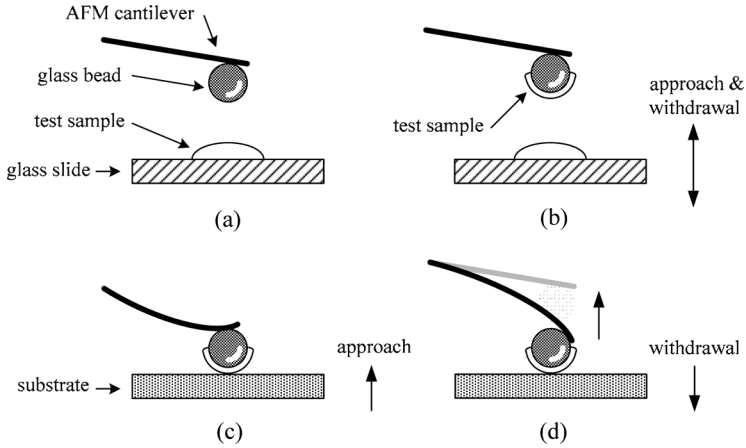



**FIGURE 4** Conceptual diagram of experimental steps for measuring the adhesive force by applying the sample to the bead directly.

sufficient accuracy since the sample may be applied on the micro-bead under various conditions. For example, it can be applied at the small region on the micro-bead or all over it. Therefore, even though the measured adhesive forces for one sample are not scattered in a wide region, they may be considerably different from one sample to another one of the same kind. A method should be found to control the contact area more precisely since the adhesive force per unit area is believed to be the meaningful number. We propose to introduce a substrate (or slide) with circular topographical patterns.

### 2.2.3. A Substrate with Circular Patterns

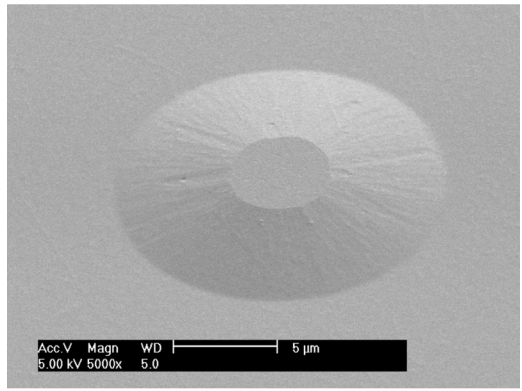
In order to precisely control the contact area between the sample and the glass slide surface, the best way is to prevent any other area except a certain region from contacting the sample. We devised a substrate with circular patterns as illustrated in Figure 5. The circular columns are made on the flat glass surface so that only the top-end surfaces of these cylinders could make contact with the micro-glass bead's bottom surface. If the top area of the truncated cone is sufficiently small, its area determines the contact area since the micro-bead cannot contact the bottom surface of the patterns due to geometrical constraints. Therefore, by choosing the radius of the top circular area properly, the contact area may be precisely controlled around the top area of the circular patterns.



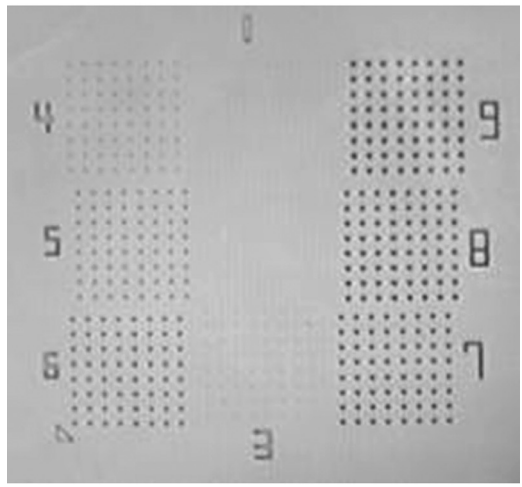
The diameter of the cylindrical patterns is determined by the following procedures. Let $D$ be the separation distance between the top surface of the cylindrical pattern and the bottom surface of the micro-bead of radius, $R$. The effective area, $A$, of interaction between the micro-sphere and the flat top surface of the cylindrical pattern may be expressed as follows [16]:

$$A = \frac{2\pi RD}{(n-5)}, \tag{1}$$

where $n = 6$ for van der Waals forces. Obviously, the contact area is expected to be larger than the area when $n = 6$ because the adhesive layer exists between the bead and the top surfaces of the pattern. In the experiments, $R$ is approximately $10\,\mu m$ and $D$ may be set to be $0.5\,\mu m$ since the thickness of the protein layer applied on a flat surface was measured to be less than $0.5\,\mu m$. By use of the formula given in Eq. (1), it is easily concluded that the contact area between the top surface of the circular pattern and the bottom surface of the micro-glass bead is determined by the top surface of the circular pattern if the radius of the cylinder patterns is determined to be less $3\,\mu m$.




(a)




(b)

**FIGURE 5** SEM image of fabricated substrate: (a) isotropic view of a circular pattern and (b) top view of pattern array with various diameters.

The base slide with circular patterns is fabricated through a micromachining process. Figure 6 shows the fabrication processes schematically. The Photo Resist (PR) is coated on the surface of a (Pyrex) glass wafer and exposed to UV light through a prepared mask. Only the regions for the top surfaces of the cylindrical patterns are made to remain through the PR removal process. The desired patterns can be obtained by wet-etching (with aqueous HF solution/buffered HF 6:1). Figure 5 shows the patterned substrate. The topographical image

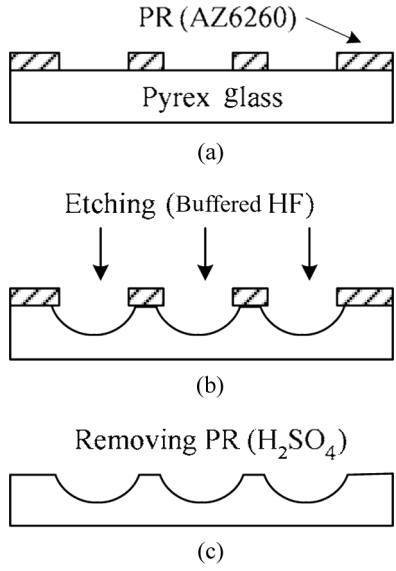



**FIGURE 6** Fabrication of substrate with micro machining process.



by SEM is shown in Figure 5a. Since the wet-etching removes glass isotropically, the pattern looks somewhat different from a cylinder. However, we can obtain more reliable data on the adhesive force repeatedly with these patterns.

As shown in Figure 5b, the fabricated patterns are categorized in the seven groups by the sizes of their top surfaces. Representative values of the top surface areas were calculated from the contact mode AFM measurement and the substrates of Pattern #7 with a mean diameter of 3.83 μm and an area of 11.5 $\mu m^2$ were used in the experiments.

In Figure 7, the conceptual diagram of measurement procedures is illustrated when the patterned substrates are used. The adhesive force can be obtained by figuring out the force-distance curve with AFM. The details of the processes are almost the same as those described in Section 2.2.2. However, since the patterned substrate is used instead of the glass slide, the following procedures must be executed after Procedure (a) described in Section 2.2.2.

(a-i) Obtain the topographical information (or image) of the substrate by scanning the surface with the modified AFM cantilever using the contact mode AFM operation.
(a-ii) Identify the shapes, locations, and areas of the circular patterns on the substrate surface.

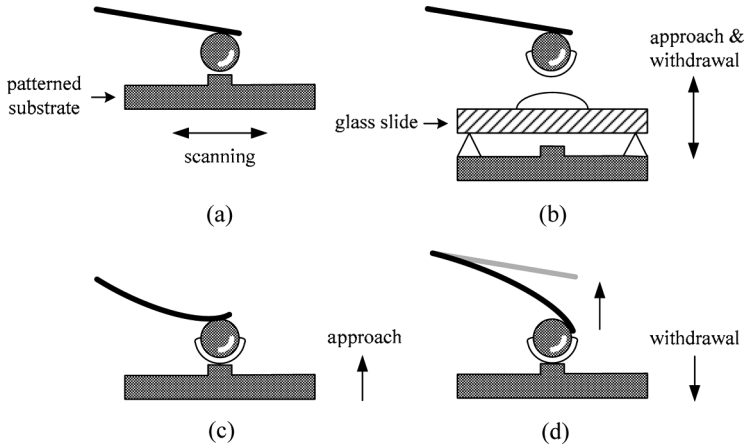



**FIGURE 7** Conceptual diagram of experimental steps for measuring the adhesive force with the modified cantilever and the patterned substrate.



It is important to precisely position the center of the bottom surface of the micro-bead at the center of the top circular surface of a cylindrical pattern. An image obtained by contact mode AFM imaging is shown in the left-hand side of Figure 8. The top circular planes are sufficiently large to position the probe on their centers with adequate accuracy. The measured force-deformation curves are shown in the right-hand side of Figure 8.

## 3. RESULTS AND DISCUSSION

The adhesive force measurements are performed for several kinds of available mussel adhesive proteins: Cell-Tak and the recombinant Mgfp-5 (Mytilus *galloprovincialis* foot protein type 5). Recombinant Mgfp-5 is a mussel adhesive protein produced from *Escherichia coli.* It is used for adhesion force measurement and was prepared as described in a previous paper of the authors [6]. To endow adhesion ability, tyrosines in recombinant Mgfp-5 should be converted to 3,4-duhydroxyphenyl-L-alanine (DOPA) which plays a key role in adhesion of mussel adhesive. Thus, tyrosine residues of recombinant Mgfp-5 (0.144 mg/ml) were converted to DOPA by 50 μg/ml of mushroom tyrosinase (Sigma-Aldrich, St. Louis, MO, USA). Then, it showed superior adhesion abilities *versus* Cell-Tak, a commercialized mussel adhesive protein [6].

The modified probes used in the experiments are made by attaching a micro-bead at the end of the silicon AFM probe (ZEILR, Nanosensors, Neuchatel, Switzerland), with a nominal stiffness of 1.6 N/m.

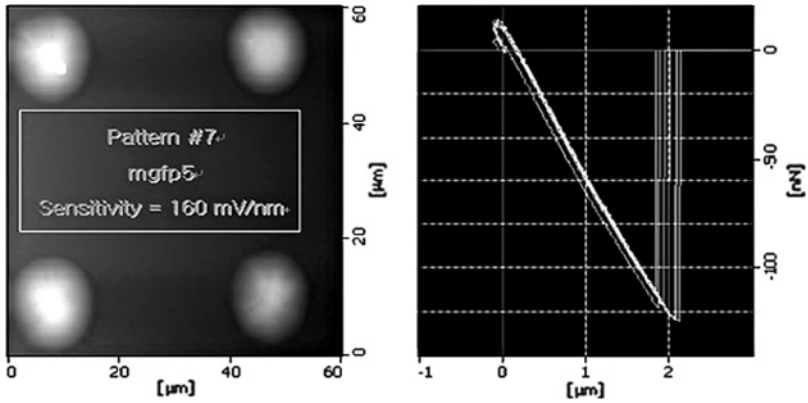



**FIGURE 8** AFM image of patterns and force-distance curve at the projection part. (The represented force at the F-D curve is raw data before multiplication by a magnifying factor.)



One modified probe is used to measure the adhesive forces of one sample only even when several samples of the same kind are tested. The adhesive force of one sample is measured many times to make sure of its repeatability and many samples of one kind are also tested in the same manner. The sample is attached at the bead by immersing the bead into the protein solution of 5 μl volume so that it is applied only at the bottom region of the micro-bead. During this process every step is monitored by use of a CCD camera with a magnifying lens. After the sample is applied at the desired region of a micro-bead, it is cured for 20 minutes in the air. During all the experimental processes, the temperature and the relative humidity are maintained at 24°C and at 40%, respectively. It takes about 5 seconds to obtain one force-distance curve for one sample by moving the modified probe (down to up) one cycle.

Figure 8 shows the force-distance curves obtained during measurements for one sample (on the right-hand side) and the AFM topographical image of the patterned substrate (on the left-hand side) obtained using the modified probe before the adhesive sample is applied on its tip. The topographical image can provide the effective contact area of the measured adhesive forces. The force-distance curves shown on the right-hand side of Figure 8 are obtained from iterated measurements for one adhesive sample.

In Figure 9 the adhesive forces per unit area are presented as a bar graph. The adhesive forces per unit area may be the meaningful quantity for representing the adhesive property of a material. It can

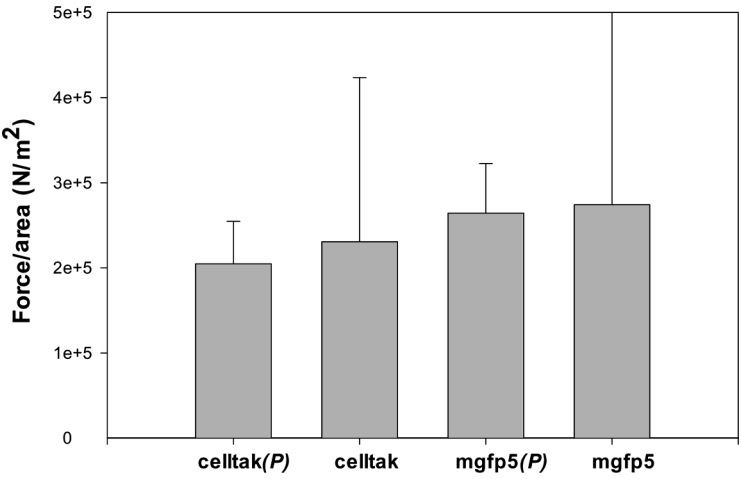



**FIGURE 9** Adhesive forces measurement using the substrate with and without patterns (bar-mean, error bar-standard deviation, (p)-with pattern).



be estimated from the force-distance curve and the contact area. If the real contact area varies from one measurement to the next, the estimated adhesive force per unit area will be scattered over various numerical values even though the measured adhesive forces are accurate. This trend can be observed in the data obtained from the measurements using a substrate without patterns. As can be seen in Figure 9, the adhesive forces of the recombinant Mgfp-5 measured with a flat substrate are sometimes out of the range of the AFM equipment used. However, all data obtained from the measurements with a patterned substrate are in a reasonable range. This is easily explained if the contact area is assumed to be properly controlled by the circular pattern on the substrate. In other words, the patterned substrate should be used to obtain reliable data. The effects of the pattern on the data obtained from measurements are obvious from the statistical point of view. If one reviews the error bars in Figure 9 carefully, it can be seen that the mean values of adhesive force per unit area are not substantially different while the error bars are remarkably reduced by adopting a patterned substrate for measurements. For example, in the case of Cell-Tak, uses of the patterned substrate reduce the standard deviation from 84 to 24%.

As expected, based on the chemical properties of Mgfp-5 and Cell-Tak, the adhesive force of the recombinant Mgfp-5 is measured to be somewhat higher than that of the Cell-Tak. The average value

of the measured adhesive forces for Cell-Tak is about 205 K $N/m^2$ while that for Mgfp-5 is about 260 K $N/m^2$.

As mentioned in Section 2.2.1, we seek to measure the average adhesive force per unit area that is compatible with the values obtained from the macro-scale adhesive force measurements. Since the macro-scale measurement provides the average adhesive force per unit area in the presence of defects or voids on the contact area, it can be significantly different from that obtained by the technique using the nanometer scale contact area. The developed approach is a micro-scale measuring scheme, so we claim that it may give an adhesive force value close to that obtained by the macro-scale scheme. This is why we tried to compare the data obtained from our experiments with those from the macro-tests. Therefore, we tried to show the validity of our approach by ascertaining the order of magnitudes of the adhesive force values of the adhesive obtained by the two testing techniques: our micro-scale and the conventional macro-scale measuring schemes.



Unfortunately, the adhesive force per unit area for the material measured here is not available by the conventional macro-scale test due to the high cost of testing; thus, we measured the adhesive force of a cheaper commercially available material. The selected material for these tests is SP600 (Susan Polymer, Hwasung, Korea). A commercialized base polymer (primarily an elastomer) for the pressure sensitive adhesive (PSA). It was deposited uniformly on a flat glass substrate and its thickness was measured as 50 μm. The adhesive force per unit area of this material is measured according to ASTM test D2972 using a TA.XT2i (Stable Micro Systems, Surrey, UK), a conventional texture analyzer. Since the developed method is believed to measure the tack [17], the probe tack test is performed using TA.XT2i. It provides 0.96 MPa as the adhesive force per unit area for SP600.

In our micro-scale test, since SP600 can be deposited uniformly on the flat glass surface, the adhesive force can be properly measured using the developed method without a patterned glass substrate. However, since the thickness of the sample layer is 50 μm, the contact area should be properly estimated based on the adequate theory. Since the material is primarily an elastomer, the theoretical calculations of the contact area should be done carefully considering the viscoelasticity and the adhesion. However, Tsukruk *et al.* measured the elasticity of an elastomer by scanning force microscopy (SFM) and the Hertz contact model [18]. Also, Mahaffy *et al.* expanded the Hertz model to include viscoelastic contributions, where the DC or quasi static component of the deforming force according to the indentation depth

is simply the contributions of the original Hertz model [19]. Thus, the viscoelastic effect was not considered here because it takes about 5 seconds for one measuring cycle.

When the adhesion exists, contact mechanics such as the JKR or DMT theories can give more accurate contact area (JKR is the more appropriate model for low modulus materials) [18,20]. Also, it is well known for that the JKR contact area is larger than the Hertz's due to the adhesive force. However, the difference between the contact areas which are calculated based on the above theories gets smaller as the deformation load (indentation force) increases [20]. Also, it is roughly estimated that the Hertz contact area does not exceed 100% of the JKR contact area in our experimental conditions. Therefore, adoption of a more accurate model does not make any difference to our conclusion. Thus, the contact area ($A$) between the sphere and the flat surface is easily found as follows (Hertz model) [21]:

$$A = \pi a^2$$

$$a^3 = \frac{PR}{K}$$

$$R = \left(\frac{1}{R_1} + \frac{1}{R_2}\right)^{-1}$$

$$K = \frac{4}{3}\left[\frac{(1-\nu_1^2)}{E_1} + \frac{(1-\nu_2^2)}{E_2}\right]^{-1}$$

where $P$ and $a$ are the load and the contact radius, respectively, and $R_i$, $E_i$, and $v_i$ are the radius of curvature, the Young's modulus, and the Poisson's ratio of the $i$-th body, respectively. Obviously, the $i$-th body can be a sphere or a flat surface (if $R_i$ is infinite).

The Young's modulus value for the SP600 should be known for complete estimation of contact area. Since our purpose is to ascertain the order of magnitudes, as mentioned earlier, we believe that adoption of its approximate value is enough for our purpose. The mechanical properties of SP600 are known to be close to those of polyisoprene rubber (elastomeric polymer). Hence, applying $1 \sim 3$ MPa, the range of its Young's modulus to the calculation [22], we obtained the value of $0.76 \sim 1.62$ MPa as the average adhesive force per unit area from the data measured using the developed micro-scale scheme. Obviously, this range includes the measured value of 0.96 MPa obtained by the conventional macro-scale measuring scheme. Also, it is of the

same order of magnitude even though the contact area is somewhat overestimated according to the JKR model.

## 4. CONCLUSIONS

In this study, a reliable method is developed to measure adhesive force of a material using the AFM with an extremely small amount of a sample. It is shown that the contact area is the first that must be controlled to obtain repeatable data for the adhesive forces. New measurement procedures are proposed to improve the reliability of measurements and introduction of a patterned glass substrate is proposed to define the contact area precisely. The developed method is applied to measure the adhesive forces of the very expensive bio-adhesive materials, Cell-Tak and Mgfp-5. The measurement data for the two materials obtained through the proposed experimental procedures using the proposed method are compared with those obtained by methods reported previously by other researchers. It is found that the standard deviations (or error bars) of the measured data are enormously reduced by adopting the proposed procedures and methods. In addition, it is also shown that the numerical value of adhesive force per unit area obtained from the developed method is the quantitatively correct one for the adhesive property of a material that is usually measured by the conventional probe tack test.



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