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Formation of a carbonaceous film on the surface of Cu in a bovine serum albumin solution

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ABSTRACT

The tribochemical reaction between metal ions and proteins can result in the formation of a carbonaceous tribo-layer on the surface of metal-on-metal (MOM) artificial joints. This carbonaceous film can lubricate the tribo-pair and reduce the corrosion of the metal. The purpose of this work is to investigate the catalysis efficiency of Cu and Co ions in the formation of carbonaceous films at the contact regions. A ball-on-disc (Al_2O_3-on-Cu and Al_2O_3-on-Co alloy) friction mode in bovine serum albumin (BSA) solution was chosen for the wear test experiments. After the wear test, the tribo-layer formed on the wear spot (on Al_2O_3 ball) and wear track (metal disc) were collected and characterized. The results show that the Cu ions released upon friction promoted the adsorption of BSA on the wear track of Cu to give a protein film which was further transformed into a carbonaceous tribo-layer under the shear force between the Al_2O_3-Cu tribo-pair. When the Al_2O_3 ball and Co alloy were chosen as the tribo-pair, however, a carbonaceous tribo-layer was not observed on the Co alloy after the wear testing. This means that the catalysis efficiency of the Cu ion for the formation of a carbonaceous tribo-layer is stronger than that of the Co ion.

1. Introduction

Total hip arthroplasty (THA) is one of the most successful treatments for hip joint disease in the United States [1,2]. Metal-on-metal (MOM) bearing have been widely employed in THAs over the past decade due to their toughness and good anti-wear properties [2–4]. For example, in 2007 America, approximately 31% of primary THA implants were MOM [1]. At present, however, MOM hip replacements are not recommended since many reports reveal that the release of heavy metal ions (Co^{2+}, Cr^{3+}, *etc.*) and metallic debris [5–8] from MOM result in patient complications such as allergies and pseudotumor [9,10].

Martin et al. [11] discovered that the release of metal ions from CoCrMo can promote the adsorption of protein onto the surface of the substrate to form a protein layer. Liao et al. [12] inferred that this protein layer can transform into a carbonaceous layer – *via* interactions between the transition metal ions (probably Co^{2+}, Cr^{3+}) and the protein – with the potential to reduce the friction and improve the corrosion resistance of MOM implants. Such a carbonaceous layer was reported by Liao et al. [12] on the surface of MOM prostheses retrieved from patients.

What needs to be pointed out is that, for the more commonly used CoCrMo alloy MOM bearings, the transformation process of the protein layer into the carbonaceous layer will occur over a long time in the body. Therefore, finding a metal ion with a high catalysis efficiency to transform the protein layer into a carbonaceous film will be a significant advancement for the continued use of MOM artificial joints. Erdemir et al. [13] reported that carbonaceous film can be formed on the surface of friction interface by synergism of the catalytic abilities of Cu and the shear force between the MoN-Cu film coated tribo-pairs, when the pure poly-alpha-olefin (PAO) 10 oil was applied as the friction medium. The aim of this work is to compare the catalysis efficiency of Cu and Co ions for the formation of a carbonaceous film during friction in the presence of a protein (bovine serum albumin, BSA) solution. A ball-on-disc tribometer comprising of an alumina ball (Φ 6 mm) and select Cu and Co alloy metal discs (Φ 10 mm × 1.5 mm) was used to simulate the friction environment.

2. Experimental

2.1. Materials and wear tests

The CoCrMo alloy (Co alloy, elastic modulus 177 (± 2) GPa, microhardness 568 (± 8) $HV_{0.01}$) and copper (Cu, 99.9%, elastic modulus 102 (± 1) GPa, microhardness 135 (± 1) $HV_{0.01}$) discs, with

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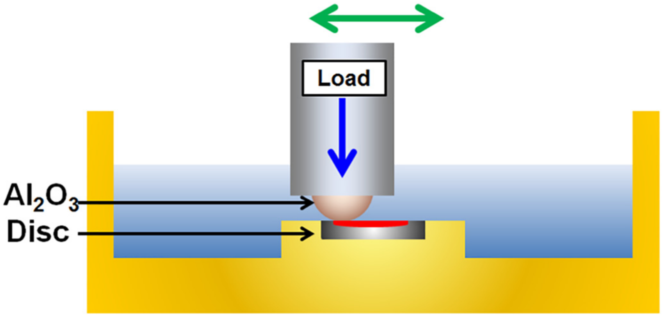

Fig. 1. Schematic view of the tribology experiment.

dimensions Φ 10 mm × 1.5 mm, were polished to a surface roughness (R_q) of ~10 nm. After polishing, the discs were ultrasonically cleaned in acetone and then in alcohol for 10 min each before being subjected to wear testing.

Wear tests were performed using a ball-on-disc reciprocating tribometer (CSEM, Switzerland) under a load of 1 N for 100,000 cycles. Al_2O_3 balls of 6 mm diameter were used to create the friction pairs against the Co alloy and Cu discs in a total volume of 50 mL bovine serum albumin solution (BSA solution, composition: BSA 20 mg/mL, NaCl: 9 mg/mL, EDTA: 2 mg/mL, sorbic acid: 0.2 mg/mL) at a room temperature of around 25 °C. The length of the wear track was about 6 mm and the sliding frequency was 1 Hz. The schematic view of the tribology experiment setup is shown in Fig. 1.

2.2. *Characterization of BSA adsorption*

The adsorption of fluorescein isothiocyanate (FITC) modified BSA molecules onto the wear track was determined from fluorescein images captured using a Fluoresce Microscope (IX51, Olympus, Japan).

2.3. *Morphology of the wear track*

After the wear tests, the morphology of wear tracks and spots were investigated using an optical microscope (AX10, ZEISS, Germany). The profile of the wear tracks was determined using a profilometer (XP2, AMBIOS, USA).

2.4. *Characterization of the structure of tribological layer*

A Raman microscope (λ = 532 nm, InVia Raman Microscope, RENISHAW, Britain) and an Attenuated Total Reflection Fourier Transform Infra-Red (ATR-FTIR, Nicolet 8500, USA) were used to investigate the structure of tribo-layer formed on the wear track. An Electron Energy Loss Spectrometer (EELS, JEOL 2100F, Japan) and Time-of-Flight Secondary Ion Mass Spectrometer (TOF-SIMS, PHI TRIFT III, Japan) were used to characterize the structure of the tribo-layer formed on the surface of wear spot.

3. Results

3.1. *Surface morphology and profile of wear track*

The microscope images of the wear tracks on the discs and the associated wear spots on the Al_2O_3 balls in contact with the respective surfaces, as well as the profiles of wear tracks are shown in Fig. 2. After the wear test experiments with alumina balls in the BSA solution, obvious furrows appeared on the surfaces of the metal discs (Fig. 2a and b). Notably, a 'black tribo-layer' appeared in the wear track of the Cu disc (Fig. 2a) that was absent in the wear track of the Co alloy (Fig. 2b). The microscope images of the wear spot on the alumina worn with the Cu and Co alloy discs are shown in Fig. 2c and d, respectively. The profiles of the wear tracks on the Cu and Co alloy discs are presented in Fig. 2e. As shown, the depth of the wear track on the Cu disc is smaller than that formed on the surface of Co alloy disc. This indicates that the wear resistance of the Cu disc in the presence of the BSA solution is better than that of Co alloy in the BSA solution. We infer that the "tribo-layer" observed in the wear track of the Cu disc may have an important influence on the wear resistance of Cu.

3.2. *Raman and ATR-FTIR spectra of tribo-layer*

In order to investigate the structure of the tribo-layers formed on the wear tracks, micro-Raman spectra were acquired of the wear track contact regions of the Al_2O_3-Cu and Al_2O_3-Co alloy tribo-pairs. The results are shown in Fig. 3a and b. The Raman spectrum of the tribo-layer formed inside wear track of the Cu disc (black area in Fig. 2a, denoted with a yellow circle) showed strong Raman activity with a D peak around 1380 cm^{-1} and a G peak around 1580 cm^{-1} (shown in Fig. 3a). This indicated that the structure of the tribo-layer formed inside wear track of the Cu disc was comprised of graphite-like materials; *i.e.*, that it was a carbonaceous film. On the other hand, the Raman spectrum of the wear track on the Co alloy (area in Fig. 2b, denoted with a yellow circle) showed no Raman activity, similar to that of the dried BSA (shown in Fig. 3b). That indicated that, under the conditions employed in this work, the friction between alumina and the Co alloy did not transform the BSA molecules in the wear track into a carbonaceous film.

In order to identify changes in the bonding of the BSA molecules during friction, the Infra-Red spectra of the tribo-layers formed inside wear tracks of the Cu and Co alloy discs were collected using Attenuated Total Reflection Fourier Transform Infra-Red (ATR-FTIR) spectroscopy. Dried BSA used as a control. The results, presented in Fig. 3c and d, show that dried BSA contained two main peaks: the amide I band at 1645 cm^{-1} and the amide II band at 1530 cm^{-1}. Fig. 3c shows that the spectrum of outside wear track on the Cu disk is similar to that of the dried BSA. The spectrum of the tribo-film formed inside wear track on the Cu disk, however, was obviously different from that of the dried BSA. A new absorption peak appears at 1600 cm^{-1}, which represents the formation of a $-C{=}C-$ bond (shown in Fig. 3c). This clearly indicates that the structure of tribo-layer inside wear track on the Cu disk was different to that of dried BSA, likely due to the denaturing of the BSA molecules under the cyclical shear force between the alumina ball and the Cu disk to form the carbonaceous film. The spectrum of tribo-layer formed inside wear track on the Co alloy (shown in Fig. 3d) showed a mere blue-shift of the amide I (from 1645 cm^{-1} to 1657 cm^{-1}) and amide II (from 1530 cm^{-1} to 1546 cm^{-1}) bands. This indicated a change in the highly-ordered structure of BSA upon adsorption onto the Co alloy.

3.3. *TOF-SIMS of tribo-layer*

Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is a versatile method to reveal, with high resolution, the chemical characteristics of a surface. The TOF-SIMS spectra and mapping of the tribo-layers from inside the wear spot on the Al_2O_3 after contact with the different metals, as well as from the areas surrounding the wear spot (*i.e.*, outside the wear spot) are shown in Fig. 4.

As shown in Fig. 4a, the SIMS spectrum of the tribo-layer obtained from inside the wear spot of the Al_2O_3 ball after wear contact with the Co alloy in the BSA solution is similar to that of obtained from outside the wear spot. The mapping of the wear spot of the alumina-Co alloy pair (Fig. 4b) also shows that there is no obvious difference between the inside wear spot and outside wear spot. This reveals that the chemical nature of tribo-layer appearing on the wear spot of the Al_2O_3-Co alloy is similar to that of the materials outside of the wear spot, indicating further that the BSA molecules were not denatured or transformed into a carbonaceous layer under friction between the alumina and the Co

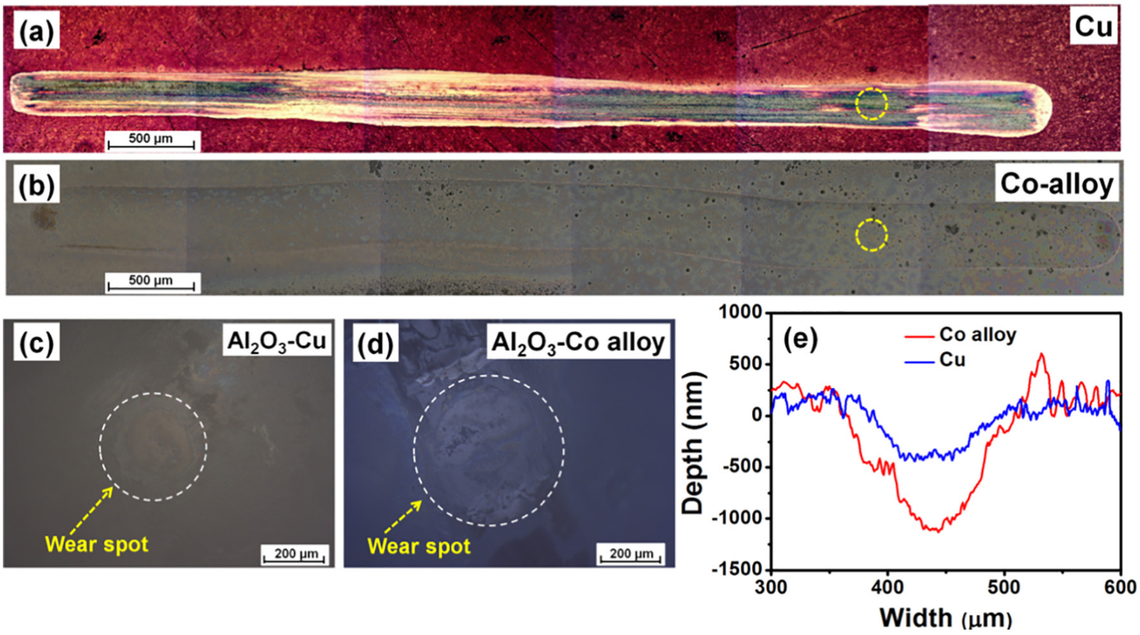

Fig. 2. Microscope images of the wear tracks on the Cu (a) and Co alloy (b); the wear spot on the alumina from contact with the Cu (c) and Co alloy (d) discs; and the profiles of both wear tracks (e).

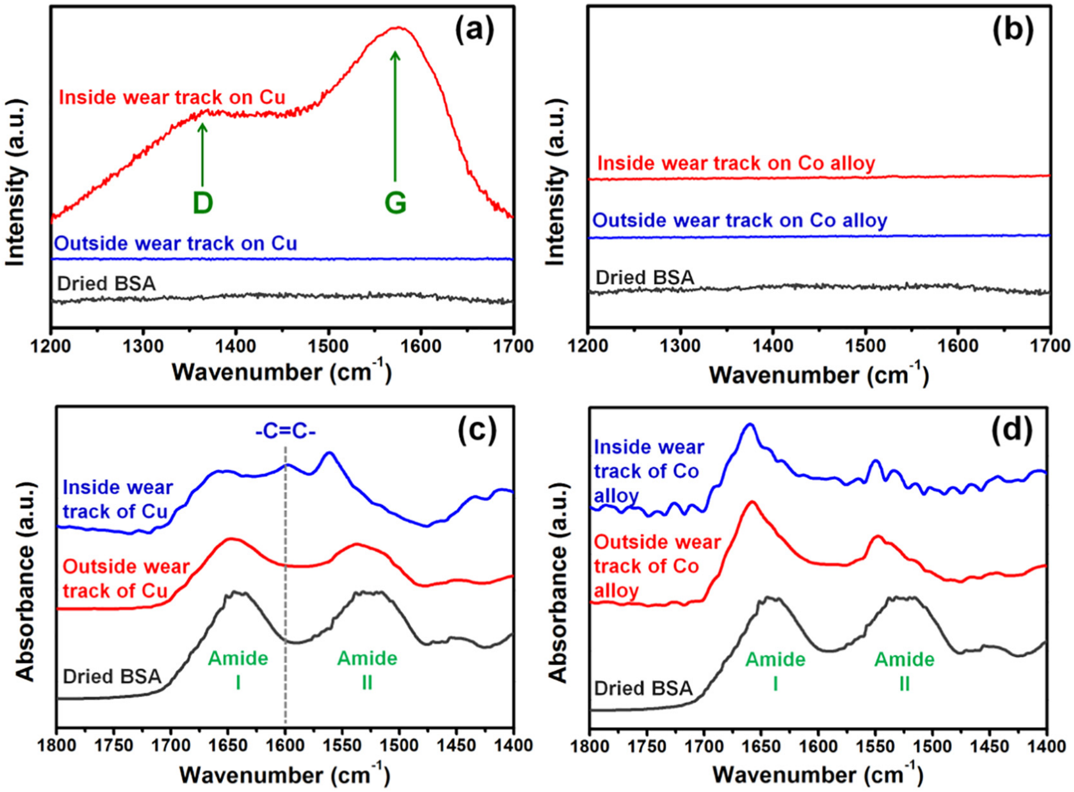

Fig. 3. Raman spectra of dried BSA, inside and outside the wear track of the Cu (a) and the Co alloy (b) discs; and the ATR-FTIR spectra of dried BSA, the inside and outside the wear track of the Cu (c) and the Co alloy (d) discs.

alloy. On the other hand, after the wear testing between the Al_2O_3 ball and the Cu disc in the BSA solution, the intensity of the SIMS spectrum of the tribo-layer obtained from inside the wear spot is much higher than that obtained outside of the wear spot (Fig. 4c). Further, the mapping (such as C, C_2, CH, *etc.*) of the tribo-layer from the inside wear spot is much lighter than that obtained from outside of the wear spot (Fig. 4d). This reveals that the tribo-layer from inside the wear spot of the Al_2O_3-Cu pair contained more hydrocarbon and carbon fractions than the surrounding areas of the wear spot. This further indicates that the carbonaceous film was formed on the wear spot upon friction between the Al_2O_3 and Cu surfaces in the presence of BSA.

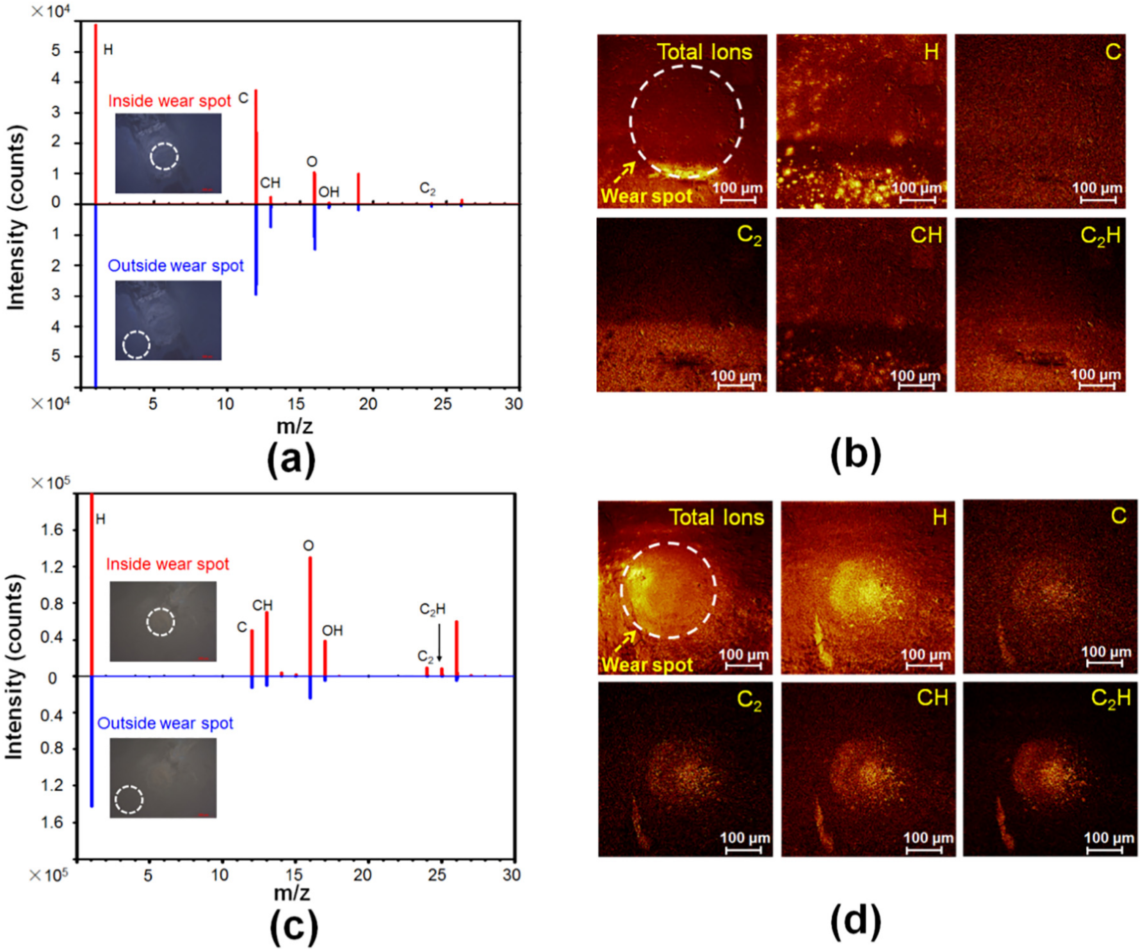

Fig. 4. The TOF-SIMS spectra of materials from inside and outside the wear spots of the Al_2O_3 with the Co alloy (a) and the Cu (c) discs; and the TOF-SIMS mapping of materials from inside and outside the wear spots of the Al_2O_3 with the Co alloy (b), and the Cu (d) discs.

3.4. EELS spectra of tribo-layer

The electron energy loss spectroscope (EELS) is an excellent method to confirm the formation of a carbonaceous film on the wear spot of Al_2O_3. After the wear test experiments in the BSA solution, a tungsten probe was used to collect the sample (tribo-layer) from the wear spot and then the EELS was used to investigate the structure of the tribo-layer. The EELS results of a BSA control sample and the tribo-layers obtained from the wear spots of the Al_2O_3-Cu and Al_2O_3-Co friction pairs are shown in Fig. 5. The EELS spectrum of the tribo-layer from the Al_2O_3-Co alloy is similar to that of dried BSA, indicating that the structure of the tribo-layer from the Al_2O_3-Co alloy pair is similar to that of BSA. That is, the friction between alumina and the Co alloy did not denature the BSA molecules to form a carbonaceous layer. Compared with the spectrum of the dried BSA, however, the spectrum of the tribo-layer obtained from the wear spot of the Al_2O_3-Cu shows an obvious π^* peak signal. This indicated that the tribo-layer formed on the wear spot of the Al_2O_3-Cu friction pair contained graphitic carbon; *i.e.*, the friction between alumina and Cu can denature the BSA molecules at the contact region to form a carbonaceous layer.

3.5. Adsorption of BSA on Cu

Fluorescein isothiocyanate (FITC) was used to investigate the adsorption of BSA molecules onto the surface of Cu. The results are shown in Fig. 6. Fig. 6a shows that the adsorption of BSA on the Cu surface before wear testing is dispersive. After being subjected to wear testing for 4 h, however, the adsorption of BSA intensified on the wear tack of the Cu surface (Fig. 6b). It is expected that the friction between the Al_2O_3-Cu tribo-pair resulted in the release of Cu ions which promoted the adsorption of the BSA molecules onto the wear tack of Cu surface.

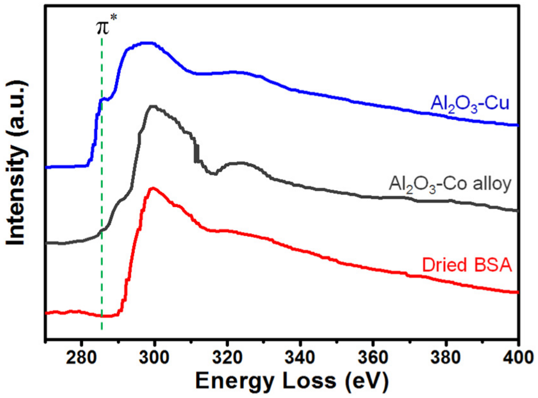

Fig. 5. The EELS spectra of dried BSA and the tribo-layers on the wear spots of the Al_2O_3-Cu (denoted with white circle in Fig. 2c) and Al_2O_3-Co alloy (denoted with white circle in Fig. 2d) pairs.

In order to investigate the changes in the molecular weight of BSA on the wear track of the Cu disc, the western-blot (WB) method was used to characterize the molecular weight of the "tribo-layer" collected from the wear track of the Cu disc. Dried BSA molecules were used as the control sample. The result of the WB experiment is shown in Fig. 7. The molecular weight of dried BSA is about 68 kDa (Fig. 7). There is an

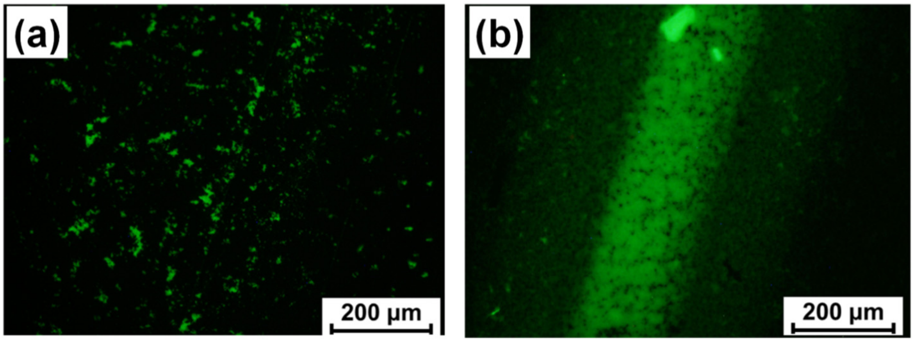

Fig. 6. Fluorescence images of BSA labeled with fluorescein isothiocyanate on a Cu surface before wear testing (a), and after wear testing in a BSA solution for 4 h (b).

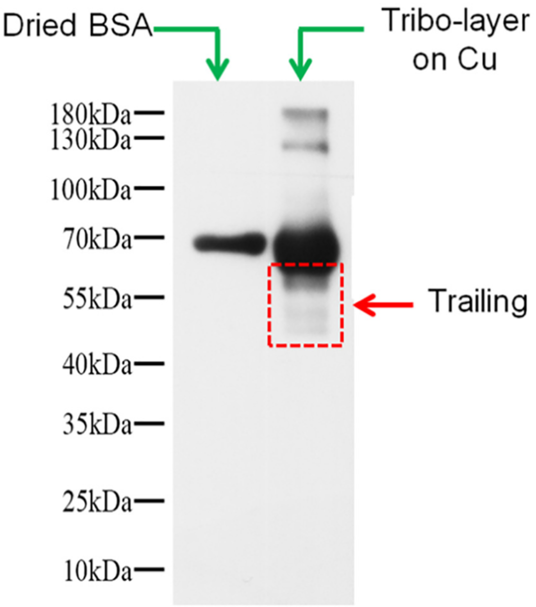

Fig. 7. The WB testing of dried BSA and tribo-layer on Cu. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

obvious trailing behind this weight for the tribo-layer (denoted with a red box). It is well-known that the smaller the molecule, the faster its movement is in the separation gel (used in WB testing). This indicates that some BSA molecules in the tribo-layer were denatured into smaller molecules. That is, the friction between the alumina and the Cu surfaces is sufficient to denature BSA, promoting its transformation into smaller molecules.

4. Discussion

Carbonaceous films can lubricate the tribo-pair in an artificial joint and suppress the ion releasing mechanism of metal ions [12]. Researchers infer that this kind of carbonaceous film can be formed on the surface of metals by the synergism of shear force and the catalytic abilities of transition metals such as Cr and Co [12,13]. Cu is a transition metal and there are many researches that investigate its application in the biomedical field [14–17]. The results of this work show that the catalysis efficiency of the Cu ion is stronger than that of the Co ion with regard to the formation of the carbonaceous layer on the surface of wear contact regions. The result of adsorption experiments with fluorescein isothiocyanate (FITC, Fig. 6) shows that the BSA molecules are obviously adsorbed onto the wear track of the Cu after wear testing with an Al_2O_3 ball in a BSA solution. This is due to the release of Cu ions which, in turn, is caused by the friction between the tribo-pair (Al_2O_3-Cu). The released Cu ions would bond with the BSA molecules in

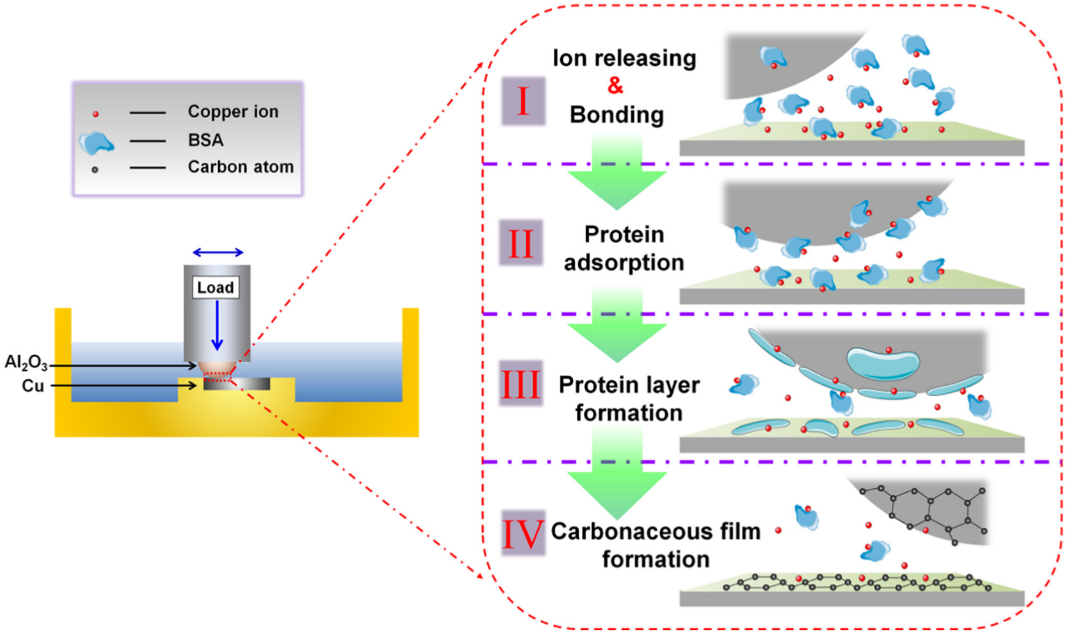

Fig. 8. The schematic of formation of a carbonaceous film on the wear track of Cu in BSA solution.

solution to promote the adsorption of the BSA molecules onto the wear track to form one "protein layer". The schematic of this process is shown in Fig. 8 (phases I, II, and III). This protein layer could subsequently transform into a carbonaceous film under the cyclic shear force between the tribo-pair (shown in Fig. 8, phase IV). The wear resistance of the tribo-pair would thus be improved and the further release of metal ions would be suppressed. When the wear testing was performed with the Al_2O_3-Cu tribo-pair in the BSA solution, a 'black tribo-layer' would appear in the wear track (shown in Fig. 2a) during the friction process. The western-blot (WB) testing of this 'black tribo-layer' shows that the molecular weight of the BSA collected from the wear track has been denatured into smaller molecules (Fig. 7). This means the shear force between the Al_2O_3-Cu tribo-pair was sufficient to denature the BSA molecules in wear track. The structure tests of the tribo-layer formed in the wear track of Cu show that there is an absorption peak at 1600 cm^{-1} of the Attenuated Total Reflection Fourier Transform Infra-Red (ATR-FTIR) spectra. This indicates that the structure of the BSA molecules in the wear track have been denatured into molecules which contain $-C{=}C-$ bonds (Fig. 3c). Both the Raman spectrum (Fig. 3a) and the electron energy loss spectroscopy (EELS; Fig. 5) spectrum of the tribo-layer formed on the Cu disc showed an obvious graphitic carbon signal. The results of the Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS, Fig. 4) present similar results to the wear track experiments; the carbon content inside the wear spot was much higher than that outside the wear spot, indicating further that the tribo-layer in the wear contact spot was a carbonaceous film. Thus, these results corroborate that the release of Cu ions from the Cu surface could promote the protein adsorption onto the wear track to form a protein layer, and the subsequent transformation of this protein layer into a carbonaceous film under the shear force between the tribo-pair (Fig. 8, phases I to IV).

When the wear testing was performed with the Al_2O_3-Co alloy tribo-pair in the BSA solution, the ATR-FTIR spectrum of the tribo-layer formed in the wear track showed a mere blue-shift of the amide I and amide II bands (Fig. 3d). This indicated that the highly-ordered structure of the BSA molecules on wear track of the Co alloy was denatured. However, this tribo-layer possessed no Raman activity (Fig. 3b). The EELS spectrum of the tribo-layer showed no obvious π^* peak signal which was similar to that of the dried BSA. That indicated that the carbonaceous film cannot, form in the wear track during the friction between the alumina and the Co alloy. This was further corroborated by the Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) experiments which showed that the carbon content inside the wear spot was similar to that of the areas surrounding the wear spot (Fig. 4). That is, the structure of the tribo-layer formed at the contact spot was similar to that of the BSA adsorbed outside of the contact spot layer (not a carbonaceous film). These results indicate that the shear force between the Al_2O_3-Co alloy tribo-pair can denature the BSA molecule, but cannot transform the protein-layer into a carbonaceous film under the conditions of this work. The resulting outcome is a poorer wear resistance of the Al_2O_3-Co alloy compared to that of the Al_2O_3-Cu friction pair (Fig. 2e).

5. Conclusion

The ball-on-disc mode was used to investigate the formation of a carbonaceous film in the contact regions of different tribo-pairs (Al_2O_3-Co alloy and Al_2O_3-Cu) in a BSA solution. The release of metal ions upon friction between tribo-pair can promote the adsorption of BSA onto the wear track to form a "protein-layer". This "protein-layer" can transform into a carbonaceous film under friction conditions of the Al_2O_3-Cu tribo-pair, and this carbonaceous film can in turn lubricate the Al_2O_3-Cu tribo-pair against further wear and ion release. Under the same conditions, however, this carbonaceous film cannot be obtained in the Al_2O_3-Co alloy tribo-pair. This reveals that Cu ions are more efficient catalysts for the formation of the graphitic carbon on the contact regions of the tribo-pair compared to the metal ions released from the Co alloy.

What needs to be pointed out is that, pure Cu is not an ideal metal to be applied in the production of artificial joint, due to the toxicity [18] and the poor mechanical properties of pure Cu. The results of this work show that the Cu ions are more efficient catalysts for the formation of the carbonaceous film than Co ions. In the future research work Cu doped films, such as Cu-TiN, Cu-DLC et al. can be fabricated for metal-on-metal (MoM) artificial joint surface modification. When the Cu doped film modified MoM joint servicing in human body, the Cu ions can be released from the films under the friction between the artificial joint tribo-pairs, and bond with the proteins in body fluid to promote the formation of protein-layer on the surface of the friction interface. And then this kind of protein-layer would transform to be carbonaceous film by the synergism of the catalytic abilities of Cu and the shear force between the tribo-pairs. This carbonaceous film can lubricate and heal the wear of the Cu doped film. As a result, the wear resistance of the tribo-pair would be increased and the lifetime of the modified artificial joint in human body would be prolonged.

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