Tribological process induced conformational transformation of protein may change the friction of cartilage

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Received 5 October 2006; accepted 13 November 2006
Available online 5 December 2006

Abstract

In-vitro testing procedures have been successfully developed to investigate the effects of tribological process induced transformation of protein-based lubricant on the friction change of articular cartilages. Serum and albumin solutions were the biological lubricants used in this study. The results indicated that the lubricating ability for cartilages deteriorates after the biological lubricants were articulated between polyethylene and stainless steel materials. In addition, the secondary structure change of the albumin molecule has been characterized after the molecules were articulated by the artificial joint materials. We have provided evidence that the conformational change of protein lubricants leads to the friction increase of articular cartilage.

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Keywords: Partial joint arthroplasty; Cartilage; Friction; Albumin; Tribochemistry; UHMWPE

1. Introduction

Partial joint arthroplasty surgeries have been widely applied to patients with unicompartmental knee joint osteoarthritis or those who suffered from the femoral neck fracture. Unicompartmental knee arthroplasty (UKA) and bipolar hemiarthroplasty are two representative treatments. Coexistence of artificial joint materials and articular cartilages in the joint area constitutes the unique feature and it may play a critical role of the subsequent progression of the cartilage degeneration.

The most common failure mechanism of UKA is component loosening, polyethylene wear and progression of disease in the un-resurfaced compartment [1]. Degeneration in the opposite compartment is a common cause of failure after unicompartmental arthroplasty [2–7]. Some authors have suggested that overcorrection of joint deformity results in the transfer of increased forces to the uninvolved compartment and accelerating degeneration [4,8]. However, the complete explanation of the progression of patello-femoral arthritis is still not provided. In addition, Elsaid et al. [9] further suggested that the decreased synovial fluid lubrication might be related to cartilage damage. Unipolar hemiarthroplasty can be a successful treatment for displaced femoral neck fractures in selected low demand elderly patients. However, bipolar hemiarthroplasty also is associated with groin pain, acetabular cartilage deterioration related to time and activity as well as polyethylene wear at the bearing surface of the femoral head and the mobile acetabular implant, which can lead to osteolysis, implant loosening, and early revision surgery [10,11]. The influence of bipolar hemiarthroplasty on acetabular cartilage had been discussed in the researches comparing bipolar hemiarthroplasty to total hip arthroplasty in patient with femoral head osteonecrosis [11]. Evaluations of the acetabular cartilages during the hemiarthroplasty revision surgeries have indicated that degenerative changes of the cartilages were present [12,13]. They concluded that even healthy cartilage could not tolerate the
abnormal stress created by a metallic femoral head after hemiarthroplasty.

Thus, we hypothesize that the interactions between the articulation of artificial joint materials and articular cartilages may contribute to the subsequent degeneration of cartilages. In the situations of partial joint arthroplasty, the influence of tribological transformed fluids released from the joint implant interface on the lubricating ability of the natural cartilage may become one of the most critical issues as shown schematically in Fig. 1. Once the association of the tribochemistry of biological lubricants due to the arthroplasty implant articulation and the degeneration of the cartilage is identified, further development can be made to enhance the durability of partial joint arthroplasty. Thus, the objective of this study is to develop the testing protocols to (1) identify the tribological transformation of lubricating biomolecules due to the articulating process, and to (2) evaluate the effects of transformed biomolecules on the friction of the articular cartilages. By further performing systematic investigations with the testing model, the reasons contributing to the progression of cartilage degeneration in the partial joint arthroplasty can be possibly understood.

2. Experimental

An in-vitro testing procedure has been developed to investigate the effects of tribological transformation of lubricating biomolecules on the friction change of articular cartilages. Bovine serum and albumin solutions were chosen as lubricants in this study. Bovine serum was used to simulate the function of synovial fluid. On the other hand, albumin represents the most abundant composition in the synovial fluid. “Post-friction” lubricants retrieved from the articulation of artificial joint materials were analyzed for possible transformation. By further applying both “fresh” and “post-friction” biological lubricants in cartilage-stainless steel friction tests, the coefficients of frictions were compared. The detailed procedures are described in the following sections.

2.1. Preparation of “post-friction” lubricant

Highly-crosslinked GUR1050 ultra-high molecular weight polyethylene (UHMWPE) obtained from United Orthopaedic Corporation, Taiwan was used in this study. 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 (Ra) of UHMWPE pins’ end surface is 0.82 μm. Bovine serum (AKH12367, HyClone), human serum albumin solution (12.6 mg/mL by desolving albumin powders (Sigma, AG-1653) in to saline solution), and saline solution were used as lubricants in the experiments. All UHMWPE pins were presoaked in the solution for at least 15 days so as to become completely saturated with the lubricants. A linear reciprocating wear test was designed to carry out the articulating of UHMWPE and 316 stainless steel.
materials. Linear reciprocating wear tests were run under a nominal contact pressure of 3 MPa, a stroke length of 19 mm, a frequency of 1.5 Hz, and an average sliding speed of 57.2 mm/s for 24 h. After the wear tests, lubricants were collected for the cartilage friction tests and biochemical analysis.

2.2. Cartilage tissue sample

Full-thickness articular cartilage was harvested aseptically from adult porcine knee joints within 12 h after slaughter. We used mosaiculty chisel to harvest osteochondral tissue. The diameter of the cylindrical tissue sample was 4.5 mm. The chisel was hammered into the femoral condyle of the porcine knee joint with the depth of 5 mm (here is 5 mm). Then we gently shook the chisel to break the cancellous bone. The chisel was removed and the osteochondral tissue was pushed out from the chisel. The pusher was applied from the part of cancellous bone, to avoid injury to the cartilage surface. The cylindrical tissue was cut to a minimize the height of osteochondral bone and fixed on the top surface of a UHMWPE pin of 6.35 mm in diameter and 25.4 mm in length by epoxy glue.

2.3. Measurement of cartilage friction

The cartilage tissue sample and the polished 316 stainless steel were mounted on the tester. Linear reciprocating frictional tests of cartilages articulating 316 stainless steels were carried out with 20 mm/s speed, 5 mm stroke length for 20 cycles. Various compressive displacements between 300 and 800 μm were applied in the friction tests. Fresh and post-friction biological lubricants of bovine serum, human serum albumin solution, and saline solution were used as lubricants. Normal forces and frictional forces were recorded and analyzed by the Labview software.

2.4. 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-helix content of proteins in solution. The wavelength at $\lambda_1 = 208$ nm and $\lambda_2 = 222$ nm are sensitive indicators of α-helix content. As the protein denatures, the ellipticity of the α-helical domains decreases toward zero. CD was employed to analyze the temperature-dependent conformational change of human serum albumin. Furthermore we compare the conformation of albumin before and after wear test. By doing so, we are able to understand the wear properties dependence on the structure of the protein lubricants. The sample solution was about 400–500 μl to fill into the cuvette. In this study, CD spectroscopy was not applied to bovine serum solution due to its complex compositions. In addition, SDS-PAGE of albumin before and after wear was performed to monitor the possible change of molecular weight of protein.

2.5. Statistical analysis

Differences in friction coefficient between the experiments using fresh and post-friction lubricants 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

3.1. Friction measurements

The friction coefficients under various lubricants and compressive displacements have been measured. Fig. 2 shows the friction coefficients under saline, serum, and albumin solutions, respectively. No significant difference of friction coefficient was observed between the experiments.
The adsorption of human serum albumin on the articulating surface that may affect the friction force [14]. Heuberger et al. [14] indicated that the unfolded protein with less α-helix content tends to occupy a larger surface area of polyethylene than is the case with fresh protein. The adsorbed layer of denatured protein effectively passivates the surface and prevents adsorption of more proteins. Therefore, a larger friction force in polyethylene–ceramics articulation was observed with denatured protein [14]. The behavior of the serum adsorption on the materials surface has also been studied by Blanchet et al. [15]. Their results proved the hypothesis that the articulation of arthroplasty implants induced tribochemical reaction of biological lubricants. In this study, we further provided evidence that the articulated biomolecules including albumin proteins also lead to the increase of friction force in a cartilage-steel sliding test. However, synovial fluids consist of a variety of compositions such as albumin, globulin, hyaluronic acid, lipids, etc. Each composition may behave differently while under an articulating process. Further experiments should be carried out to investigate the mechanism of tribochemical reactions of individual composition. The transformation of the biological molecules during the tribochemical process could be identified. By doing so, the combinational influence from tribochemistry of lubricating biomolecules on the friction between natural cartilages can be understood. Currently, there remains a difficulty on measuring the friction force with the curve surfaces of the natural cartilages. Efforts should also be made by contact mechanics analysis in order to precisely quantify and calculate the friction coefficients. Thus, identification of the failure mechanism of partial joint arthroplasty system might be expected based on the development from this study.

Acknowledgement

The authors are pleased to acknowledge the financial support from the US Air Force (A0ARD-05-4081) and the Far Eastern Memorial Hospital (FEMH-94-C-040), Taiwan.

References