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Association of polyethylene friction and thermal unfolding of interfacial albumin molecules

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Abstract

Under the articulation of artificial joints, ultra-high molecular weight polyethylene (UHMWPE) acts as a bearing surface under the lubrication of synovial fluid containing various proteins. Albumin is the most abundant composition and acts as the interfacial molecule in the boundary lubrication regime. The dissipated energy including thermal energy from the tribological process may lead to the conformational change of albumin molecules.

In this study, a series of experiments were designed and carried out to investigate the association of thermal unfolding albumin and the frictional characteristics of highly-crosslinked UHMWPE (x-UHMWPE). An accelerated oxidation experiment was used to prepare x-UHMWPE with an oxidized surface. Analysis of the albumin protein by circular dichroism (CD) spectroscopy was performed to detect the conformational changes during a thermal process. In addition, a molecular simulation was performed to understand the structural change of albumin at various temperatures and the exposed hydrophobic contact areas. Linear reciprocating frictional tests were carried out to obtain the start-up friction coefficients. The results indicate that a decrease of α-helix content and an unfolding of the secondary structure of albumin were observed with increasing temperatures which may come from the frictional heat of joint articulation process. The conformational change of albumin differentiates the frictional characteristics for x-UHMWPE with different oxidation levels. A model, describing that the properties of the lubricating molecules and articulating surfaces may affect the adsorption of the boundary lubrication thin film which is critical to the tribological behavior, is proposed.

Keywords: UHMWPE; Albumin; Friction; Conformation

1. Introduction

1.1. Total joint replacement

Total joint replacements have been successfully implanted clinically to replace damaged human joints for several decades [1]. A total joint replacement system comprises of a pair of material surfaces articulating against each other to simulate the functions of the human joints. The current articulating materials used clinically include metal–polymer, metal–metal, ceramics–ceramics, and ceramics–polymer pairs. From years of research and development, an artificial joint system consisting of Co–Cr alloy sliding on an ultra-high molecular weight polyethylene (UHMWPE) surface has been proven to function well and is widely used clinically. Earlier problems of the total joint system include the fracture and fixation problem of the joint implant. Optimizing of the material strength has resolved most of the fracture problems [2,3]. The introduction of bone compatible porous surface coatings to encourage bone in-growth on the stem to provide another choice for fixation has helped to almost eliminate the initial fixation problem [4–6]. Currently, wear of UHMWPE and wear particle induced osteolysis and bone resorption which lead to the loosening of the prosthesis are the major factors causing the failure of the total joint replacement.

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In the past several years, wear of UHMWPE has been dramatically reduced by using highly cross-linked UHMWPE (x-UHMWPE) [7–9]. However, the effects of smaller x-UHMWPE wear particles on the biological responses are not clear. Therefore, there still exists feasibility to further improve the lubrication and to reduce the wear of the artificial joints in vivo. To do this, further understanding of the lubrication mechanism of synovial fluid on the articulation of artificial joints is needed.

1.2. Biological lubrication

Based on the above reasons, further measurements of the interactions of synovial fluid and its compositions with the articulation of artificial joints are necessary. We need to further understand what happens to the most abundant protein in synovial fluid-albumin under a tribological process of the artificial joint materials. While under the high load and low speed condition of joint articulation, the squeezed molecules at the interface are responsible for protecting the articulating materials [10]. Therefore, the synovial mediated lubrication can be classified as a boundary lubrication mechanism [11]. Many researchers studied the friction behaviors of artificial joint under various biological lubricants [12–16]. It was also indicated that the frictional heat might lead to the chemical reactions of protein lubricants [17]. Typical pin-on-disk wear tests were also performed to investigate the effect of biological lubricants on the wear rate of UHMWPE [18–20]. Fang et al. [21–23] further designed an accelerated wear testing procedure to compare the wear of UHMWPE under different biological lubricants. This accomplishment enabled the bioactivity evaluation of different sizes and shapes of UHMWPE wear particles [24]. The influence of biological lubricant on the morphology of UHMWPE wear particles with surface textures have been reported [22]. From an engineering viewpoint, synovial fluid can be viewed as a lubricant containing albumin proteins, globulin proteins, lipids, hyaluronic acid, etc. Among which, 56% are albumin. It is also indicated that the albumin contents in the synovial fluid decreased to 42% in rheumatoid arthritis patients [25]. Therefore, it is necessary to systematically test the lubrication behaviors of the various biological molecules in the synovial fluid, especially, the most abundant composition-albumin.

1.3. Albumin molecules

Human serum albumin which is the most abundant protein in the synovial fluid is a multi-functional non-glycosylated, negatively charged plasma protein, with ascribed ligand-binding and transport properties, antioxidant functions, and enzymatic activities [26]. It consists of 585 amino acids with a molecular mass of 65 kDa [27]. Based on the X-ray analyses performed by Carter and Ho [27], albumin is a heart-shaped molecule with a high α-helical content but no β-sheet. The tertiary structure of albumin is composed of three repeating domains and each of these is comprised of two subdomains with common structural elements [28,29]. The role of albumin molecules in the lubrication of cartilage and artificial joint materials, especially, for the UHMWPE bearing surface, is critical. The protein adsorption behavior on the bearing surface has been indicated to affect the wear of UHMWPE [13]. Thermal induced unfolding of albumin and its thermodynamic features were reported previously [30]. The role of thermal unfolding albumin-mediated boundary lubrication was investigated for the artificial joint articulation system [31]. Elsaid et al. [32] suggested that the decreased synovial fluid lubrication might be related to the cartilage damage. Therefore, it is hypothesized that the frictional energy dissipated during the arthroplasty articulation may lead to the temperature rise and energy transfer to the protein molecules. Molecular dynamics simulations were widely used to provide insights into protein folding/unfolding process in atomic level [33,34]. By applying both experimental and molecular dynamics simulation approaches, it is expected the critical properties of albumin can be obtained to evaluate its lubrication performance.

1.4. Objective and approaches

The objective of this study is to evaluate the friction behavior of x-UHMWPE influenced by the thermal process induced structural change of interfacial albumin molecules. The experimental and simulative approaches were designed to understand the conformational change of albumin induced by heat. A linear reciprocating friction test was then carried out to measure the friction coefficients. By building up such a testing platform, it is expected to establish the relationship between frictional heat induced structural change and the lubricating ability under articulation of x-UHMWPE and metal for albumin molecules.

2. Materials and methods

An accelerated oxidation experiment was used to prepare x-UHMWPE with different oxidized surfaces. Analysis of the albumin protein was performed using circular dichroism (CD) spectroscopy to detect the conformational changes during a thermal process. In addition, a molecular simulation procedure was performed to understand the structural change of albumin at various temperatures. Linear reciprocating frictional tests were then carried out to obtain the friction coefficients.

2.1. UHMWPE materials

Highly-crosslinked GUR1050 UHMWPE (x-UHMWPE) materials were obtained from United Orthopaedic Corporation, Taiwan. UHMWPE cylinder pins were machined to 6.35 mm in diameter and 25.4 mm in length with diamond turning on both end surfaces without polishing. The mean roughness (R_a) of UHMWPE pins' end surface is 0.82 μm. 316 stainless steel plates were prepared as the articulating materials and the surfaces were polished ($R_a = 0.11$ μm). The surface roughness of the stainless steel plates was slightly higher than that expected for artificial joints ($R_a \sim 0.01$ μm).

To obtain the oxidized x-UHMWPE for comparison, x-UHMWPE was aged at elevated temperature to accelerate oxidation of the material. The ASTM F2003-02 [35] was used as a guideline for the experiments. x-UHMWPE pins were placed in a thermal chamber under 80 °C and 1 atm for 23 days. The materials were then stored in a vacuum drier before the friction tests were performed. Before and after the accelerated oxidation process, Fourier transform infrared-attenuated total reflectance (FTIR-ATR Specturm, GX2000 PERKIN-ELMER) was applied to determine the oxidation level of UHMWPE. An oxidation index (OI) is defined as the ratio of the area of the absorption peaks between 1650 and 1850 cm^{-1} (C=O) to the area of the absorption peaks between 1330 and 1396 cm^{-1} (C–H). The measured oxidation index for the materials taken from the shelf (x-UHMWPE) and the one undergoing the accelerated oxidation process (oxidized x-UHMWPE) are 0.92 and 1.58, respectively.

2.2. Albumin analysis

Albumin powders (Sigma, AG-1653) were dissolved in the saline solution to prepare the human serum albumin (HSA) solution of 12.6 mg/ml. Six milliliters albumin solution was poured in a caped vial and placed in a 95 °C constant temperature water bath for 5 min during a thermal process. The vial was then put in the room temperature (25 °C) for another 5 min. The above procedures were repeated until the total period of 30 min in the 95 °C water bath was reached [31].

Fresh and thermal processed albumin solutions were sampled for protein analysis. The conformation of albumin in solution was monitored using a circular dichroism spectroscopy (CD Spectropolarimeter, J-810, Jasco). CD is particularly well suited to determine the α-helical content of proteins in solution. The wavelength at $\lambda_1 = 208$ nm and $\lambda_2 = 222$ nm are sensitive indicators of α-helical content. As the protein denatures, the ellipticity of the α-helical domains decreases toward zero.

2.3. Molecular simulation

Molecular simulation of albumin was applied to understand the conformation change of the molecule induced by a temperature increase in this study. Molecular minimization and dynamics simulations were performed with the InsightII/Discover 2000 package, distributed by Accelrys Inc. (San Diego, CA), using the consistent valence force field (CVFF) [36] with a Morse potential for the bonded atoms. The coordinates of the non-hydrogen atoms used for the initial trial geometries were taken from the experimental results obtained by single-crystal X-ray analysis deposited in the Protein Data Bank (human serum albumin, 1AO6) [37]. The protonation state of protein was adjusted in order to mimic a pH of 7.0. This structure was then optimized with backbone HSA fixed to remove any bad contacts of side chains. The optimized structure of HSA was further solvated with water molecules with inner and outer layers being 15 and 10 Å, respectively, keeping the outer water layer fixed to avoid the diffusion of the water molecules within the inner layer. In order to arrange the soaked water molecules of inner layer randomly, water molecules of inner layer alone were submitted to 10,000 iterations by conjugate gradient minimization, keeping the protein atoms and outer water layer fixed. Then, 1 ns MD simulations with 15 ps equilibrium step were carried out at 300, 400, 500, and 600 K. The integration step in all simulations was 1 fs and the trajectories of HSA structures were saved every 2.5 ps for further analysis. The range of cut-off radius was set as 12 Å for both non-bonded electrostatic and van der Waals interactions.

2.4. Friction measurement

A schematic of the friction test setup and the photo of the contacting materials are shown in Fig. 1. A multi-directional wear tester equipped with a three-axes force sensor was set up. Three servo-motors and one piezo-elecrical force sensor were integrated and controlled by a Labview software to synchronize the sliding motion and data acquisition. This configuration makes the simulation of any motion pattern possible. In this study, a linear reciprocating motion was designed for the friction tests. The x-UHMWPE sample and the polished 316 stainless steel plate were mounted on the tester. Linear reciprocating wear tests of x-UHMWPE articulating 316 stainless steels were carried out with 20 mm/s speed, 5 mm stroke length for 50 cycles. Various compressive displacements

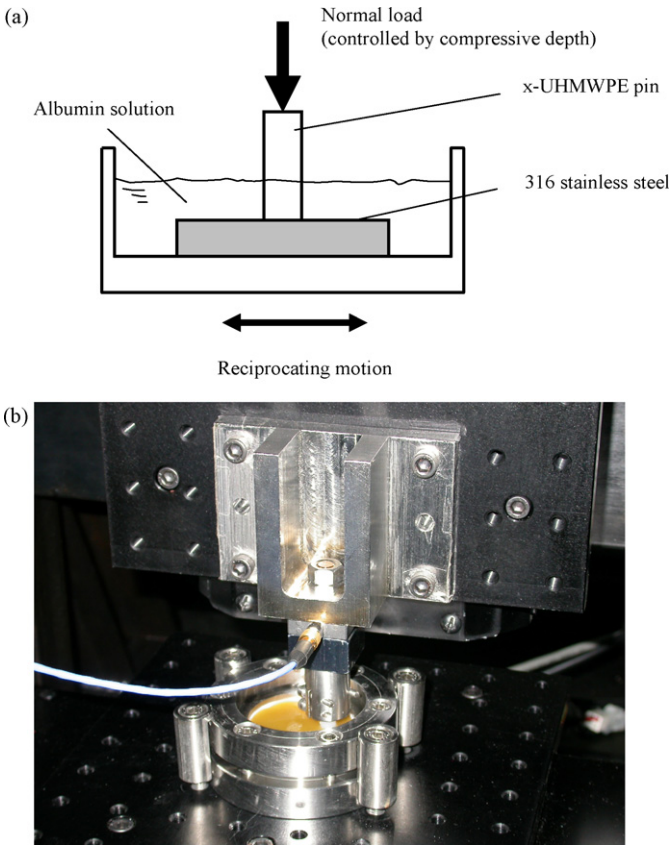

Fig. 1. (a) Schematic of the linear reciprocating friction test. (b) Photo of the contacting UHMWPE and 316 stainless steel under the lubrication of albumin solution.

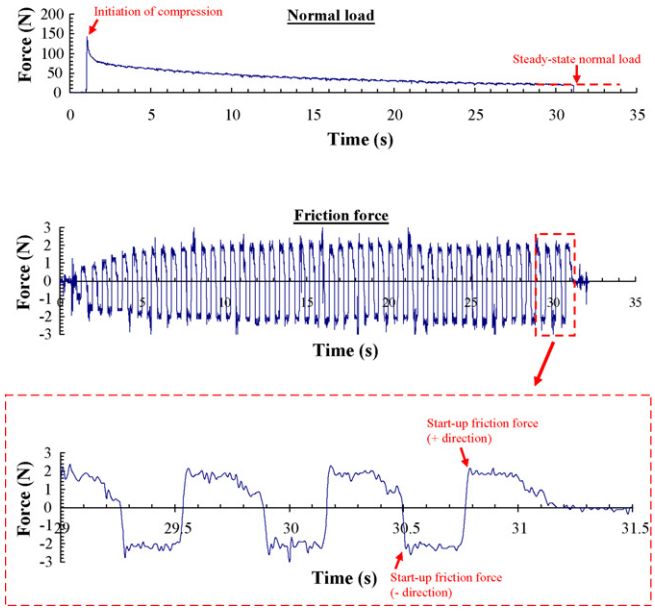

Fig. 2. An example plot of the normal load and frictional force curve for the linear reciprocating sliding process. Start-up friction forces for the last six cycles were chosen to calculate the average friction coefficients for comparisons.

between 300 and 600 μm were applied in the friction tests. Fresh and thermal processed albumin solution and saline solution were used as lubricants. Normal forces and frictional forces were recorded and analyzed by the Labview software. Fig. 2 shows an example plot of the normal load, frictional force, and start-up friction coefficient for the linear reciprocating sliding process. The positive and negative frictional force and friction coefficient indicate the opposite moving direction during the linear reciprocating process. It is seen that the normal force decays rapidly at initial linear reciprocating motions due the stress relaxation of polymer. Average value of normal loads and friction force were calculated by retrieving the measurements of the last six sliding process when a steady normal load was achieved. Friction measurement tests were repeated three times. The steady normal loads under various compressive displacements during the sliding are listed in Table 1.

2.5. Statistical analysis

Differences in friction coefficient between the experiments were assessed by one-way analysis of variance (ANOVA) to make allowance of comparisons. A value of $p < 0.05$ was considered significant. Statistical analyses were performed using SAS software on a personal computer.

3. Results and discussion

3.1. Thermal unfolding of albumin

The CD spectra of 12.6 mg/ml albumin solution were obtained as shown in Fig. 3. It is shown that the peak of α-helix secondary structure at $\lambda_1 = 208$ nm and $\lambda_2 = 222$ nm was decreased after the thermal process. The results indicated that albumin may lose its secondary structure and lead to the unfolding of this protein. The results suggest that the frictional energy generated from the articulation of artificial joint materials may be dissipated in the joint space. The energy may contribute to the temperature rise of the system, chemical reactions of the synovial fluid, or the wear of the arthroplasty or cartilage.

Molecular dynamics simulations were also performed to understand the thermal effect on the structural change of albumin molecules. The secondary structure analysis was carried out using the Kabsch and Sander algorithm [38] incorporated in their DSSP program. Fig. 4 shows the snapshots of average structure of albumin at different temperatures. The red areas shown in Fig. 4 represent the α-helices regimes. It is seen that the α-helix content decreases with increasing temperatures. HSA is composed of 38 α-helices without β-sheet. To determine the structural stability of each α-helix in HSA at different temperatures, the averaged helicity was calculated during the 1 ns MD simulations and the results are given in Fig. 5(a). It is obvious that the averaged helicity of HSA decreased linearly as temperature increased from 300 to 600 K, indicating that higher temperature destroys the native structure of HSA, leading to the loss of α-helical content. This result is consistent with the findings of previous experiments, in which an elevated temperature caused the loss of α-helix by measuring the ellipticity at 208 and 222 nm using circular dichroism [31]. Furthermore, Wang et al., [39] have investigated thermal stability of HSA in aqueous solution by applying Fourier transform infrared spectroscopy (FTIR) and indicated that the maximum peaks at 1652 and 1547 cm^{-1} (α-helix) shifted to 1647 and 1542 cm^{-1} (random coil), respectively, with the increase of temperature.

3.2. Hydrophobicity of albumin and UHMWPE

Fig. 5(b) shows that the averaged hydrophobic accessible surface area (ASA) increases linearly with increasing simulation

Table 1
The steady normal loads (N) for the x-UHMWPE and oxidized x-UHMWPE sliding on 316 stainless steels under various compressive displacement and lubricants

Compressive displacement (μm)	x-UHMWPE on steel			Oxidized x-UHMWPE on steel		
	Albumin (fresh)	Albumin (thermal)	Saline	Albumin (fresh)	Albumin (thermal)	Saline
300	25.4	16.8	12.5	17.7	12.5	16.3
400	57.5	26.6	18.9	33.8	27.8	25.6
500	89.9	45.5	30.5	64.5	47.9	44.6
600	124.8	45.0	58.4	100.0	82.0	76.8

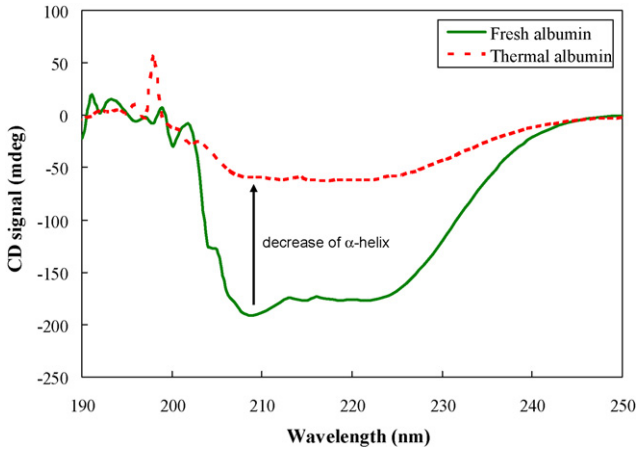

Fig. 3. CD spectra for fresh and thermal processed (95 °C, 30 min) albumin solutions.

temperature. This result provides atomic evidences in favor of the previous molecular model, in which the unfolded HSA preferentially adsorb onto hydrophobic polymeric surfaces via hydrophobic interactions due to the exposure of the interior hydrophobic surface area of protein [31]. The results suggest that elevated temperatures result in the loss of the hydrophobic contacts and the decrease of intramolecular hydrophobic interactions within HSA, leading to the exposure of hydrophobic ASA and the increase of intermolecular hydrophobic interactions.

3.3. Friction coefficients

Fig. 6(a) indicates that the friction coefficient of x-UHMWPE sliding over steel in the thermal processed albumin solution is higher than the friction coefficients measured in fresh albumin solution under various compressive displacements ranging from

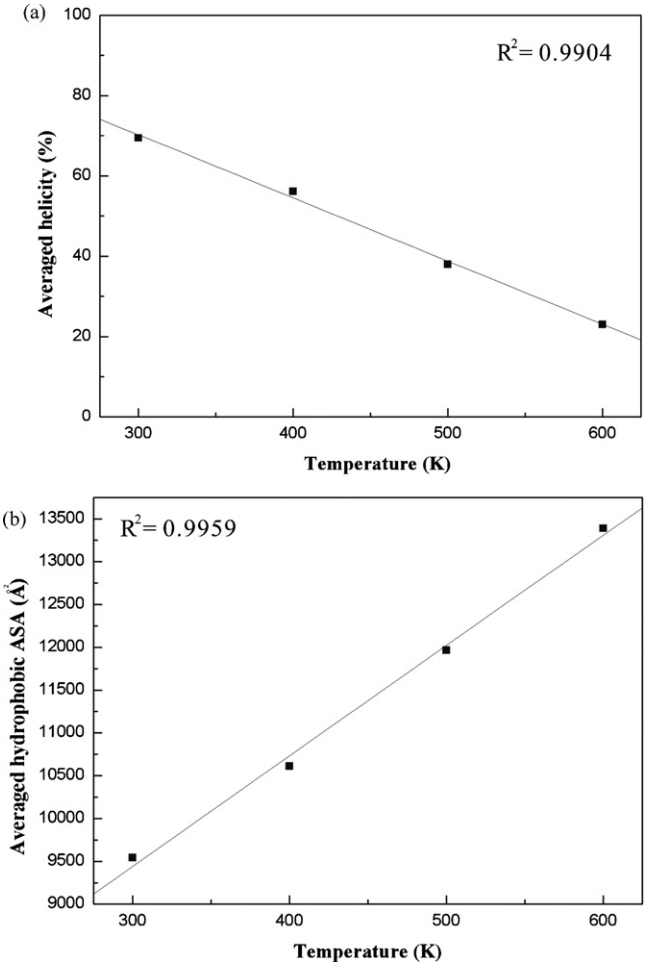

Fig. 5. (a) Linear relationship between the averaged helicity of albumin and temperature; (b) linear relationship between the averaged hydrophobic ASA of albumin and temperature.

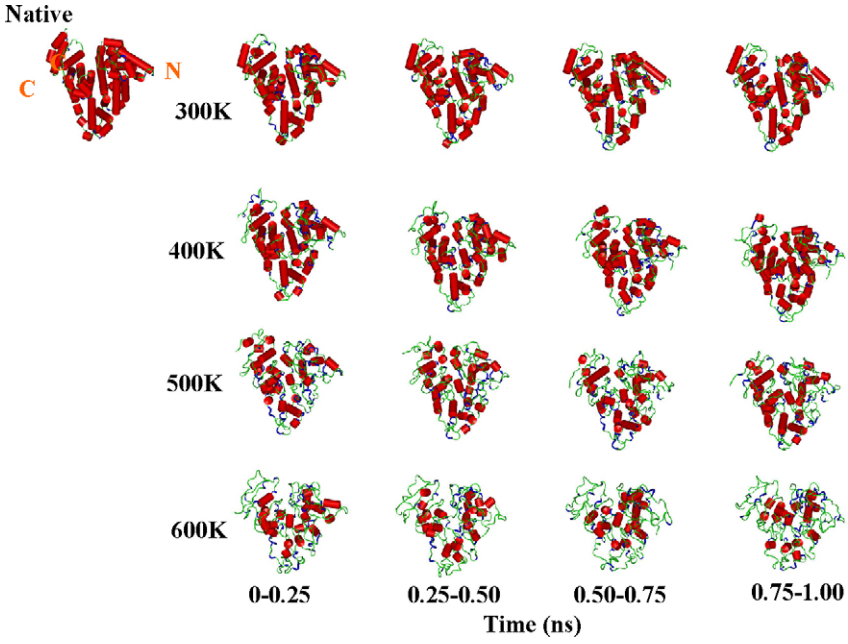

Fig. 4. The snapshots of average structure of albumin at different temperatures.

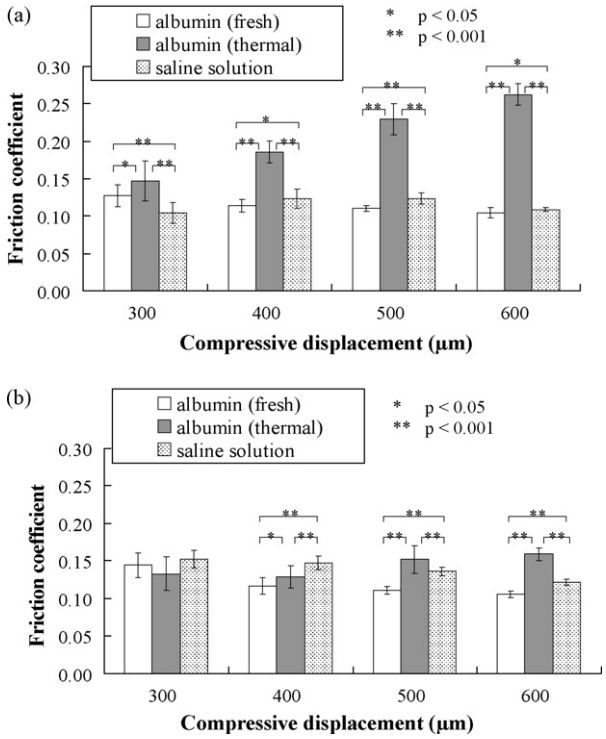

Fig. 6. (a) Start-up friction coefficients for x-UHMWPE-316 stainless steel sliding experiments; (b) start-up friction coefficients for oxidized x-UHMWPE -316 stainless steel sliding experiments.

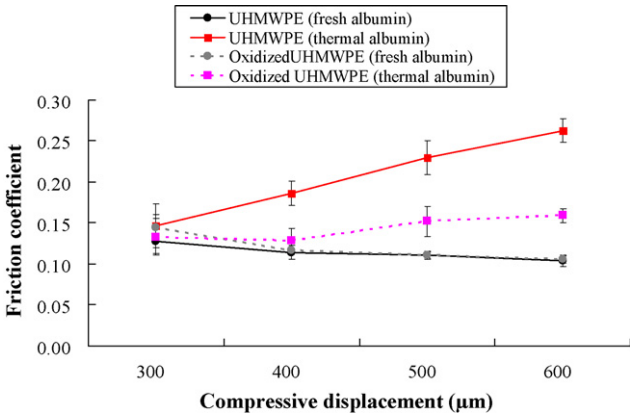

Fig. 7. Curves of the friction coefficients for x-UHMWPE with different oxidation level under the lubrication of fresh and thermal processed albumin solutions.

300 to 600 μm ($p < 0.05$). Thermal processed albumin solution contains a measurable amount of unfolded albumin as shown in Fig. 3. Compared to native albumin, unfolded albumin has a less regular structure and exposes more hydrophobic side chains of amino acids, resulting in more hydrophobic interaction between the unfolded albumin and material surfaces. The unfolded albumin forms a compact layer of protein that tends to adhere to the hydrophobic x-UHMWPE surface and results in the increase of friction coefficient. However, the friction coefficients for thermal processed albumin solutions increase with increasing compressive displacements (loads). With increasing normal load, the unfolded albumin may be pressed onto surfaces to a greater amount, leading to increasing the friction coefficients. When the hydrophobic x-UHMWPE is replaced by a less hydrophobic oxidized x-UHMWPE, the friction coefficients are shown in Fig. 6(b). Comparing the friction coefficients under fresh and thermal processed albumin solutions for oxidized x-UHMWPE, no difference is observed under a compressive displacement of 300 μm ($p = 0.065 > 0.05$). Under the compressive displacements of 400, 500, and 600 μm, thermal processed albumin solution results in higher friction coefficients than fresh albumin solution ($p < 0.001$). When the compressive displacement is the same, fresh albumin solution, compared with thermal processed albumin solution, leads to smaller friction coefficients as shown in Fig. 7. The unfolded albumin has a less regular structure than native albumin and may rearrange its irregular conformation on the surface more readily, causing a larger amount of protein adsorption on the surface. The compacting structure of the unfolded albumin then leads to the increase of friction coefficient. With increasing normal load, the unfolded albumin is also pressed to a greater amount, leading to a trend of increase of the friction coefficients as shown in Fig. 7.

3.4. A hypothesis model

As discussed above, the thermal unfolding albumin tends to form a compact layer. With increasing normal load using thermal processed albumin, the friction coefficients increase for both x-UHMWPE and oxidized x-UHMWPE. The articulation of x-UHMWPE and steel with thermal processed albumin leads to the highest friction coefficient. The x-UHMWPE gave considerably higher friction coefficients than the oxidized x-UHMWPE using the thermal albumin. Comparing to oxidized x-UHMWPE, x-UHMWPE has a more hydrophobic surface property due to a more symmetric molecular structure. The hydrophobic unfolding albumin having stronger bonding strength with the more hydrophobic x-UHMWPE [31] may explain the result. When the fresh albumin solution was applied, the smaller friction coefficients are always obtained. Under the small normal load of 300 μm, oxidized x-UHMWPE results in a larger friction coefficient under fresh albumin solution ($p < 0.001$). While under the compressive displacements of 400, 500, and 600 μm, the difference of friction coefficients under fresh albumin solutions between x-UHMWPE and oxidized x-UHMWPE is not obvious ($p > 0.05$).

Based on the experimental results of friction tests and the computation results of albumin helicity and averaged hydrophobic ASA, we propose a hypothesis that accounts for the effects of albumin conformational change on the start-up friction coefficient as shown in Fig. 8. The unfolding of albumin induced by the thermal process leads to the increase of the hydrophobic contact area of albumin. The unfolded albumin tends to form a compact layer of protein under contact pressure and leads to the increase of friction coefficient in comparison to the native albumin structure. x-UHMWPE has a relative hydrophobic surface. The unfolded albumin forms a compact layer of protein that tends to adhere on the hydrophobic x-

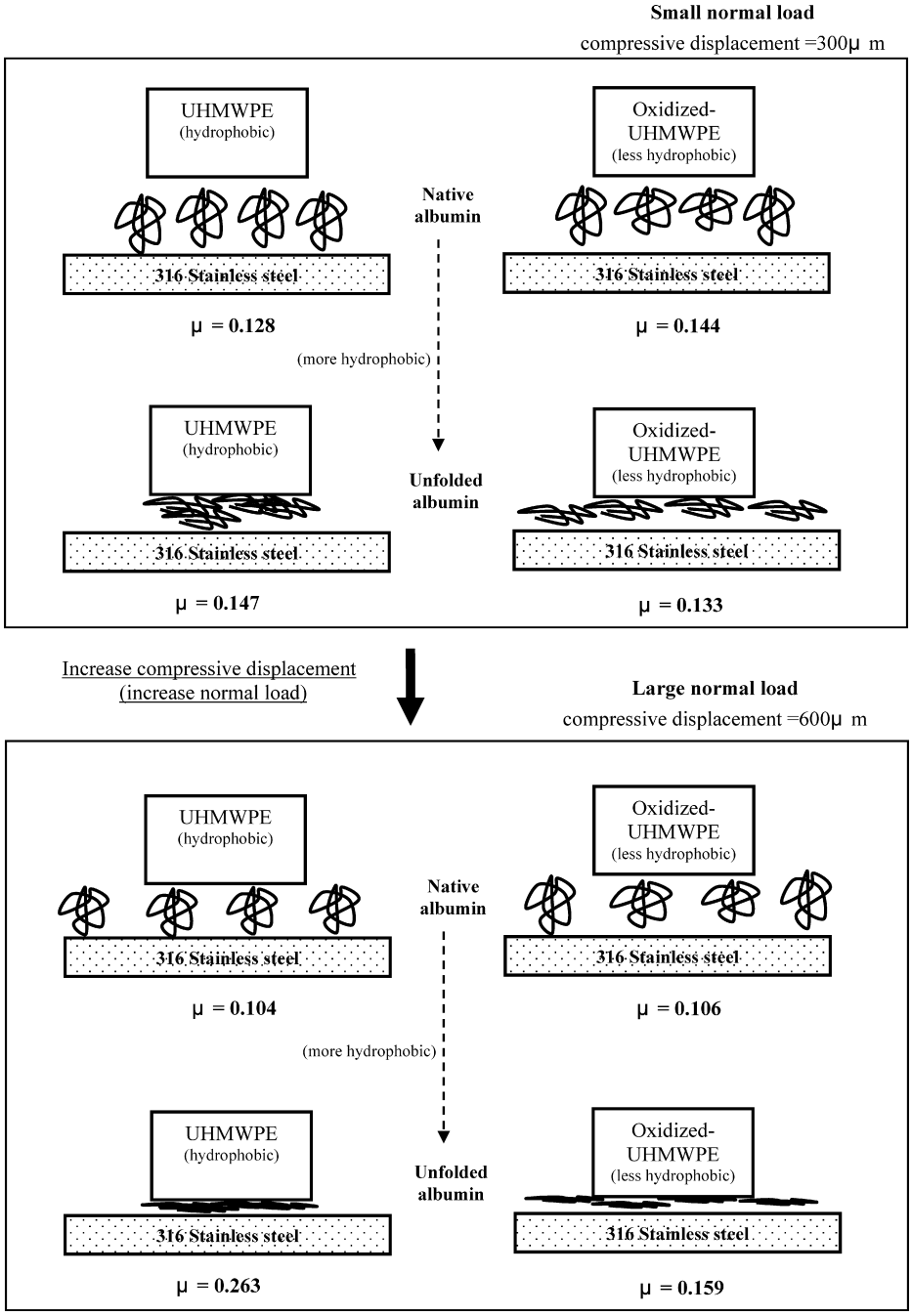

Fig. 8. Schematic illustrations for the effects of albumin conformational change on the start-up friction coefficients for x-UHMWPE and oxidized x-UHMWPE sliding over 316 stainless steel materials.

UHMWPE surface and results in the increase of friction coefficient. In addition, native albumin acts as a lubrication enhancer for oxidized x-UHMWPE. Compared to x-UHMWPE, oxidized x-UHMWPE is more hydrophilic and increase of normal load brings more concentrated native albumin layer at the interface and reduces the friction coefficient.

The mechanism of the thermal unfolding was investigated by both experimental and computational approaches. Previous researches suggested that the decrease of hydrophobic contact is simultaneously associated with the decrease of the structural integrity of α-helices during thermal unfolding of albumin. Furthermore, the kinetics of adsorption and desorption of albumin on the articulating surface play a critical role on the effects of boundary lubrication. Further investigation shall be designed and carried out to quantify the amount of protein adsorption on the articulating surfaces. By obtaining this information, further strategies can be developed to enhance the lubrication of joints in the biological system.

4. Conclusions

In this study, the thermal unfolding of albumin molecules was characterized. The interactions of the conformational

change of albumin with x-UHMWPE and oxidized x-UHMWPE surfaces were investigated through a series of friction tests in order to point out their impacts on boundary lubrication. The effects of the relationship between the hydrophobicities of albumin and articulating materials on their frictional characteristics were also indicated. The results indicate that a decrease of α-helix content and an unfolding of the secondary structure of albumin were observed with increasing temperatures which may come from the frictional heat of joint articulation process. The conformational change of albumin differentiates the frictional characteristics for UHMWPE with different oxidation levels. A model describing that the properties of the lubricating molecules and articulating surfaces may affect the adsorption of the boundary lubrication thin film which is critical to the tribological behavior was proposed.

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