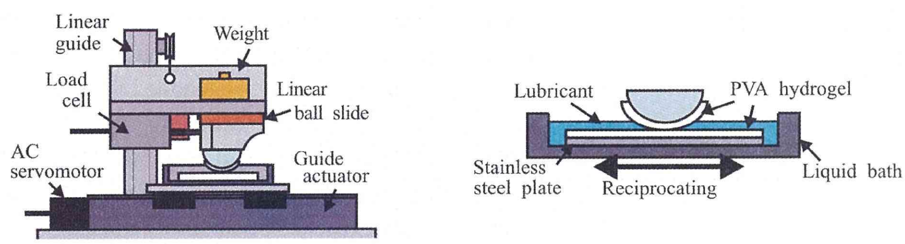
Lubricant compositions used in this study are shown in Table 1. Normal saline solution (Otsuka Pharmaceutical Factory, Inc.) was used as solvent for lubricants. L-α dipalmitoyl phosphatidylcoline (DPPC) (Wako Pure Chemical Industries, Ltd.) was selected as a phospholipid constituent. DPPC is a main constituent 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).



(a) Schematic view (b) Detailed view

Fig.1 Schematics of reciprocating friction tester

Fig. 2 PVA hydrogel prepared by freezing-thawing method

Table 1 Composition of lubricants

Lubricant	DPPC [wt%]	Albumin [wt%]	γ-globulin [wt%]
A	0	0	0
B		0.7	0
C		0	0.7
A_{P1}	0.01	0	0
B_{P1}		0.7	0
C_{P1}		0	0.7
A_{P2}	0.02	0	0
B_{P2}		0.7	0
C_{P2}		0	0.7

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 A_{P1} and C_{P1} with comparing lubricants A and C that contained no DPPC as shown in Fig.3 (b). Lubricant B_{P1} 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))

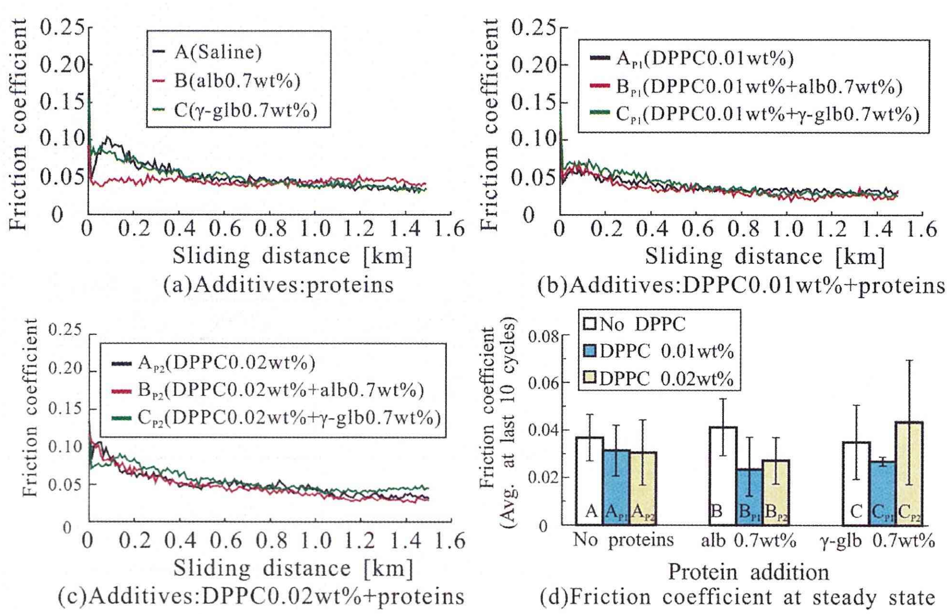


Fig.3 Friction coefficient during the reciprocating friction test and at steady state

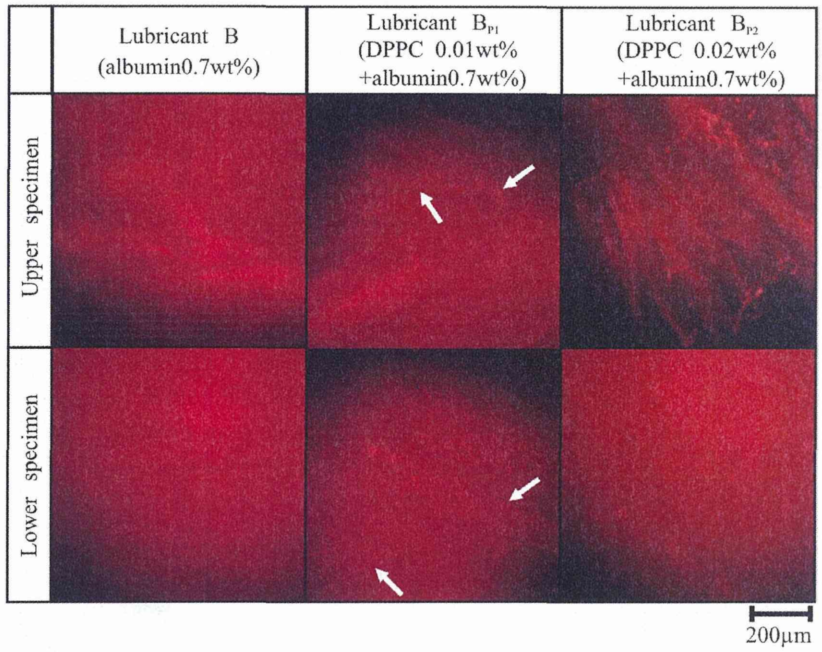


Fig.4 Fluorescent images of albumin film on PVA hydrogel

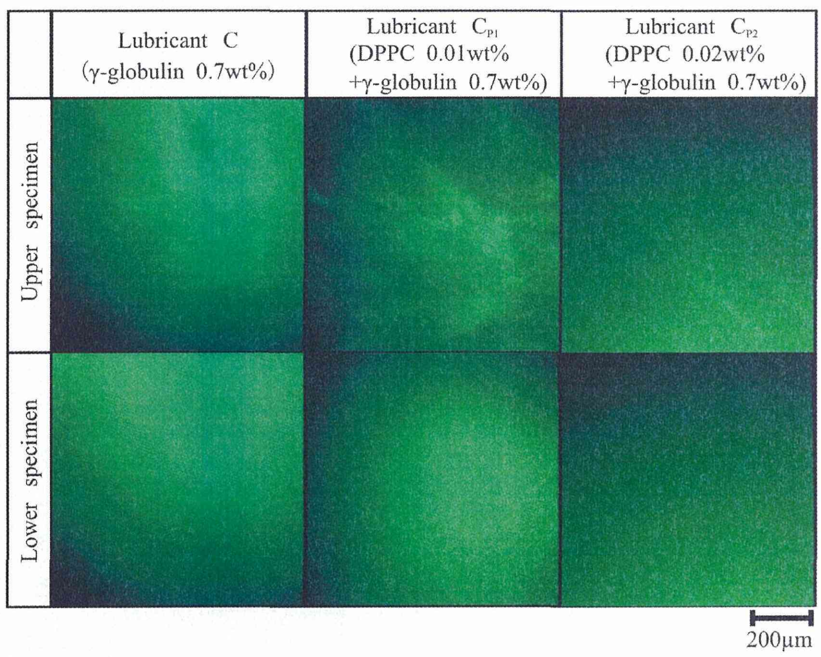


Fig.5 Fluorescent images of γ-globulin film on PVA hydrogel

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 γ-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.

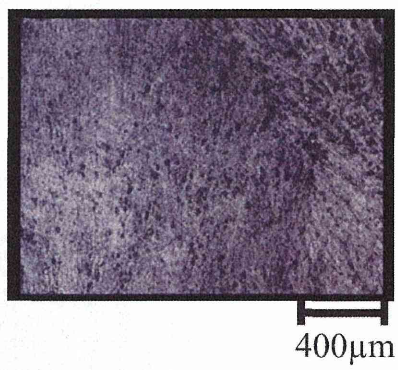


Fig.6 Intact surface of PVA hydrogel

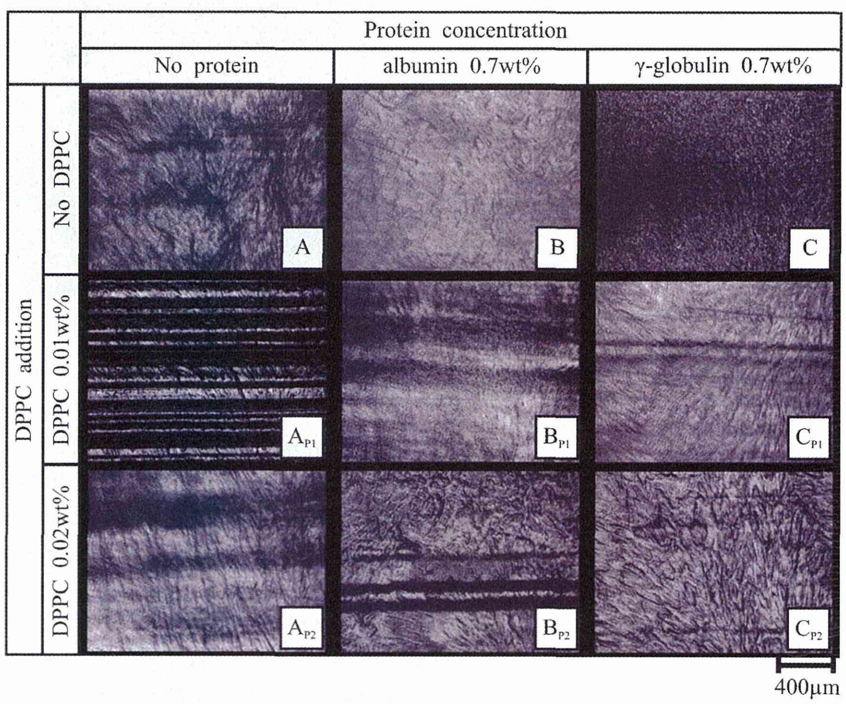


Fig.7 Worn surface of PVA hydrogel as lower specimens

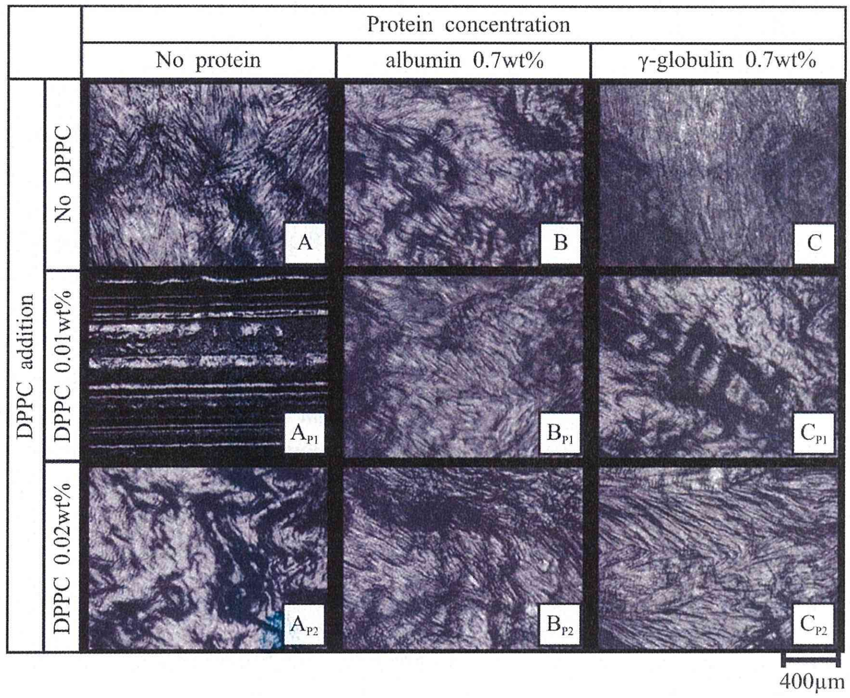


Fig.8 Worn surface of PVA hydrogel as upper specimens

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.

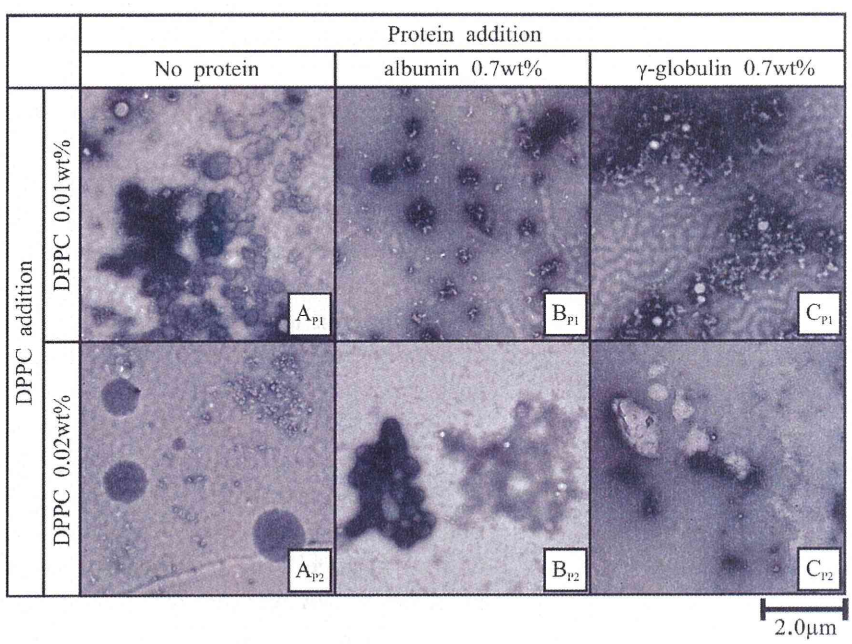


Fig.9 TEM images of lubricant components

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(28), and the authors indicated the lubricating mechanisms of multi-lamellar film formed by friction-induced spread of liposomes(24). 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(29) 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(24). It was reported that the liposomes made by neutral phospholipids such as DPPC have high affinity to albumin (30) and there are influences of DPPC/protein concentration on maintaining and collapsing the structure of liposomes and phospholipid bilayers(31).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 (32) and friction coefficient of PVA hydrogel as biphasic material increases with increase of loading time due to the exudation of internal water (33). 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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Poly(2-methacryloyloxyethyl phosphorylcholine)-grafted highly cross-linked polyethylene liner in primary total hip replacement: one-year results of a prospective cohort study

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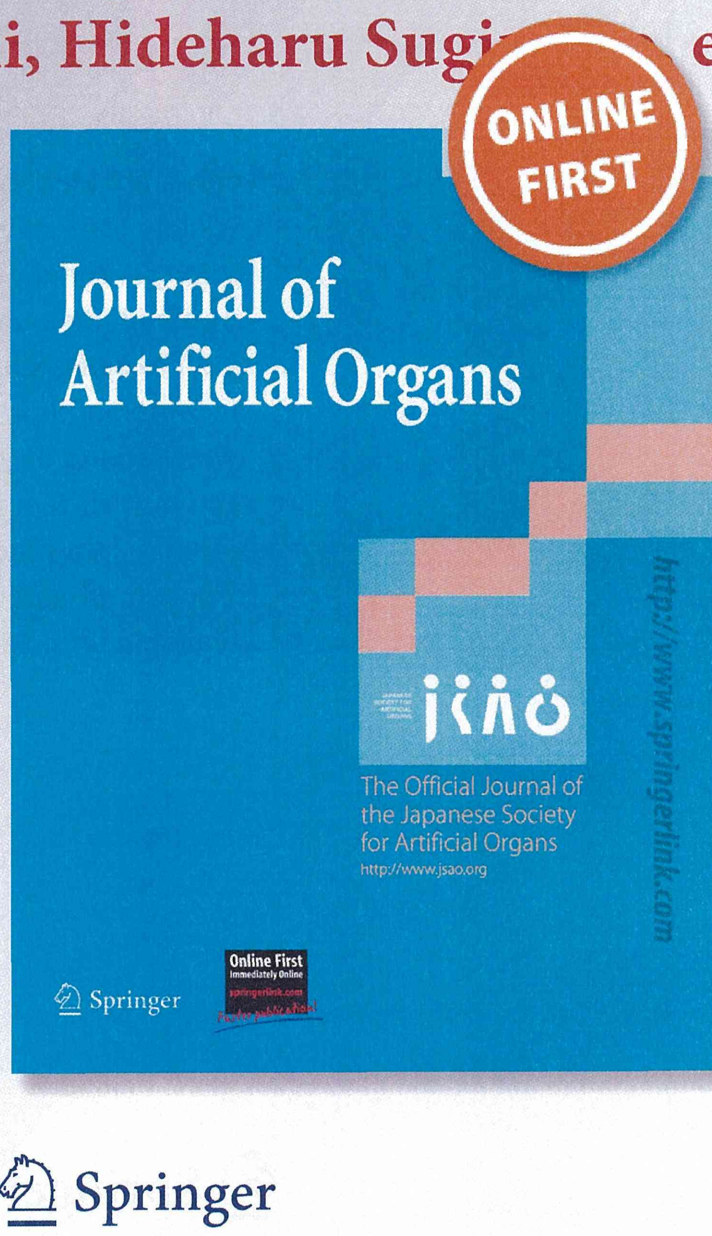

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