Available online at www.sciencedirect.com
SCIENCE DIRECT®
Colloids and Surfaces B: Biointerfaces 32 (2003) 1–10
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Competitive protein adsorption studied with atomic force microscopy and imaging ellipsometry

Peiqing Ying, Yong Yu, Gang Jin *, Zulai Tao

Institute of Mechanics, Chinese Academy of Sciences, Beijing 100080, PR China

Accepted 15 July 2002

Abstract

The adsorption and competitive adsorption of collagen and bovine serum albumin (BSA) were directly visualized and quantified using atomic force microscopy (AFM) and imaging ellipsometry. Chemically modified silicon surfaces were used as hydrophilic and hydrophobic substrates. The results showed that collagen and BSA in single component solution adsorbed onto a hydrophobic surface two times more than that onto a hydrophilic surface. The competitive adsorption between collagen and BSA showed that serum albumin preferentially adsorbed onto a hydrophobic surface, while collagen on a hydrophilic surface. In the binary solution of BSA (1 mg/ml BSA) and collagen (0.1 mg/ml), nearly 100% of the protein adsorbed onto the hydrophobic surface was BSA, but on the hydrophilic surface only about 6% was BSA. Surface affinity was the main factor controlling the competitive adsorption.

Keywords: Competitive protein adsorption; Surface hydrophobicity; Imaging ellipsometry; Atomic force microscopy

1. Introduction

Competitive protein adsorption is important in many interfacial phenomena such as hemocompatibility of biomaterials and cellular adhesion and growth on substrates [1–3]. For example, the adhesion of human vein endothelial cells onto polystyrene-based culture surfaces is affected by the competitive adsorption between fibronectin and vitronectin in serum [4]. As the most abundant protein of the extracellular matrix, collagen is often used as a pre-coated protein to support cell growth in vitro [5–7]. Albumin is one of the most abundant proteins in serum or plasma and is often used as a passivating agent to prevent the adhesion of cells [8]. The competitive adsorption between collagen and serum albumin is of great importance in biomaterial design. The competitive adsorption between the two kinds of proteins on hydrophobic or moderately hydrophobic surfaces has been investigated, and collagen adsorption is measured

* Corresponding author. Tel.: +86-10-62631816; fax: +86-10-62561284.
E-mail address: gajin@imech.ac.cn (G. Jin).

0927-7765/02/$ - see front matter © 2002 Elsevier B.V. All rights reserved.
doi:10.1016/S0927-7765(02)00133-9

with isotope labeling [9–11]. Dewez et al. [9] studied the effects of human serum albumin (HSA) on the adsorption of collagen on polystyrene substrates and showed that the presence of HSA reduced collagen adsorption, especially on the more hydrophobic substrates. Boissonnade and co-workers [10,11] investigated the competitive adsorption of albumin against collagen at solution–air and solution–polyethylene interfaces and demonstrated that albumin was the only adsorbing protein on both interfaces within a large range of collagen concentration. Surface hydrophobicity is a key factor that affects competitive protein adsorption. In order to demonstrate this, competitive protein adsorption on surfaces with various hydrophobicity should be studied. In this paper, silicon wafers chemically modified to be highly hydrophilic or hydrophobic were chosen as substrates to study the competitive adsorption between collagen and BSA on the two surfaces. Two microscopic techniques, namely atomic force microscopy (AFM) and imaging ellipsometry, were used for the visualization and quantification of competitive protein adsorption.

Imaging ellipsometry is a recently developed optical technique which can directly visualize the lateral thickness distribution of protein adsorption layers on surfaces with no labeling. Its main advantage is that every point on a surface is measured at the same time with high sampling speed, thus avoiding the effect of singularity on measurement. This method has a high spatial resolution in the order of micron laterally and 0.1 nm vertically [12]. A linear relationship has been deduced theoretically between the square root of the intensity and the surface concentration or the thickness of the protein adsorption layer [13]. The difference in gray-scale between the protein adsorption layer and its substrates shows the thickness of the layer or the surface concentration of adsorbed proteins.

AFM is a powerful technique used to investigate surface topography on a scale of nanometers [14]. Collagen fibrils and subfibrillar structure on mica have been observed with AFM [15,16]. BSA adsorbed on organosilane monolayer surface and on self-assembled monolayers (SAMs) were also investigated with AFM [17,18].

2. Materials and methods

2.1. Proteins

Bovine serum albumin (BSA) and its antibody were purchased from Sigma. Calf skin purified collagen, was purchased from Boehringer Mannheim Biochemica (Collagen S).

2.2. Substrates

Silicon wafers (thin film 7×20 mm^2) with a natural silicon dioxide layer on an optically polished plane were used as substrates. The hydrophilic wafer surface was prepared by washing it in both TL1 solution (H_2O:30% H_2O_2:25% $NH_4OH = 5:1:1$) and TL2 solution (H_2O:30% H_2O_2:37% $HCl = 6:1:1$). The reactions of TL1 and TL2 with basic and acidic solutions, and the oxidation of hydrogen peroxide not only removed contaminants from the silicon surface, but also improved the number of silanol groups on the surface, thus making the surface hydrophilic. The hydrophobic surface was prepared with the silanization of the hydroxylated surface. After being rinsed in distilled water and ethanol, the hydroxylated surfaces were incubated in a mixture of dichlorodimethylsilane (20%, v/v) and trichloroethylene (80%), after which they were rinsed in ethanol and trichloroethylene in sequence. All chemicals used were of analytical grade. The 18.3 MΩ cm water was produced by ion exchange demineralization, then passed through a Milli-Q plus system from Millipore (Millipore, Bedford, MA).

2.3. Contact angle measurement

Water contact angles were measured at 25 °C for dried wafers and glass with the sessile drop method. Deionized water (4 μl) was gently dropped on the surfaces and the contact angle was read directly using a goniometer. The contact angles for non-modified, hydrophilic and hydrophobic silicon wafers were about 40, 5 and 80°, respectively.

2.4. Protein and competitive adsorptions

Protein and competitive adsorptions were carried out in a PBS solution (8 mM $Na_2PO_4 \cdot 2H_2O$, 2.68 mM KCl, 1.14 mM KH_2PO_4, 137 mM NaCl; pH 7.2). Single or binary solutions containing collagen, BSA or a mixture of the two in various concentrations were used. For non-real-time adsorption, silicon wafers were incubated in a protein solution for 30 min, then washed with PBS and deionized water, and dried with nitrogen. The adsorption was measured with imaging ellipsometry and AFM. In competitive adsorption, adsorbed proteins on the surfaces formed a BSA and collagen co-adsorption layer. The co-adsorption layer was immersed in an anti-BSA solution. The anti-BSA in the solution bound with the BSA in the layer to form a protein complex of BSA/anti-BSA and resulted in varied of surface concentrations (Fig. 1). The more BSA adsorbed in the co-adsorption layer, the more anti-BSA bound onto the protein complex layer resulting in a large increase of the surface concentration. In this way, the amount of BSA adsorbed in the co-adsorption layer could be deduced from the surface concentration variation, thus determining the competitive adsorption between BSA and collagen.

For real-time adsorption, silicon wafers were incubated in a cell containing a protein solution. The protein adsorption on substrates and the protein binding process between proteins were measured with time.

2.5. Imaging ellipsometry analysis

A homemade ellipsometric imaging system was used for the visualization and quantification of the thickness distribution of protein adsorption layer [19]. Its capacity for lateral spatial resolution can distinguish the effects of singularities (local abnormal variations in the image introduced by contamination) appearing on the surfaces, such as a speck of pollution in an otherwise uniform adsorption layer. The basic experimental setup was a conventional polarizer-compensator-sample-analyzer (PCSA) null ellipsometer. It consisted of a Xenon arc-lamp and a specific collimating system used as a light source to provide an expanded parallel probe beam with a diameter of about 40 mm. In order to illuminate a large area of the sample surface, the expanded probe beam and a charge-coupled device (CCD) for imaging were used to replace the narrow beam and the photodetector of a conventional ellipsometer. A 633 nm interference filter was placed in the incident optical path to increase the ellipsometric contrast of the image. However, for a sample on which there was a lateral distribution in layer thickness, null ellipsometry could not be carried out the entire surface at the same time due to the fact that different areas would yield different polarization changes. One possible solution was to use the off-null mode in an ordinary null ellipsometer, so the combined null and off-null ellipsometry was used at an incident angle close to the pseudo-Brewster

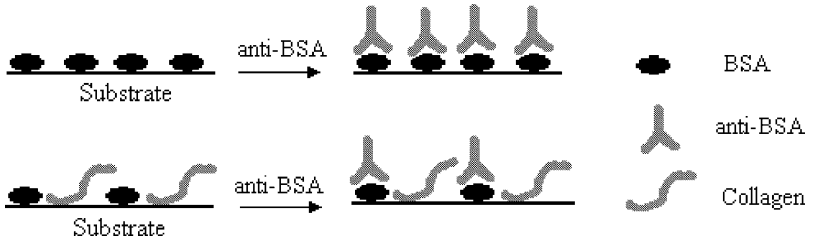

Fig. 1. Schematic figure showing the detection of protein adsorption and competitive adsorption. A substrate with a BSA adsorption layer or a BSA–collagen co-adsorption layer was immersed in an anti-BSA solution. Based on the specific interaction between BSA and anti-BSA, the anti-BSA in the solution bound with BSA in the layer to form a protein complex of BSA/anti-BSA, resulting in a variation of surface concentration.

angle of the substrate. An image of 7×15 mm^2 of the surface was focused onto the CCD video camera for intensity measurements. The spatial resolution of the system is in the order of micron laterally and 0.1 nm vertically. Normally, the detected protein adsorption layer was rather thin and transparent at the probe wavelength, with a thickness was in the range of 0.5–10 nm. In studies of protein adsorption layers on silicon substrates with native oxide, the compensator was fixed at an azimuthal position of 45°, and the analyzer and polarizer were held constant. The optical components in the system were adjusted to fulfill the null conditions on a silicon substrate without adsorbed layers and the off-null ellipsometric principle was used to measure the adsorption layer thickness [19]. The video signal corresponding to the thickness distribution was captured, digitized and stored in gray-scale format in a computer. Under these conditions, the detected intensity I was related to the thickness of the layer according to the formula:

$$I = kd^2,$$

which gives a linear relation between the intensity and the square of the thickness of the adsorbed protein layer or the square of the surface concentration of the proteins [13]. This proportionality showed a deviation of less than $\pm 2\%$ up to $d \approx 5$ nm. Under the same protein and the same ellipsometric conditions, k is constant and can be determined by the protein layer with known gray-scale and thickness. The relationship between surface concentration and film thickness was:

$$\text{Surface concentration } (\mu g/cm^2) \approx K \times d \text{ (nm)},$$

where $K \approx 0.12$ [20].

In this paper, the absolute thickness of the protein layer was calibrated by conventional ellipsometry and the results of imaging ellipsometry shown in gray-scale were processed to give the surface concentration of the adsorption layer.

2.6. Atomic force microscopy

An atomic force microscope (AFM AutoProbe CP Research Scanning Probe Microscope, Park Scientific Instruments, CA) was used for surface observation. Images were obtained with an IC-AFM mode (Ultrolevers20) at 25 °C, 30–40% relative humidity.

3. Results and discussion

3.1. Mono-adsorption of collagen and BSA

Figs. 2 and 3 showed the real-time adsorption of collagen and BSA on hydrophilic and hydrophobic substrates, respectively. The surface concentration of protein was obtained by real-time imaging ellipsometry measurements. In Fig. 2, the adsorption of collagen onto hydrophobic surfaces showed a gradual increase of the adsorption of the collagen with time; the time elapsed until 80% saturation adsorption was about 30 min. The adsorption of collagen onto hydrophilic surfaces showed a sharp rise followed by a slow increase; the time to reach the 80% adsorption saturation was about 10 min. The surface concentration of collagen at saturation adsorption was about 0.9 and 0.3 μg/cm^2 on hydrophobic and hydrophilic, respectively, showing that the adsorption on hydrophobic surfaces was three times of that on hydrophilic surfaces.

The BSA adsorption exhibited the same behavior as collagen adsorption (Fig. 3). BSA adsorbed faster but less on a hydrophilic surface than on hydrophobic surface. Compared with collagen adsorption, the surface concentration of BSA at

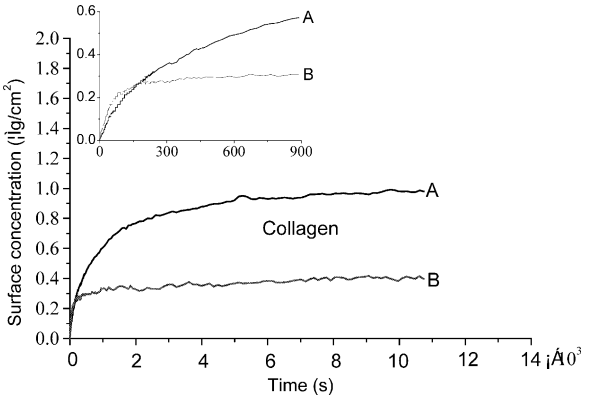

Fig. 2. Time courses of the real-time adsorption of collagen (0.1 mg/ml) on hydrophobic (A) and hydrophilic (B) surfaces.

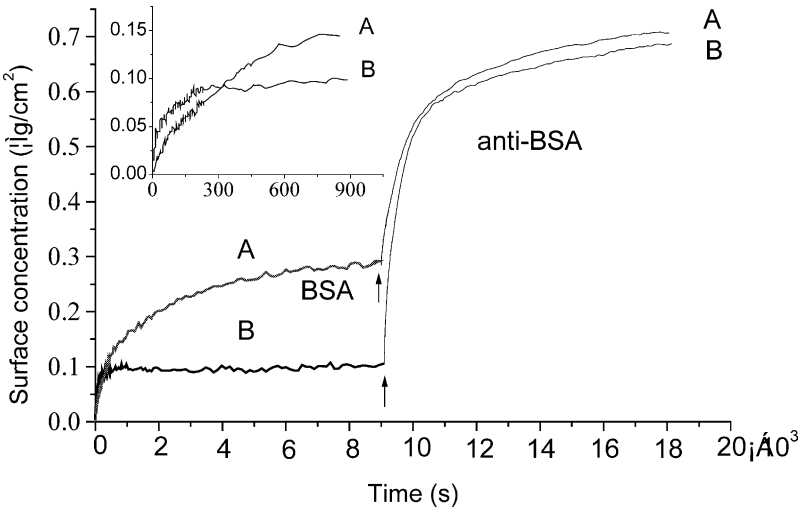

Fig. 3. Time courses of the real-time adsorption of BSA (1.0 mg/ml) and the interaction of anti-BSA and BSA on hydrophobic (A) and hydrophilic (B) surfaces. Arrows show the time at which the BSA solution was changed into an anti-BSA solution. The anti-BSA in the solution interacted with the BSA adsorbed on the substrates resulting in the second increase of surface concentration.

saturation adsorption was as low as about 0.3 and 0.1 μg/cm^2 on hydrophobic and hydrophilic, respectively, which should be due to the low molecular weight of BSA. The surface concentration of BSA on a hydrophobic surface was about three times of that on a hydrophilic surface. When the BSA adsorption layers were incubated in an anti-BSA solution, BSA retained its affinity with anti-BSA on both surfaces.

Collagen is a triple helix about 300 nm long and 1.5 nm in diameter with a molecular weight of about 300,000, while BSA is much smaller with a molecular mass of about 66,200 and a size of about 14×4 nm^2 [21]. The surface concentration corresponding to the close-packed layer of molecules in a 'side-on' configuration is 0.1 μg/cm^2 for collagen and 0.2 μg/cm^2 for BSA. In the experiment, the surface concentration in the saturation state for collagen adsorption was about 0.9 and 0.3 μg/cm^2, that is, nine and three times that of 'side-on' packing, on hydrophobic and hydrophilic surfaces, respectively. The results suggested that some of the adsorbed collagen molecules exhibited 'end-on' configuration or the collagen molecules partly overlapped. The same phenomenon occurred in BSA adsorbed on hydrophobic surfaces (Fig. 4).

3.2. Competitive adsorption of collagen and BSA studied with imaging ellipsometry

Real-time competitive adsorption of collagen and BSA were investigated with imaging ellipsometry as shown in Fig. 5. The variation of surface concentration for the competitive adsorption of collagen and BSA on the hydrophilic surface resembles that of the pure collagen adsorption. The surface concentration of the co-adsorption layer on the hydrophilic surface was about 0.3 μg/cm^2, which was close to the surface concentration resulted from the pure collagen adsorption. The subsequent incubation of the co-adsorption layer

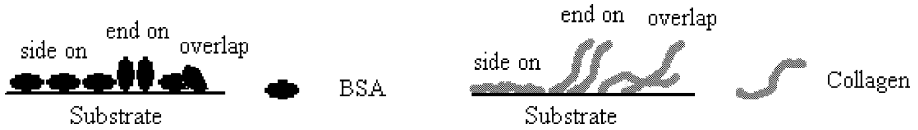

Fig. 4. Schematic figure showing BSA and collagen molecules exhibiting 'side on', 'end on' and 'overlap' adsorption on substrates.

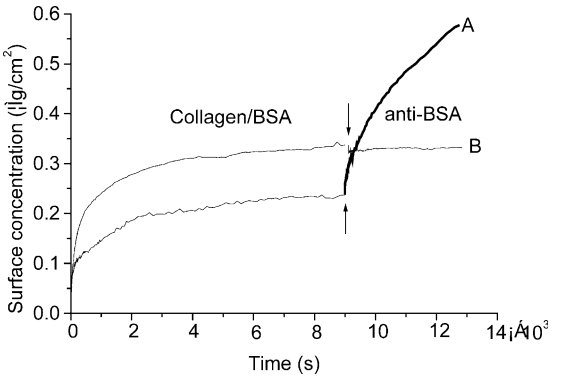

Fig. 5. Time course of the co-adsorption of collagen (0.1 mg/ml) and BSA (1.0 mg/ml) and the interaction of anti-BSA/BSA on hydrophobic (A) and hydrophilic (B) surfaces. Arrows show the time that the collagen/BSA solution was changed into an anti-BSA solution, causing an interaction between the anti-BSA in the solution with the BSA adsorbed on the substrates.

in anti-BSA solution resulted in little increase of the surface concentration, which indicated that less little BSA adsorbed in the competitive adsorption layer on the hydrophilic surface; however, much more BSA adsorbed in the competitive adsorption layer on the hydrophobic surface.

Real-time adsorption of collagen and the following co-adsorption of collagen and BSA were also investigated. Fig. 6 showed that the subsequent incubation of the collagen-adsorption layer in the binary solution resulted in the a decrease in the surface concentration decreasing on the hydrophobic surface, but almost no change on the hydrophilic surface. The final surface concentration on the hydrophobic surface approached the saturation state of the pre BSA adsorption. This showed that when the hydrophobic surface adsorbed with collagen was incubated in the binary solution of collagen and BSA, most collagen molecules on hydrophobic surface desorbed and were displaced by BSAs; however, collagen on the hydrophilic surface was not displaced.

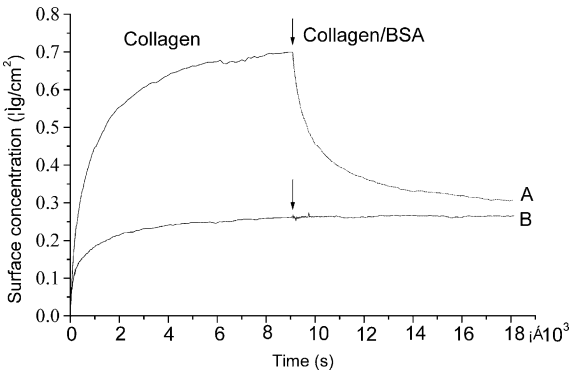

Fig. 6. Time course of the adsorption of collagen (0.1 mg/ml) and then co-adsorption of collagen (0.1 mg/ml) and BSA (1.0 mg/ml) on hydrophobic (A) and hydrophilic (B) surfaces. Arrows show the time that the collagen solution was changed into the binary solution of collagen and BSA (collagen/BSA).

The competitive adsorption of different concentrations of BSA (1, 10, 50, 100, 1000 μg/ml) was also studied with non-real-time measurement with the collagen concentration fixed at 0.1 mg/ml. After 30 min competitive adsorption, the adsorption layers were immersed in an anti-BSA solution for another 30 min. Based on the specific interaction between BSA and anti-BSA, the increase of surface concentration caused by BSA/anti-BSA complex indicated the amount of BSA adsorbed on the co-adsorption layer. The greater the increase in surface concentration, the more BSA adsorbed. Fig. 7 showed the surface concentration for competitive protein adsorption on hydrophobic and hydrophilic surfaces. On hydrophobic

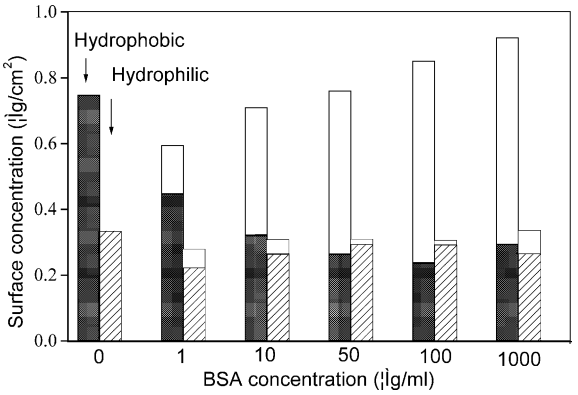

Fig. 7. Competitive protein adsorption on hydrophobic (black) and hydrophilic (slashed) surfaces between collagen (0.1 mg/ml) and BSA (0–1000 μg/ml). Substrates were incubated in the binary solution of collagen and BSA for 30 min, washed with PBS and de-ionized water, and then incubated in anti-BSA solution for 30 min to form the anti-BSA/BSA complex through the interaction of anti-BSA with BSA adsorbed on the substrates. The increases of surface concentrations caused by the anti-BSA/BSA complex were shown in the figure as white columns.

surfaces, the surface concentration of the co-adsorption layer decreased was obviously reduced with the introduction of BSA, but the BSA/anti-BSA complex after incubating in anti-BSA increased with the higher concentrations of BSA. When the concentration of BSA reached 100 μg/ml or higher, the complex layers became constant. On hydrophilic surfaces, the variation in the surface concentration of the co-adsorption layer was not so obvious even with an increase in the BSA concentration; the complex layers were almost constant, which indicated that collagen adsorption was the main phenomenon in the studied concentration range. Supposed that the affinity between the anti-BSA and the BSA from the binary solution was the same as BSA from single component solution, the percentage of BSA in the co-adsorption layer (collagen 0.1 mg/ml and BSA 1 mg/ml) can be deduced from the variation in thickness caused by the anti-BSA binding with the BSA. Nearly 100% of the protein adsorbed on hydrophobic surface was BSA, but only about 6% on hydrophilic surface.

Fig. 8 showed the thickness distribution of competitive protein adsorption on a substrate surface that was half hydrophilic and half hydrophobic. Fig. 8A showed the silicon substrate with hydrophilic (right part) and hydrophobic (left part) surfaces. The adsorption layer on the hydrophilic surface was thicker than that on the hydrophobic surface (Fig. 8B). After the whole surface was incubated in anti-BSA (Fig. 8C), the layer thickness increased obviously on the hydrophobic surface, but not so obviously on the hydrophilic, which showed that more of the anti-BSA/BSA complex formed on the hydrophobic surface, but almost no complex formed on the hydrophilic surface, showing that BSA from the mixed solution preferentially adsorbed onto the hydrophobic surface.

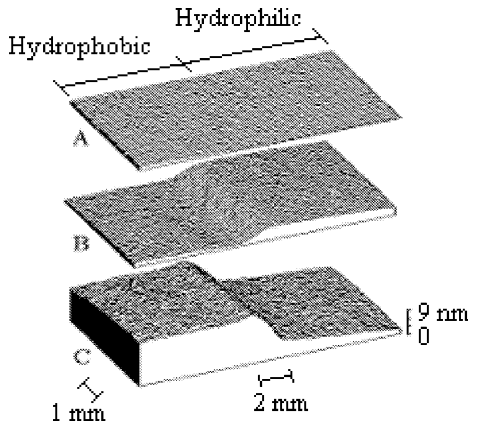

Fig. 8. Ellipsometric images showed: (A) the substrate surface with hydrophobic and hydrophilic regions; (B) the layer thickness distribution of the competitive adsorption after the substrate was incubated in the binary solution of collagen (0.1 mg/ml) and BSA (1 mg/ml) for 30 min; (C) the layer thickness variation caused by the anti-BSA and BSA binding after the layer shown in B was incubated in anti-BSA solution for 30 min.

3.3. AFM investigation of competitive protein adsorption

Collagen and BSA adsorption as well as their co-adsorption were also visualized by AFM. Fig. 9A and B represented the AFM images of hydrophilic and hydrophobic surfaces, respectively. Collagen homogeneously adsorbed on the two surfaces, as shown in Fig. 9C and D. The surface concentration of collagen on the hydrophobic surface was obviously higher than that on the hydrophilic surface, which was consistent with the results of imaging ellipsometry. BSA adsorbed on both surfaces as a grain shape with an average width of 14–18 nm (Fig. 9E and F). The difference of BSA adsorption on the two kinds of surface visualized with AFM was not as obvious as that of collagen.

The two kinds of surfaces were incubated in the mixed BSA and collagen solution (1 and 0.1 mg/ml in PBS) for 30 min, and then visualized with AFM, as shown in Fig. 9G and H. Collagen adsorbed on the hydrophilic surface, similar to the pure collagen solution, but was hardly adsorbed on the hydrophobic surface. These results were consistent with the observation with imaging ellipsometry that collagen adsorbed preferentially onto hydrophilic surfaces, but BSA onto hydrophobic surfaces in competitive adsorption.

Factors that affect competitive protein adsorption include protein transport rates, binding affinities and protein unfolding rates [22]. The diffusion coefficients of collagen and BSA are

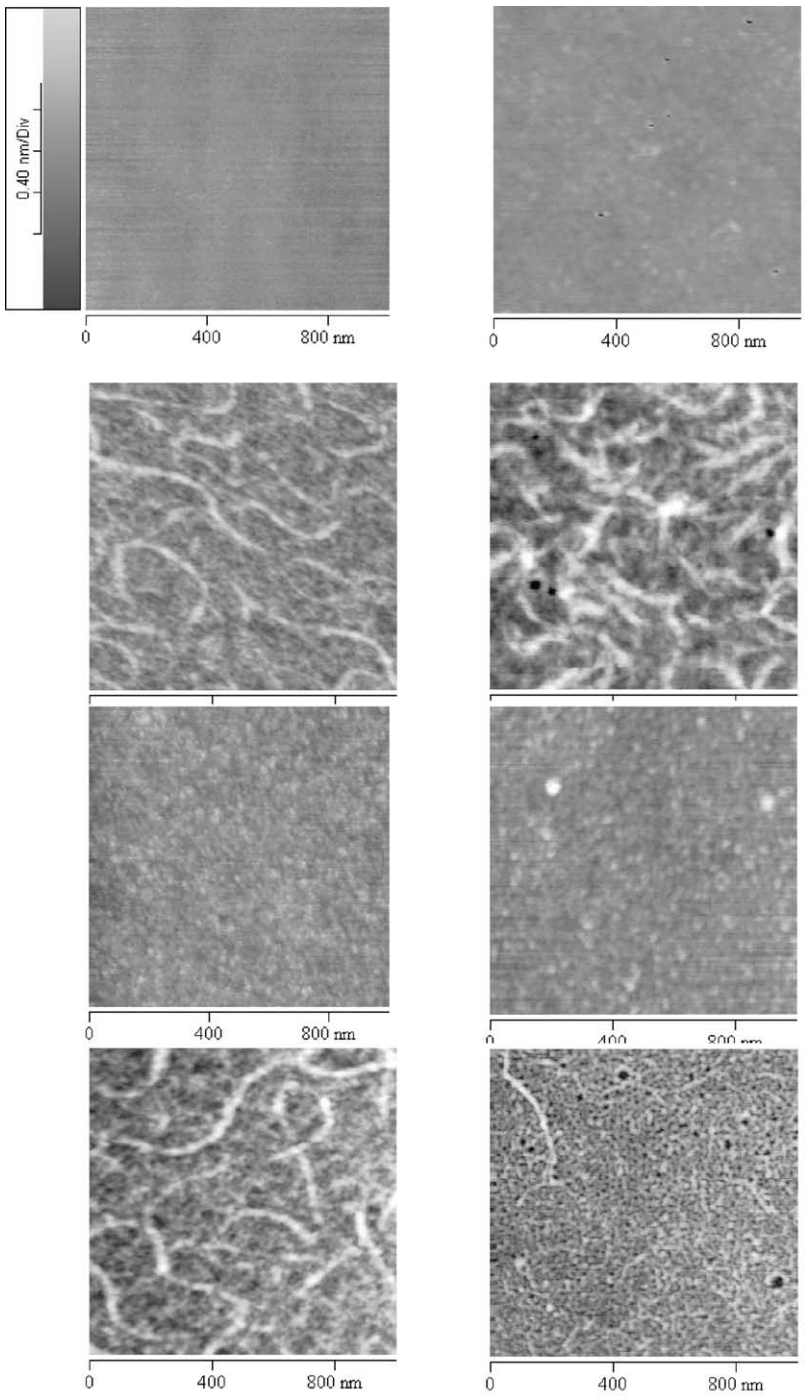

Fig. 9. Protein adsorption and competitive adsorption of collagen (0.1 mg/ml) and BSA (1 mg/ml) investigated with AFM. (A) Hydrophilic substrate; (B) hydrophobic substrate; (C) collagen adsorption on hydrophilic surface; (D) collagen adsorption on a hydrophobic surface; (E) BSA adsorption on hydrophilic surface; (F) BSA adsorption on a hydrophobic surface; (G) BSA and collagen competitive adsorption on a hydrophilic surface; (H) BSA and collagen competitive adsorption on a hydrophobic surface. Adsorption time was 30 min.

7.8×10^{-8} [23] and 6.1×10^{-7} cm^2 s^{-1} [24], respectively, and the protein transport rates mainly affect the initial competitive adsorption. The binding affinities for collagen and BSA are hydrophobic interaction on hydrophobic surfaces and electrostatic interaction and hydrogen bonding interaction on hydrophilic surface. BSA is a globular and rather flexible protein that is easily denatured after adsorption [25,26] while collagen is non-flexible and rather rigid [10]. If the protein transport rates and protein unfolding rates were the main factors controlling collagen and BSA competitive adsorption, BSA would be the main protein adsorbed on both kinds of surfaces, contrary to the results of these experiments. The competitive adsorption of collagen and BSA on hydrophilic and hydrophobic surfaces showed that binding affinity must be the key factor. On the hydrophobic surface, the hydrophobic interaction between the surface and the flexible BSA molecules was more than that of the rather rigid collagen molecules. On the hydrophilic surface, the electrostatic interaction and hydrogen bonding interaction between the surface and the collagen molecules was stronger than that of the negatively charged BSA molecules, which may be due to electrostatic repulsion between the negatively charged surface and BSA molecules. The high affinity between collagen molecules and hydrophilic surfaces resulted in the preferential adsorption of collagen on hydrophilic surfaces.

4. Conclusion

The microscopic observation with AFM and the quantitative visualization of surface concentration distribution of adsorbed protein with imaging ellipsometry showed that collagen and BSA adsorbed on the hydrophobic surfaces two times more than on hydrophilic surfaces. in the competitive adsorption between collagen and BSA in the mixed solution of BSA (1 mg/ml BSA) and collagen (0.1 mg/ml), nearly 100% of the protein adsorbed on the hydrophobic surface was BSA, but only about 6% on the hydrophilic surface. Binding affinity was the key factor affecting competitive adsorption of collagen and BSA.

Acknowledgements

The authors wish to thank Z.H. Wang and Y.H. Meng for their help in imaging ellipsometry measurement. The Chinese Academy of Sciences and the National Natural Science Foundation of China are acknowledged for their support of this work.

References

[1] B. Lassen, M. Malmsten, Competitive protein adsorption at radio frequency plasma polymer surfaces, J. Mater. Sci.: Mater. Med. 5 (1994) 662–665.
[2] J.L. Dewez, A. Doren, Y.J. Schneider, P.G. Rouxhet, Competitive adsorption of proteins: key of the relationship between substratum surface properties and adhesion of epithelial cells, Biomaterials 20 (1999) 547–559.
[3] A.S.G. Curtis, J.V. Forrester, The competitive effects of serum proteins on cell adhesion, J. Cell Sci. 71 (1984) 17–35.
[4] J.G. Steele, B.A. Dalton, G. Johnson, P.A. Underwood, Adsorption of fibronectin and vitronectin onto Primaria and tissue culture polystyrene and relationship to the mechanism of initial attachment of human vein endothelial cells and BHK-21 fibroblasts, Biomaterials 16 (1995) 1057–1067.
[5] J. Gerlach, P. Stoll, N. Schnoy, E.S. Bucherl, Membranes as substrates for hepatocyte adhesion in liver support bioreactors, Int. J. Artif. Organs 13 (1990) 436–441.
[6] V. Rocha, D.L. Ringo, D.B. Read, Casein production during differentiation of mammary epithelial cells in collagen gel culture, Exp. Cell Res. 159 (1985) 201–210.
[7] C.A. Rezmolpff, L.J. Loretz, D.M. Pesciotta, T.D. Oberley, M.M. Ignjatovic, Growth kinetics and differentiation in vitro of normal human uroepithelial cells on collagen gel substrates in defined medium, J. Cell Physiol. 131 (1987) 285–301.
[8] M. Taborelli, L. Eng, P. Descouts, J.P. Ranieri, R. Bellamkonda, P. Aebischer, Bovine serum albumin conformation on methyl and amine functionalized surfaces compared by scanning force microscopy, J. Biomed. Mater. Res. 29 (1995) 707–714.
[9] J.-L. Dewez, V. Berger, Y.-J. Schneider, P.G. Rouxhet, Influence of substrate hydrophobicity on the adsorption of collagen in the presence of pluronic F68, albumin, calf serum, J. Colloid Interface Sci. 191 (1997) 1–10.
[10] A. Baszkin, M.M. Boissonnade, Competitive adsorption of albumin against collagen at solution–air and solution–polyethylene interfaces, J. Biomed. Mater. Res. 27 (1993) 145–152.
[11] M. Deyme, A. Baszkin, J.E. Proust, E. Perez, G. Albrecht, M.M. Boissonnade, Collagen at interfaces II: competitive

adsorption of collagen against albumin and fibrinogen, J. Biomed. Mater. Res. 21 (2000) 321–328.
[12] G. Jin, R. Jansson, I. Lundström, H. Arwin, Imaging ellipsometry for biosensor applications, The 8th international conference on solid state sensors and actuators, and Eurosensors IXStockholm, Sweden, June 25–29, 1995.
[13] H. Arwin, S. Welin-Klintström, R. Jansson, Off-null ellipsometry revisited: basic considerations for measuring surface concentrations at solid/liquid interfaces, J. Colloid Interface Sci. 156 (1993) 377–382.
[14] G. Binnig, C.F. Quate, C. Gerber, Atomic force microscope, Phys. Rev. Lett. 56 (1986) 930–933.
[15] M.F. Paige, J.K. Rainey, M.C. Goh, Fibrous long spacing collagen ultrastructure elucidated by atomic force microscopy, Biophys. J. 74 (1998) 3211–3216.
[16] D.R. Baselt, J.P. Revel, J.D. Baldeschwieler, Subfibrillar structure of type I collagen observed by atomic force microscopy, Biophys. J. 65 (1993) 2644–2655.
[17] A. Takahara, S. Ge, K. Kojio, T. Kajiyama, In situ atomic force microscopic observation of albumin adsorption onto phase-separated organosilane monolayer surface, J. Biomater. Sci. Polym. Ed. 11 (2000) 111–120.
[18] K. Wadu-Mesthrige, N.A. Amro, G.Y. Liu, Immobilization of proteins on self-assembled monolayers, Scanning 22 (2000) 380–388.
[19] G. Jin, P. Tengvall, I. Lundström, H. Arwin, A biosensor concept based on imaging ellipsometry for visualization of biomeolecular interactions, Anal. Biochem. 232 (1995) 69–72.
[20] M. Stenberg, H. Nygren, The use of the isoscope ellipsometer in the study of adsorbed *proteins* and biospecific binding reactions, Journal de Physique 44 (1983) 83–86.
[21] T. Peters, Jr., Serum albumin, Adv. Protein Chem. 37 (1985) 161–245.
[22] J.L. Brash, T.A. Horbett, Proteins at Interfaces II, Fundamentals and Applications, ACS, Washington DC, 1995, pp. 1–23.
[23] M. Deyme, A. Baszkin, J.E. Proust, E. Perez, M.M. Boissonnade, Collagen at interfaces II. In situ collagen adsorption at solution/air and solution/polymer interfaces, J. Biomed. Mater. Res. 20 (1986) 951–962.
[24] P. Van Dulm, W. Norde, The adsorption of human plasma albumin on solid surfaces with special attention to the kinetic aspects, J. Colloid Interface Sci. 91 (1983) 248–255.
[25] C.E. Giacomelli, W. Norde, The adsorption–desorption cycle. Reversibility of the BSA–silica system, J. Colloid Interface Sci. 233 (2001) 234–240.
[26] W. Norde, C.E. Giacomelli, BSA structural changes during homomolecular exchange between the adsorbed and the dissolved states, J. Biotechnol. 79 (2000) 259–268.