Influence of Phospholipid and Protein Constituents on Tribological Properties of Artificial Hydrogel Cartilage Material*

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Abstract
In this study, the influence of phospholipid and protein constituents on friction and wear behavior of artificial hydrogel cartilage was investigated. A sliding pair of an ellipsoidal specimen of poly (vinyl alcohol) (PVA) hydrogel and a flat specimen of PVA hydrogel was evaluated in simplified reciprocating friction test. Dipalmitoylphosphatidylcholine (DPPC) was selected as a phospholipid constituent and was dispersed in saline as liposome. Fluorescent-labeled albumin and γ-globulin were used as protein constituents in lubricants at concentration of 0.7 wt%. After reciprocating friction test, the boundary film formed on the surface of PVA hydrogel and the worn surface of PVA hydrogel were observed by using fluorescent microscope and confocal laser scanning microscope, respectively. When only albumin or γ-globulin was added to lubricant, adhesive wear pattern was frequently observed and large breaking-off of surface structure of PVA hydrogel occurred. Lubricants that contain both proteins and 0.01wt% DPPC showed reduction of friction and suppression of large breaking-off of surface structure of PVA hydrogel. Meanwhile, under coexistence of protein and 0.02wt% DPPC, friction increased compared to that for lubricants that contain 0.01wt% DPPC and the adhesive wear patterns became obvious. Therefore, both the concentration and the relative ratio of proteins to phospholipids are important factors to function adequately as excellent boundary lubricant for PVA hydrogel.

Key words: Artificial Hydrogel Cartilage, Phospholipid, Protein, Friction, Wear

1. Introduction
Total joint replacement has made significant contributions to better recovering of the articular joint function for patients of osteoarthritis and rheumatoid arthritis. Several materials such as biocompatible polymers, metals and bioceramics are applied as bearing materials of artificial joints. Ultra-high molecular weight polyethylene (UHMWPE) is commonly used as a bearing material for artificial joints. However, the loosening and the failure of artificial joint occur due to biological reaction to wear particles of UHMWPE(1,2). Therefore, to extend the viability of joint prosthesis, the improvement of wear resistance of UHMWPE has been widely studied(3-5). In metal-on-metal and ceramic-on-ceramic hip joints, the effective formation of fluid film was indicated(6-7). However, metal ion release from metal component and fracture of ceramic component remained to be solved. As a
consequence, the improvement of lubrication mode in artificial joint by mimicking of the excellent lubrication mechanism of natural joint is also proposed.

Application of soft materials as artificial cartilage to bearing materials of artificial joints was proposed to improve the lubrication mode of artificial joints\(^{(8,9)}\). Many researches on the artificial cartilage have been conducted\(^{(10-12)}\), and poly (vinyl alcohol) (PVA) hydrogel is one of the anticipated materials for artificial cartilage. Enhancement of fluid film formation by soft elastohydrodynamic lubrication (EHL) is expected by using PVA hydrogel as material for bearing surfaces, and it was reported that PVA hydrogel showed low friction at steady walking condition in knee joint simulator test\(^{(13)}\). However, considerable wear of PVA hydrogel occurs in severe condition like mixed or boundary lubrication due to poor wear resistance and mechanical strength\(^{(14,15)}\). To achieve the clinical application of PVA hydrogel as artificial cartilage, it is important to elucidate the wear mechanism of PVA hydrogel under the condition lubricated by body fluid. In joint cavity, synovial fluid is supplied mainly through synovial membrane and functions as lubricant for natural and artificial joint. Synovial fluid contains many biomolecules such as proteins, lipids and hyaluronic acid and it is indicated that those constituents influence on friction and wear behavior of artificial joint materials\(^{(16,17)}\). Therefore, it is important to elucidate the function of synovial fluid constituents as lubricant for PVA hydrogel to propose the improving method for wear reduction and material property modification considering the influences of synovial fluid constituents. In addition, the effect of intra-articular injection of some functional molecules such as hyaluronic acid\(^{(18)}\), phospholipids\(^{(19)}\) and proteins\(^{(20)}\) on improvement of the lubrication of natural and artificial joints have been shown. For the application of some biomolecules to intra-articular injection, it is important to elucidate the mechanisms of lubrication by synovial fluid constituents.

In the previous study, it was found that the wear of PVA hydrogel depended on both the ratio and content of albumin and \(\gamma\)-globulin being mixed into lubricants\(^{(21)}\). It was indicated that the structure of protein boundary film on PVA hydrogel surface was the key essence for wear reduction\(^{(22)}\). In addition, it was indicated that the stability of protein boundary film changes with the concentration and the addition ratio of proteins in lubricants through in situ observation on forming protein boundary film\(^{(23)}\). When phospholipid and proteins coexist in lubricants, sheet-like matters were formed on rubbing surface especially in the lubricants that contain both phospholipid and albumin, and they contributed to reduction in friction of PVA hydrogel\(^{(24)}\). However, an important influence of phospholipid constituent on wear behavior of PVA hydrogel has not been cleared yet.

In this study, phospholipid and proteins were selected as additives to test lubricants. And then, influence of phospholipid and protein constituents on friction and wear behavior of PVA hydrogel was investigated.

2. Experimental methods

2.1 Reciprocating friction test

Details of reciprocating friction tester used in this study are shown in Fig. 1. A sliding pair of an ellipsoidal (major axis: 40mm diameter, minor axis: 25mm diameter) reciprocating upper specimen of PVA hydrogel as 2mm thickness and a flat stationary lower specimen of PVA hydrogel was tested. PVA hydrogel used in this study is shown in Fig. 2. PVA hydrogel was prepared by freezing-thawing method, and number of freezing-thawing cycles was 5 times. Polymerization degree and saponification degree of PVA (Kishida Chemical Co. Ltd.) was 2000 and 98.4--99.8 mol\%, respectively. The elastic modulus of PVA hydrogel was 1.2 MPa and equivalent water content was 79\%. The applied load was 2.94 N and average contact pressure was 0.093 MPa. Sliding speed of 20 mm/s was selected and the reciprocating stroke was 35 mm. Total sliding distance in this study was 1.5km.
Lubricant compositions used in this study are shown in Table 1. Normal saline solution (Otsuka Pharmaceutical Factory, Inc.) was used as solvent for lubricants. L-α dipalmitoyl phosphatidylcholine (DPPC) (Wako Pure Chemical Industries, Ltd.) was selected as a phospholipid constituent. DPPC is a main constituent of phospholipids in natural synovial fluid. DPPC was dispersed as liposomes in saline by ultra-sonication method. Bovine serum albumin (Wako Pure Chemical Industries, Ltd.) and human serum γ-globulin (Wako Pure Chemical Industries, Ltd., Japan) were used as protein constituents. The concentrations of each constituent were within the physiological range in natural synovial fluid\(^{25,26}\). Phospholipid concentration in synovial fluid changes with the condition of synovial joints and individual differences\(^{26}\), and the concentration of DPPC in this study is within the range of healthy and postoperative synovial fluid. Albumin and γ-globulin were fluorescently labeled by rhodamine-B-isothiocyanate (Sigma Aldrich Co.) and fluorescein isothiocyanate isomer I (Sigma Aldrich Co.), respectively. Concentration of proteins in this study is within the physiological concentration but relatively low\(^{27}\).

After friction test, protein boundary film formed on PVA hydrogel was observed by using fluorescent microscope (IX 71, Olympus Corporation). Then PVA hydrogel specimens were washed with solution of surface active agent to remove the adsorbed molecules and the worn surface of PVA hydrogel was observed by using confocal laser scanning microscope (VK-8500, Keyence Corporation).

![Fig.1 Schematics of reciprocating friction tester](image1)

![Fig. 2 PVA hydrogel prepared by freezing-thawing method](image2)

<table>
<thead>
<tr>
<th>Lubricant</th>
<th>DPPC [wt%]</th>
<th>Albumin [wt%]</th>
<th>γ-globulin [wt%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.7</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0.01</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>A(_P1)</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B(_P1)</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C(_P1)</td>
<td>0.7</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>A(_P2)</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B(_P2)</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C(_P2)</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
2.2 TEM observation of lubricant components

To reveal the influence of the lubricant composition on the morphology of the complexes of DPPC and proteins, lubricant components were observed by transmission electron microscope (TEM) before friction test. The lubricants shown in Table 1 were prepared and stored at room temperature for 6 hours. They were diluted a thousand fold by pure water to observe each component clearly and these were used as sample solutions. The copper TEM grid (Nisshin EM Corporation) was hydrophilized by using Plasma Ion Bombarder (PIB-10, Vacuum Device, Inc.) to prevent the aggregation of sample molecules on TEM grid. Then TEM grid was put on the 10μL droplet of diluted lubricant for 30s. Then, the residue of sample on copper TEM grid was absorbed by using filter paper. For negative staining of adsorbed molecules on TEM grid, samples were treated by 2.0 % solution of uranyl acetate for 30s. Finally, treated TEM grids were dried in air. The samples were observed by using transmission electron microscope (HT7700, Hitachi Hi-Technologies Corporation) operated at 100kV.

3 Results

3.1 Reciprocating Friction Test

Transients of friction coefficient during friction test in lubricants that contain no DPPC were shown in Fig.3 (a). When only single protein was added to lubricants, lubricant B showed lower friction than other two lubricants at initial state. However, there were little differences between three lubricants at steady state.

When 0.01wt% DPPC was added to lubricants, friction at initial state was reduced in lubricants AP1 and CP1 with comparing lubricants A and C that contained no DPPC as shown in Fig.3 (b). Lubricant BP1 that contains 0.01wt% DPPC and 0.7wt% albumin showed lower friction than those of other lubricants, and addition of 0.01wt% DPPC significantly reduced friction with coexistence of albumin and DPPC.

When only DPPC was added at a concentration of 0.02wt%, friction coefficient at initial state increased and the effect of friction reduction at steady state was slight as compared to that of 0.01wt%. In lubricants that contain proteins and 0.02wt DPPC, addition of DPPC with high concentration led the increase of friction (Fig.3 (d))

![Fig.3 Friction coefficient during the reciprocating friction test and at steady state](image-url)
The images of protein boundary film on PVA hydrogel by fluorescent observation were shown in Fig. 4. Formation of smooth sheet-like adsorbed matters was observed in lubricant $B_{P1}$ that contained 0.01wt% DPPC and albumin (Fig. 4, arrows). However, degradation of forming sheet-like film was confirmed in lubricant $B_{P2}$ that contained 0.02wt% DPPC. When lubricants contained $\gamma$-globulin as protein constituents, no sheet-like film was formed under coexistence of DPPC (Fig. 5).

Intact surface of PVA hydrogel and worn surfaces of PVA hydrogel as lower specimen were shown in Figs. 6 and 7, respectively. In normal saline, significant wear occurred with breaking off of the surface structure (Fig. 7 (A)). In lubricant that contains albumin or
γ-globulin but no DPPC, severe wear occurred with loss of intact surface structure (Figs. 7 (B), (C)). In lubricants that contain DPPC but no protein, severe abrasive wear occurred under low DPPC concentration and the reduction of wear was observed under high DPPC concentration (Fig. 7 (A_P2)). Wear reduction of PVA hydrogel was also confirmed in lubricant that contains protein and 0.01wt% DPPC (Figs. 3 (B_P1), (C_P1)). However, both abrasive and adhesive wear patterns were observed in the lubricants that contains protein and 0.02wt% DPPC. These results indicated that DPPC contributes to reduction of friction and adhesivity and shifted the wear mode of PVA hydrogel from adhesive wear to abrasive wear. In addition, DPPC with high concentration and coexistence of DPPC with low concentration and protein reduce wear of PVA hydrogel. However, coexistence of DPPC with high concentration and protein has little effect in reducing wear of PVA hydrogel.

Worn surfaces of PVA hydrogel as upper specimen were shown in Fig. 8. In lubricant A_P1, severe abrasive wear pattern with many scratches was confirmed. In contrast, significant wear occurred with breaking off of the surface structure in other lubricants. For all cases, there was no effect of lubricant additives on surface protection of PVA hydrogel as upper specimens.

Fig. 6 Intact surface of PVA hydrogel

Fig. 7 Worn surface of PVA hydrogel as lower specimens

<table>
<thead>
<tr>
<th>Protein concentration</th>
<th>No protein</th>
<th>albumin 0.7wt%</th>
<th>γ-globulin 0.7wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC addition</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>DPPC 0.01wt%</td>
<td>A_1</td>
<td>B_1</td>
<td>C_1</td>
</tr>
<tr>
<td>DPPC 0.02wt%</td>
<td>A_2</td>
<td>B_2</td>
<td>C_2</td>
</tr>
</tbody>
</table>
3.2 TEM observation of lubricant components

TEM images of lubricant constituents are shown in Fig.9. Liposomes in lubricant A_P1 are sized in several hundreds nanometers. In lubricant A_P2 that contained twice as much DPPC as lubricant A_P1, some liposomes fused together and grew in size. When DPPC and proteins coexisted and concentration of DPPC was 0.01wt%, liposomes got distorted but remained their spherical structure. However, when the concentration of DPPC became higher as 0.02wt%, some liposomes aggregated and some lost their spherical structure and collapsed. Therefore, it was confirmed that DPPC/protein concentration in lubricant was key factor for the structure of liposomes and DPPC bilayers.
4. Discussion

When lubricants contained DPPC but no proteins, friction coefficient was reduced by DPPC addition and wear pattern was shifted from adhesive to abrasive wear. In addition, wear of PVA hydrogel was suppressed by addition of DPPC with high concentration. Therefore, DPPC has role of reduction of the adhesivity and shear resistance between PVA hydrogels. The boundary lubricating ability of multi-lamellar film of phospholipids was reported, and the authors indicated the lubricating mechanisms of multi-lamellar film formed by friction-induced spread of liposomes. In lubricant that contained 0.01wt% DPPC, small liposomes easily collapsed and formed lamellar film based on the bilayer structure. In multi-lamellar film composed by phospholipid, water layer exists between each bilayer and it is considered that this layer functions as a low shearing resistance layer. Although lubricant A_P2 that contained 0.02wt% DPPC showed wear reduction of PVA hydrogel as compared to lubricant A_P1, there was little difference in friction coefficient at steady state between lubricants A_P1 and A_P2. In general, liposomes are stabilized by fusion and enlargement. Therefore, wear of PVA hydrogel was reduced by increase of liposomes intervening between rubbing surfaces but additional effect of friction reduction was not obtained by suppression of forming multi-lamellar film due to the stabilization of liposomes.

In previous study, liposomes adsorbed on the rubbing surface were spread and formed smooth boundary film and showed low friction and the smooth sheet-like adsorbed matters that are composed of DPPC and albumin formed under rubbed condition. It was reported that the liposomes made by neutral phospholipids such as DPPC have high affinity to albumin and there are influences of DPPC/protein concentration on maintaining and collapsing the structure of liposomes and phospholipid bilayers. When concentration of liposomes in lubricant is low, the structures of liposomes and bilayers maintained and the liposomes did not become larger. And then, the liposomes were spread and formed lamellar film and functioned as boundary lubricant. When concentration of liposomes in lubricant is high, liposomes fused, grew in size and stabilized. Therefore, it is considered that liposomes in lubricants A_P2, B_P2, C_P2 could not be easily spread by frictional loading and could not form multi-lamellar films. And thus, lubricating function of phospholipid was not fully utilized and adhesive wear pattern became obvious. These results indicated that not only concentration of single constituent but also relative concentration of proteins and phospholipid are important factors for these constituents to function as excellent boundary lubricants.

The effect of additives to lubricant on suppression of wear was not confirmed on the upper specimens. The contact area of upper specimens was not changed during reciprocating friction test. PVA hydrogel is the biphasic material that contains about 80% water and has biphasic lubrication property. It is indicated maintaining of water content and interstitial fluid pressure are important to maintaining the biphasic lubrication mechanism and friction coefficient of PVA hydrogel as biphasic material increases with increase of loading time due to the exudation of internal water. There was little chance of recovery of hydration for upper specimen and it is considered that biphasic lubrication ability of upper specimens decreased during the test. Therefore, it is indicated that adsorbed film by proteins and phospholipid itself could not protect sufficiently the upper surface of PVA hydrogel with contact zone under continuous loading.

Thus, the establishment of the synergistic function of boundary lubrication by adsorbed film and biphasic lubrication is an important factor for reduction in both friction and wear of PVA hydrogel. The improvements in boundary lubrication and biphasic lubrication properties of PVA hydrogel are planned in further study.

In this study, concentration of proteins in lubricant was relatively low within
physiological concentration. In addition, natural synovial fluid contains other lubricating components such as hyaluronic acid. Therefore, influence of the addition of hyaluronic acid and protein concentration would be researched in future study.

5. Conclusion

In this study, influence of phospholipid and protein constituents on friction and wear behavior of PVA hydrogel as artificial cartilage was investigated. It was indicated that DPPC contributes to reduction of friction of PVA hydrogel and the appropriate coexistence of DPPC and proteins significantly reduces wear of PVA hydrogel. In addition, both the concentration and the relative ratio of proteins and phospholipids are important factors for these constituents to function as excellent boundary lubricants for PVA hydrogel. These findings would contribute to the elucidation of the wear mechanisms of PVA hydrogel in synovial fluid and improvement of material properties of PVA hydrogel considering the influences of synovial fluid as lubricants.

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