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Interaction of Serum Proteins with Hemodialysis Membrane: Comparison with De-adhesion Process of AFM Probe from Adhesive Tapes

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Adhesive properties of several serum proteins to the hemodialysis membrane surface previously studied by atomic force microscopy were compared with a more general case of adhesion/de-adhesion process of adhesive tapes and films. The three different tapes and film samples showed de-adhesion curves with different level of tack and flow properties. The two types of de-adhesion force curves of serum albumin from the hemodialysis membrane can be explained in terms of the difference in the physical process of de-adhesion and in a general perspective of self-cohesive force of the adhesives.

Keywords Hemodialysis membrane; serum proteins; adhesion; adhesive tapes: atomic force microscopy, de-adhesion process

1. Introduction

Hemodialysis is an important medical treatment with 50 years of history for patients with renal malfunctions [1]. The patient's blood is forced to flow through bundles of hemodialysis membrane tubes with a semi-permeable property. Small molecules such as urea are dialyzed out of blood while vital components such as red blood cells and much of serum proteins are retained and charged back to the blood vessels of the patient. Hemodialysis membrane must have a good biocompatibility not to cause blood coagulation or adsorb cells/proteins that would reduce the efficiency of dialysis and eventually stops the blood flow inside of the tubes. Adhesion and de-adhesion process of serum proteins on the hemodialysis membrane surface has provided an important guide for the selection of the basic materials for membrane manufacturing and the improvement of the quality of the final products [2, 3]. Often the membrane is formed from a mixture of hydrophobic and hydrophilic polymer materials as described [4, 5]. In this process a mixed blend of polysulfone (PS)

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and polyvinylpyrrolidone (PVP) in dimethylacetamide (DMAC) is forced through a narrow tube with a parallel flow of water/DMAC fluid mixture. Most of PVL molecules are washed away in the liquid phase except those rooted in PS phase. The remaining PS membrane has a large number of pores having diameter suitable for hemodialysis. The walls of the membrane and pores therein are considered to be partly covered with PVP with some exposed PS phase. It is important, therefore, to know the interaction of major serum proteins with the surface of hemodialysis membrane tubes.

We have previously reported the comparative study of adhesion of five different serum proteins to the surface of hemodialysis membrane tubing formed from PS and PVP according to the method as described in refs. [4, 6]. In the cited paper [5], we used an atomic force microscope (AFM) as a nanotechnological measuring tool of de-adhesion force. Atomic force microscope (AFM) has been used for creating a contour map of the sample surface by mechanically probing the surface topography with a sharp needle tip integrated on a small cantilever [7]. The cantilever deflection caused by near field interaction between the tip and the sample surface is recorded as height changes for topography data. In addition to creating topographic image of the sample surface, AFM has also been used to measure the magnitude of the physical interaction, basically in terms of force, between its variously modified tip and the sample [8]. The repulsive interaction deflects the cantilever upward whereas attractive interaction downward. Based on this simple principle, indentation of the sample surface or tensile stretching of polymer chains has been conducted both on biological and non-biological specimens [8–11]. In this paper, we adopted AFM to measure the force required to detach the cantilever tip from the sample surface after making a temporal contact between them.

In Fig. 1, application platform of AFM for the measurement of force is schematically explained. In Fig. 1A, the AFM cantilever and the sample are in their positions before mutual contact. In B, the tip is applying a tensile force to the sample to peel off an adhesive surface, and in C, the tip is indenting into the sample by compressive load.

In both cases, the downward or upward deflection of the cantilever due to tensile or compressive interaction with the sample is converted to the force after knowing the conversion factor of cantilever deflection to force (spring constant). In the case of protein-hemodialysis membrane interaction, the tip was modified with covalent cross-linkers so that the proteins could be covalently immobilized on the silicon nitride cantilever tip according to the method described in [6].

The interaction of serum proteins with the hemodialysis membrane surface can also be considered as an interesting example of more general case of adhesion/de-adhesion processes of adhesive materials. Adhesive tapes and films are commonly available with a wide range of selections for our daily use and for some special purposes. One of the common adhesive materials coated on such tapes and films are cyanoacrylate polymers of various modifications.

The adhesion and de-adhesion process of macroscopic/mesoscopic probes on the surface of adhesive samples has been studied using specially built mechanical devices [12] and resulting force curves have been analyzed as a combination of “tack and flow” properties of the samples. This interpretation of the process in terms of “tack and flow” mechanism is useful to understand the results obtained on the surface of hemodialysis membrane, Using their custom made apparatus, Takahashi et al. recorded a force curve of the entire process of probe indentation and following de-adhesion on a layered adhesive material with simultaneous observation of the cavity formation within the adhesive material [12]. The de-adhesion force curve was characterized by a concave non-linear increase of the tensile force up to a failure point which was followed by a sudden decrease of the force down

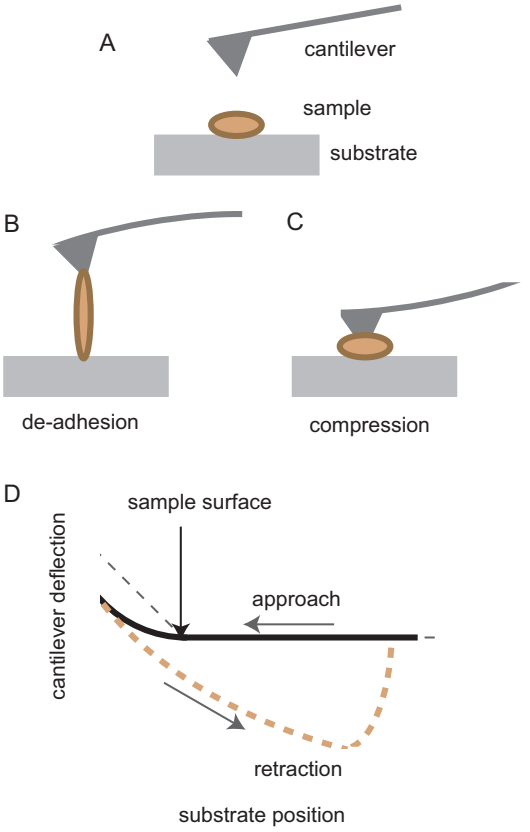

Figure 1. Application of AFM for the measurement of de-adhesion force and compressive force on a soft sample. A: before contact of AFM cantilever tip and the sample on a substrate. B: application of tensile force for de-adhesion measurement. C: application of compressive force for indentation experiment. D: recording of a force curve as a function of force against substrate movement (D). By subtracting cantilever deflection (d) from D, one obtains the deformation of the sample and re-plot the result as force-extension (*F*-*E*) curve.

to the zero level corresponding to simultaneous breakdown of multiple adhesive bonds. By careful examination, they found the final part of the force curve often had stepwise decrease of the force. We became particularly interested in the concave increase of the force up to the point of tensile failure that reminded us of the extension process of flexible molecular chains such as DNA or denatured proteins even though the sample scale is very different [13, 14]. Takahashi et al. explained the particular feature as due to formation of fibrillate structures interspersed by growing cavities in the glue that was being pulled up underneath the flat-faced probe. According to their explanation, cavity formation in the double adhesive interface (probe-glue-substrate) is a decisive factor for the appearance of the long and concave feature of the force curve.

In this paper, we show the result of similar experiments on the adhesive surface of two kinds of adhesive tapes and a specially prepared film but performed at a nano-meter scale using an AFM probe. Such a nano-scale experiment was intended so that there would be little, if any, possibility of cavity formation simply because of the small size of the probe.

The resulting curves had a convex, rather than concave, increase of the tensile force up to the tensile failure point supporting our contention that at a very small contact area between the probe and the adhesive material, fibrillation of the latter due to cavity formation was most unlikely. The two types of tensile force curves obtained during the de-adhesion process of serum proteins from the hemodialysis membrane can be explained as two extreme cases of macroscopically observed de-adhesion processes of AFM probe from adhesive tape surface.

2. Materials and Methods

2.1. Adhesive Tapes

Commercially available NW-15 Nicetack® (Nichiban, Tokyo, Japan) and Scotch 3M Mending Tape® (Sumitomo 3M, Tokyo, Japan,), both coated with cyanoacrylate adhesives, were used in this work. They will be hereafter called, respectively, Nichiban tape and 3M tape. Another thin tape sample coated with butylacrylate was also tested (to be called BA tape). Tape samples were cut into small pieces (approximately 10 mm × 10mm) and glued to the surface of separate pieces of slide glass.

2.2 Atomic Force Microscopy

For force curve measurement, a Nanowizard II AFM (JPK, Berlin, Germany) was placed on a Zeiss Axio Observer 200M optical microscope (Carl Zeiss, Jena, Germany) equipped with a CCD camera for observation of sample surface and the AFM cantilever. Olympus OMCL-AC160TS-C2 rectangular cantilever (length = 160 μm, width = 50 μm, thickness = 4.6 μm, tip height = 14 μm, tip radius = 7–10 nm as supplied from Olympus, Tokyo, Japan) was mounted on the AFM and used after sensitivity calibration. Spring constant calibration was not performed and the nominal value of 42 N/m as supplied by the manufacture was used in this work. The numerical result in this work, therefore, should be taken as semi-quantitative. The original force curves obtained on Nanowizard AFM were converted to force-extension curves after they were converted to ASCII text files by JPKSPM Data Processing software and analyzed by a custom built software based on IgorPro (Wavemetrics, Lake Oswego, OR).

3. Results

Working with adhesive materials using an AFM needs some special care because the tip-sample interaction is strong and expected to interfere with the scanning operation of the AFM. To ensure detachment of the tip from the sample surface at the end of the force curve tracing, we employed relatively stiff OMCL-AC160TS-C2 rectangular silicon cantilevers with specifications given above. A recommended correction factor with regard to the oscillation angle of the fundamental mode for this type of cantilevers is 0.817 so that the operative spring constant becomes 34 N/m (JPK Nanowizard II User Manual SPM Software Release 3.3.). We took this value in the following presentation of force data. In this paper, we are mainly concerned with the retraction regime of the force curve since the de-adhesion process of the AFM tip from the adhesive surface is represented in this regime.

In Fig. 2, typical examples of the retraction regime of the force-extension curves obtained on a Nichiban tape, 3M tape, and BA tape are given. The curves given in Fig. 2

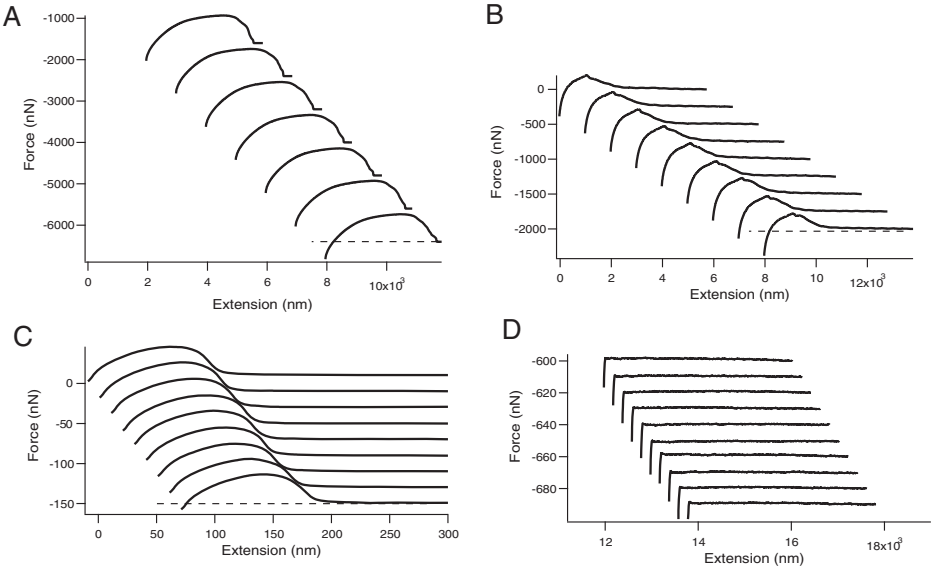

Figure 2. De-adhesion curves obtained on A: Nichiban tape in air and at 25°C as described in text (seven curves in a cascading style). The abscissa and ordinate here and in the following figures are, respectively, sample extension in nm and the tensile force sensed by the AFM cantilever (nN). B: De-adhesion curves obtained on 3M tape in air and at 25°C (nine curves in a cascading style). C: De-adhesion curves obtained on BA tape in air and at 25°C (nine curves in a cascading style), D: de-adhesion curves obtained on BA tape in water at 25°C (ten curves in a cascading style).

are those most prevalently observed and allowed straightforward interpretation for each sample. Curves with more complex overall features will be treated separately.

In the case of Nichiban tape (Fig. 2A), the non-linear increase of the tensile force from zero to the tensile failure point of ∼600 nN clearly shows a convex increase of force in contrast to the concave curve reported by Takahashi et al. in their macroscopic experiment [12]. The extension of the adhesive material up to the failure point (tack strength) was about 3000 nm ($= 3\ \mu$m) and it took further 1 μm extension before the final rupture of all the adhesive bonds. The total sample extension (flow length) was therefore close to 4 μm. The speed of tip retraction was 12 μm/s. Since all these numerical values would depend on factors such as the pulling rate, indentation depth, the tip size, additives in adhesive material, and so on, the results given in Fig. 2 should be taken as examples under the given condition in this work. The zero force level is given, as a dotted horizontal line in the bottom force curve. The results give a strong support to our hypothesis that the de-adhesion curve should have a convex curvature at a very small contact scale with little possibility of cavity formation at the adhesive interface, Under the present experimental setup, cavity formation was unlikely from the estimated size of the AFM tip in the range of 10 nm, though not completely excluded.

The depth of indentation was estimated to be ∼0.7 μm from the approach part of the force curves. The energy of de-adhesion was estimated as 1.6 ± 0.06 pJ by integrating the area under the curves.

Similar results obtained for 3M tape are presented in Fig. 2B. In the case of 3M tape, the extensions before and after the tensile failure point were approximately the same being about 1 μm in each case. Compared with the Nichiban tape, the self-cohesive property of

3M tape was weaker making the flow part after the tensile failure relatively longer. Average depth of indentation was ∼1.7 μm. The average de-adhesion work was 0.4 ± 0.02 pJ. In the case of BA tape as presented in Fig. 2C, tensile strength as well as the extension before the final failure were both smaller than the previous two cases, and consequently, the de-adhesion energy of ∼3 fJ was almost 500 times less than the case of Nichiban tape and 100 times less than 3M tape. We confirmed that the de-adhesion work was significantly reduced for all three samples when similar experiments were performed under water in accordance with our daily experience. Results for BA tape are given in Fig. 2D.

Since the thickness of adhesion layer of Nichiban, 3M and BA tapes was, respectively, 90 μm, 60 μm, and 18 μm, the tip was probing only the surface layer of the tapes. It should also be noted that a 14 μm high AFM tip was pressed into the adhesion layer up to 5% (Nichiban), 12% (3M) and 0.5% (BA) of its height under our experimental conditions ensuring that only the tip, not the cantilever spring part, was in contact with the adhesive layer.

From the results given above and the geometric information of the cantilever provided by the manufacture, we estimated the average energy of de-adhesion per unit area of contact as approximately 3 and 0.1 J/m^2, respectively, for Nichiban and 3M tapes indicating that Nichiban tape is ∼30 times stickier than 3M tape. It is a reasonable result considering the 3M tape is meant to have less sticky property so that its peeling would be easier than other types of adhesive tapes. Since the indentation of the tip in BA tape was ∼0.5% of the tip height, the contact area was small. The de-adhesion energy per unit contact area was 5 times less than the case of Nichiban tape.

4. Discussion

The probe-tack test used in macroscopic experiment is designed to control the contact pressure, contact time and separation speed, and allows accurate measurement of de-adhesion force with simultaneous observation of the separation behavior through an optical microscope [15, 16]. The AFM based method employed in this work did not have the capability of visualizing nanometer scale contact area but could probe the basic nature of adhesive tape materials with little possibility of cavity formation. The resulting force curves invariably had convex increase of tensile force up to the tensile failure point indicating that there was no fibrillation in the contact area as was observed in [12]. The following part of the force curves after the tensile failure point was different, especially between Nichiban tape and 3M tape. 3M tape showed a more gradual decrease of tensile force indicating a weaker self-cohesive force between bundled polymer molecules allowing a more "slippery" extension.

Conceptually, de-adhesion force curves have a feature as depicted by a graph in Fig. 3. The initial rapid increase of tensile force reaches a maximum value (tensile strength), then a sudden or gradual decrease of the force is observed until the final detachment of the probe from the adhesive surface. The two important parameters in such a graph are the tensile strength, strain to final failure, and the de-adhesion energy as calculated from the area under the curve. All three parameters depend on experimental factors such as, 1) pulling speed, 2) contact area between the probe and the sample, 3) contact time of the probe with the sample, and others including 4) temperature. If the contact area can be estimated, the de-adhesion energy per unit contact area is a meaningful parameter to compare the adhesive strength of different samples tested under similar conditions. In the result section, we gave such values for three different kinds of tapes assuming that the sample surface was flat in all cases.

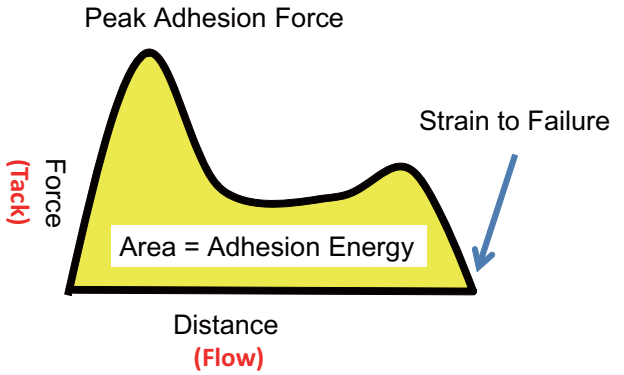

Figure 3. Schematic de-adhesion curve showing several characteristic parameters.

It was then concluded that Nichiban tape required ~30 times more de-adhesion work than 3M tape.

In our previous paper, we reported observation of two types of de-adhesion force curves when an AFM probe coated with serum proteins were detached from the surface of hemodialysis membrane [5]. In Fig. 4, we present the result of similar experiment performed

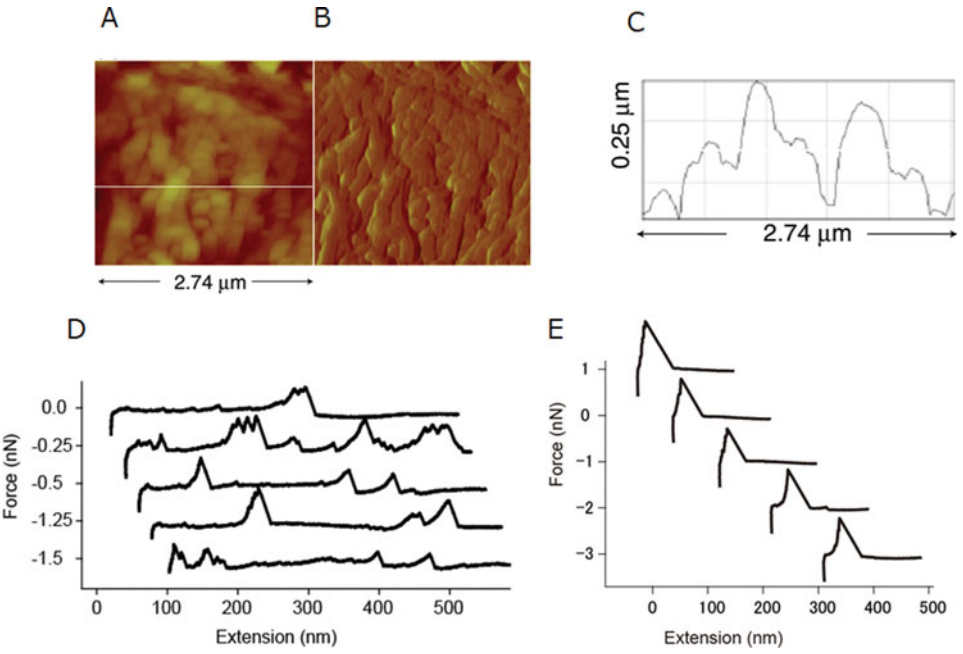

Figure 4. Result on hemodialysis membrane using an AFM probe modified with human serum albumin. A: AFM image of the hemodialysis membrane surface (height image). B: deflection image. C: cross-sectional image along the white line in A. D: curves with a long extension before tensile failure is reached. E: curves with short extension. (A, B and C were reproduced from [5] with permission).

using a probe coated with human serum albumin to be compared with the results obtained in this work on adhesive tapes.

In one type of force curves obtained in protein de-adhesion from the hemodialysis membrane surface (Fig. 4D), a long and flat flow was observed before the final rupture. This long extension was concluded as representing an extension of soft PVP chain having extremely low self-cohesive force. The other type of force curves with short extension in Fig. 4E testifies that the self-cohesive force of the sample was stronger than its de-adhesion force from the membrane surface. In this case it is most likely that the protein on the AFM tip was directly adsorbed to the membrane surface without intervening PVP chain. Moreover, the de-adhesion process of the protein was nearly a single step jump from adsorbed to completely free form suggesting the step proceeded as a two step transition without involving unfolding of the protein.

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