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Study on adsorption behaviours of bovine serum albumin on CoCrMo alloy and Al₂O₃ ceramic surfaces

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Abstract

The adsorption behaviours of bovine serum albumin (BSA) molecules on CoCrMo alloy and Al₂O₃ ceramic were considered as the main research object in this study, and atomic force microscopy (AFM) was used for investigation. The thickness of the BSA films as well as adsorption forces between the BSA molecules and samples were tested. The results indicate that the thickness of the adsorbed film is close to 2.09 nm, exhibiting lateral monolayer adsorption as the main mode of adsorption of deformed BSA molecules on the material surfaces; the adsorption force of the BSA molecules on CoCrMo alloy is greater than 85.9 nN, and the adsorption force on Al₂O₃ ceramic is greater than 68.2 nN. This difference in the adsorption force may be related to the fact that the surface positive charge of the CoCrMo alloy is greater than that of the Al₂O₃ ceramic.

1. Introduction

CoCrMo alloy and Al₂O₃ ceramic are two commonly used biomedical materials that have good biocompatibility, chemical stability, corrosion resistance, and excellent mechanical properties. In clinical medicine, as replacement materials for bone and hard tissues, they are widely used in the manufacture of artificial hip and knee joints as well as other medical devices [1–8]. When these biomaterials are implanted in the human body, the plasma proteins in vivo can spontaneously adsorb onto their surfaces and change their physical and chemical properties, which can reduce the corrosion and wear of the material surfaces [9–14]. It is commonly observed that a layer of protein film forms on the surfaces of devices implanted in humans [15–22].

Yu et al [23] studied the adsorption of bovine serum proteins on the surface of hydrophilic silicon wafer by atomic force microscopy (AFM). Their results showed that the adsorption of bovine serum albumin (BSA) molecules was in a single layer with horizontal adsorption. Tencer et al [16] studied the adsorption behaviours of BSA molecules on the surface of gold and tested the thickness of the BSA molecular layer by atomic force microscopy to be about 1.8–2.1 nm. McClellan et al [24] tested the adsorption thickness of the BSA molecular film on hydrophilic oxide silicon wafers to be in the range of 2.0–3.8 nm by the elliptic polarisation method. AFM can be used to observe not only the morphology of an object surface but also the film and adsorbate on the material surface. It can also be used to test the adhesion between molecules and films through the force–displacement curve. Wang et al [25] tested the adhesion between a straight carbon chain molecular deposition film and quartz surface by AFM. Yuan et al [26] observed the characteristics of bacterial deposition film on the surface of copper alloy and tested the adsorption force of the bacteria on the surface.

The physical and chemical properties of material surfaces have considerable influences on the adsorption of proteins. The study of protein adsorption is the basis for developing biosensors, biochips, and biomaterials. In this study, using AFM as the main research tool, the adsorption behaviours of BSA molecules on the surfaces of CoCrMo alloy and Al₂O₃ ceramic were studied; moreover, the thickness of the BSA adsorption layer was measured, and the adsorption forces of the BSA molecules on the CoCrMo alloy and Al₂O₃ ceramic were tested to analyse the reasons for the differences in the adsorption forces. This work is thus expected to provide a better theoretical basis for research and development of artificial hip joints.
2. Experiments

2.1. Materials and equipment
CoCrMo alloy samples (self-made) of size 10 mm \( \times \) 10 mm \( \times \) 3 mm and Al\(_2\)O\(_3\) ceramic samples (self-made) of size 10 mm \( \times \) 10 mm \( \times \) 3 mm were used along with diamond polishing paste (Shanghai Flash Hardware Abrasive Factory, China), calf serum albumin (Sigma, USA), and phosphate buffer saline (PBS, self-made). A three-dimensional video microscope (HK-1000, HZROX Co. Ltd., Japan), an atomic force microscope (DI-Nanoscopeiv, USA), a Taly-surf 635 pro filamenter (TAYLOR HOBSON, UK), and a polishing machine (unipol-802, MTI Corporation, China) were also used.

2.2. Experimental methods

2.2.1. Preparation of BSA solution
Approximately 500 mg calf serum albumin was weighed and dissolved in 500 ml PBS to obtain 1 mg ml\(^{-1}\) BSA-PBS solution.

2.2.2. Preparation of material surface
The CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples were polished with 500\# and 800\# sandpaper followed by polishing with W5, W2.5, W1.5, W1.0, and W0.5 polishing pastes stepwise to obtain smooth surfaces.

2.2.3. BSA adsorption on material surface
The polished CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples were ultrasonically cleaned with absolute ethanol and deionised water; then, they were immersed in 1 mg ml\(^{-1}\) BSA-PBS solution for 30 min, after which they were taken out and washed with PBS solution and deionised water before vacuum drying. The CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples with BSA adsorbed on the surface were thus obtained.

2.2.4. Measurement of surface morphology, thickness, and adsorption force
The morphologies of the CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples before and after BSA adsorption were observed by AFM, and the thickness of the BSA adsorption layer was measured. The adhesion of BSA to the CoCrMo alloy and Al\(_2\)O\(_3\) ceramic was tested by selecting the BSA molecules on the surfaces of the materials.

3. Results and discussion

3.1. Surface roughness
The surface roughness of polished CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples is shown in figure 1. It can be seen that the surface obtained by polishing has few macroscopic scratches and a smooth surface, which is suitable for the next experiment.

3.2. Morphology and thickness of the film surface

3.2.1. Polished sample surface
The surface morphologies of the CoCrMo alloy and Al\(_2\)O\(_3\) ceramic samples are shown in figure 2. Compared with Al\(_2\)O\(_3\) ceramic, the hardness of the CoCrMo alloy was low, so it was easier to polish. The polished surface was smooth without scratches. While the hardness of the Al\(_2\)O\(_3\) ceramic was larger, which was difficult to process during polishing, the grooves and scratches on the polished surface were obvious.

The height distribution curves of the surface are shown in figure 3. It can be seen from figures 2(a) and (b) that the height distribution curves of the CoCrMo alloy surface were ‘slender’, indicating that their surface height distribution was relatively concentrated and height deviation was small. The height distribution curves of the Al\(_2\)O\(_3\) ceramic surface in figures 3(c) and (d) were relatively ‘coarse and short’, illustrating that their surface distribution was highly uncentrated and height deviation was large. This also further suggests that there were many grooves and scratches on the surface of the Al\(_2\)O\(_3\) ceramic.

3.2.2. Adsorption behaviour of BSA
BSA is a biological macromolecule, the monomolecular model is 14 nm \( \times \) 4 nm \( \times \) 4 nm [23, 27], as shown in figure 4.

The volume of the BSA monomolecule can be calculated by the following formula:

\[
V = (4/3)\pi r_1 r_2 r_3 = (1/6)\pi d_1 d_2 d_3
\]

According to the characteristics of the BSA molecules, there are usually four theoretical morphological models for the BSA monolayer adsorption on a material surface [16], as shown in figure 5. No deformations...
occurred during the adsorption of the BSA molecules on the material surfaces; when the BSA molecules are
closely arranged, there are two kinds of adsorption, namely lateral adsorption shown in figure 5(a) with an
adsorption thickness of about 4 nm and end-face adsorption shown in figure 5(c) with an adsorption thickness
of about 14 nm.

When the deformed BSA molecules are adsorbed on the material surface in a lateral manner, as shown in
figure 5(b), assuming that the contact surface between each BSA molecule and the material is a rectangle, the area
is $S_1 = d_1 \cdot d_2$, and volume of each BSA molecule does not change, such that the theoretical adsorption thickness
of the BSA can be calculated as follows:

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**Figure 1.** Surfaces ($\times 400$) and roughness of the CoCrMo alloys (a), (b) and Al$_2$O$_3$ ceramics (c), (d) after polishing. a: $Ra = 0.0091 \mu m$, b: $Ra = 0.0153 \mu m$, c: $Ra = 0.0147 \mu m$, d: $Ra = 0.0248 \mu m$.

**Figure 2.** Surface topographies of the CoCrMo alloys (a), (b) and Al$_2$O$_3$ ceramics (c), (d).
When the deformed BSA molecules are absorbed on the material surface in an end-face manner, as shown in figure 5(d), assuming that the contact surface between each BSA molecule and material is a square, the area is 
\[ S_2 = d_2 \cdot d_3 \] and volume of each BSA molecule does not change, such that the theoretical adsorption thickness of the BSA can be calculated as follows:

\[
h_t = \frac{V}{S_1} = \frac{1}{6} \pi d_3 = 2.09 \text{ nm}
\]
After being soaked in the BSA solution for 30 min, the surface morphologies of the CoCrMo alloy and Al2O3 ceramic samples are as shown in figure 6, and their surface height distribution curves are as shown in figure 7.

Based on a comparison of figures 2 and 6, it can be seen that the sample surfaces soaked in the BSA for 30 min completely adsorb the BSA molecules. The surface height distributions of the CoCrMo alloy and Al2O3 ceramic are basically normal, and the distribution curves of the two materials were 'slender'; these results show that the BSA molecules on the sample surfaces form a thin film of uniform thickness.

The AFM images in figures 2 and 6 were analysed using Nanoscope software, and the average height and roughness of the CoCrMo alloy and Al2O3 ceramic surfaces before and after adsorption of BSA were obtained, as shown in table 1. The surface roughness of the CoCrMo alloy samples (a) and (b) were small after BSA was adsorbed on the surface, and the average height of the surface significantly increased. Further, the surface roughness of the Al2O3 ceramic samples (c) and (d) were large, and there were many gullies; after the BSA was adsorbed on the surface of the sample, a part of the gully was filled, so that the average height of the surface

\[ h_2 = \frac{1}{6}\pi d_1 = 7.33 \text{ nm} \]

Figure 6. Surface morphologies of the CoCrMo alloys (a), (b) and Al2O3 ceramics (c), (d) after adsorbing BSA molecules.

Figure 7. Height distributions of the surfaces of the CoCrMo alloys (a), (b) and Al2O3 ceramics (c), (d) after adsorbing BSA molecules.
reduced. Meanwhile, it can be seen from (c) and (d) that the height difference was negative, indicating that the deep gullies or scratches could not be filled by the BSA molecules. As can be seen from the surface roughness after adsorption of BSA molecules, in a small range, the roughness of the BSA film was about 1.1–1.3 nm. The four height differences obtained from the experiments were 2.413 nm, 3.266 nm, −2.743 nm, and −1.523 nm, and it can be concluded that the thickness of the BSA adsorption film was about 1.5–3.2 nm, which was close to 2.09 nm. Therefore, the adsorption mode of the deformed BSA molecules on the surfaces of the CoCrMo alloy and Al2O3 ceramic was by the lateral monolayer.

3.3. Measurement of surface adsorption force

3.3.1. Testing principle

An A-type probe was used for testing: \( F_0 = 40–75 \text{ kHz}, K_e = 0.58 \text{ N m}^{-1}\), noncontact mode, and scanning range of 2 \( \mu \text{m} \times 2 \mu\text{m} \). The testing principle of the surface adsorption force is as shown in figure 8. The order 1 → 2 → 3 is the process by which the tip of the probe and sample surface approach gradually to a sudden jumping contact. The order 3 → 4 → 5 is the loading process after the probe touches the surface. The order 5 → 6 is the unloading process when the tip of the probe gradually leaves the sample surface. The order 6 → 7 → 8 is the process by which the probe suddenly jumps off the sample surface and returns to its initial position. The jump-off point 6 is a key point on the force–displacement curve, and the adhesion force can be determined through the bending amount of the probe microcantilever at this point.

The straight line 5′–6′ is the unloading section under actual conditions. Because of the displacement error in the loading process and elastic recovery, the loading and unloading lines do not coincide. The force–displacement curves thus show the magnitude of the force between the sample and probe tip when the fixed end of the AFM microcantilever probe approaches vertically, contacts, and leaves the sample surface. As long as the force–displacement curve was measured, the adhesion force on the sample surface could be analysed \([26, 27]\).

The adhesive force can be obtained as

\[
F = -k_e \frac{s_s}{s_i} \Delta x
\]

where \( k_e \) is the elastic modulus of the probe, \( s_s \) is the slope of the return straight line 5′–6′, and \( s_i \) is the slope of the forward straight line 5–6; \( \Delta x \) is the distance between the intersection 4 of the return line and baseline to the intersection 7′ of the jump-off line and baseline.

Table 1. Average heights and roughness of CoCrMo alloy and Al2O3 ceramic surfaces before and after adsorption of BSA.

|                     | CoCrMo-a | CoCrMo-b | Al2O3-c | Al2O3-d |
|---------------------|----------|----------|----------|----------|
| Average height/nm   | 1.968    | 0.575    | 7.068    | 7.428    |
| Ra/nm               | 7.806    | 1.045    | 1.268    | 1.717    |
| Before adsorption h | 4.381    | 1.164    | 11.072   | 5.905    |
| After adsorption H  | 2.413    | —        | 3.266    | —        |
| Height difference \( h = H - h \) | —        | —        | —2.743   | —        |
| Height difference \( h = H - h \) | —        | —        | —1.523   | —        |

Figure 8. Typical force–displacement curve by AFM.
3.3.2. Determination and calculation of surface adsorption force

The A-type probe test results are as follows: \( F_0 = 40\text{–}75 \text{kHz}, k_e = 0.58 \text{ N m}^{-1} \), force–displacement curve between the BSA molecules on the surface of the CoCrMo alloy and probe, as well as the fitting line of the force-displacement curve shown in figure 9. When the probe is gradually loaded to the maximum value, the probe automatically returns, and the force during jumping is the adhesion between the probe and BSA molecules. It can be proved by repeated experiments that the BSA molecules do not leave the surface of the CoCrMo alloy, indicating that the adsorption force of the BSA molecules on the CoCrMo alloy was greater than that on the probe.

According to figure 9(b), the forward straight line equation is as follows:

\[
y = 149.95 - 0.65167x, \text{ slope: } S_1 = -0.65167
\]  

(2)

The return line equation is as follows:

\[
y = 155.68 - 0.61604x, \text{ slope: } S_2 = -0.61604
\]  

(3)

The intersection of the return line and baseline is \( (225.79, 18.96) \), and the intersection of the jump-off line and baseline is \( (381.27, 15.51) \). The distance between the two intersections is calculated as follows:

\[
\Delta x = 381.27 - 225.79 = 155.48 \text{ nm}
\]  

(4)

Substituting equations (2), (3), and (4) into equation (1), the adsorption force can be obtained as

\[
F = 85.2 \text{ nN}.
\]

After testing and calculation, the adhesion forces at 18 points on the CoCrMo alloy surface are shown in table 2.

| Adhesion force (nN) | Adhesion force (nN) |
|---------------------|---------------------|
| 85.2                | 70.7                |
| 82.6                | 88.1                |
| 79.7                | 80.5                |
| 82.6                | 87.6                |
| 84.2                | 79.3                |
| 91.3                | 82.9                |
| 97.9                | 82.2                |
| 75.5                | 82.1                |
| 77.8                | 78.5                |

Therefore, it can be concluded that the adsorption force of BSA on the CoCrMo alloy should be greater than 82.9 nN.

Using the same method, the force-displacement curve and fitting line between the BSA molecules on the \( \text{Al}_2\text{O}_3 \) ceramic surface and probe are obtained, as shown in figure 10.

It is seen that there is a small difference between figures 10 and 9, where the end of the forward line in figure 10 is a horizontal straight line. The reason for the difference is because the detection range of the detector is exceeded during loading, and the output value always stays within the range values to provide a horizontal straight line. Similarly, 18 points were measured on the surface of the \( \text{Al}_2\text{O}_3 \) ceramic, and the adhesion forces are shown in table 3.
Their average is computed as follows:

\[ F = \frac{1}{18} \sum F_i = 68.2 \text{ nN} \]

It can thus be concluded that the absorption force of the BSA on the Al\textsubscript{2}O\textsubscript{3} ceramic is greater than 68.2 nN. The adsorption force of the CoCrMo alloy for BSA was greater than that of the Al\textsubscript{2}O\textsubscript{3} ceramics, and the reason for this may be related to the hydrophobic interactions, electrostatic interaction, coordinated interactions, and hydrogen bonding between the materials, among others.

First, the unfolded protein can adsorb onto the material surface and react easily with the oxide and metal ions. In terms of structure, the BSA can be divided into three regions: i, ii, and iii (see figure 4(a)). At pH = 7.40, regions i and ii are negatively charged, while region iii is neutral and slightly negatively charged [23]. The isoelectric point of BSA itself is 4.7–4.9, and when mixed with PBS at pH 6.9 [28], BSA must have a large negative charge, so it is easily adsorbed on the surface of the positively charged CoCrMo alloy and Al\textsubscript{2}O\textsubscript{3} ceramic; further, the positive charge of the CoCrMo alloy is more than that of the Al\textsubscript{2}O\textsubscript{3} ceramic.

Second, there are no hydrophobic forces between the BSA and sample surfaces, and the only interaction force between the BSA and material surfaces is electrostatic force and hydrogen bonding. After the BSA reaches the material surface, the mobile positively charged groups in the protein move toward the hydrophilic surface [29, 30], causing the electrostatic interactions between the BSA and material surfaces to change from repulsive to attractive. The BSA can thus be stably adsorbed on the surfaces of hydrophilic materials.

### 4. Conclusion

In summary, after the BSA molecules are adsorbed on the surfaces of the CoCrMo alloy and Al\textsubscript{2}O\textsubscript{3} ceramic, the roughness of the adsorption film is about 1.1–1.3 nm. The thickness of the adsorbed film is about 1.5–3.2 nm, whose average is close to 2.09 nm; therefore, the deformed BSA molecules are adsorbed on the surfaces of the CoCrMo alloy and Al\textsubscript{2}O\textsubscript{3} ceramic in a lateral monolayer manner that is considered as the main adsorption mode. The adsorption force of the BSA molecules on the CoCrMo alloy is greater than 85.9 nN and that on the Al\textsubscript{2}O\textsubscript{3} ceramic is greater than 68.2 nN; the adsorption force of the CoCrMo alloy with BSA is greater than that of the Al\textsubscript{2}O\textsubscript{3} ceramic. This difference in the adsorption force may be related to the fact that the surface positive charge of the CoCrMo alloy is more than that of the Al\textsubscript{2}O\textsubscript{3} ceramic. Under the same conditions, the optimum roughness of the CoCrMo alloy is 0.0153 \( \mu \text{m} \) and that of the Al\textsubscript{2}O\textsubscript{3} ceramic is approximately 0.0248 \( \mu \text{m} \).
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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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