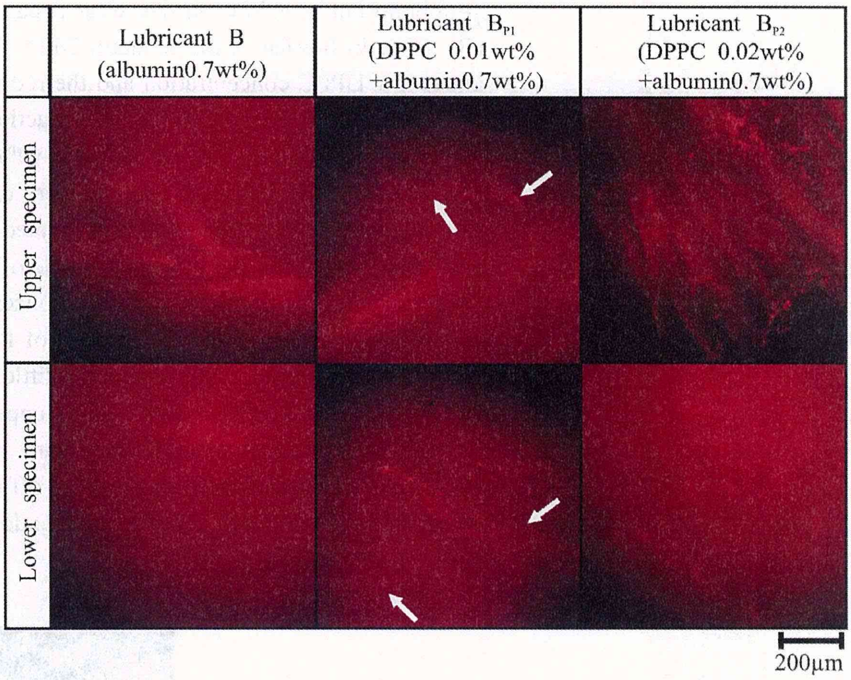

Fig.4 Fluorescent images of albumin film on PVA hydrogel

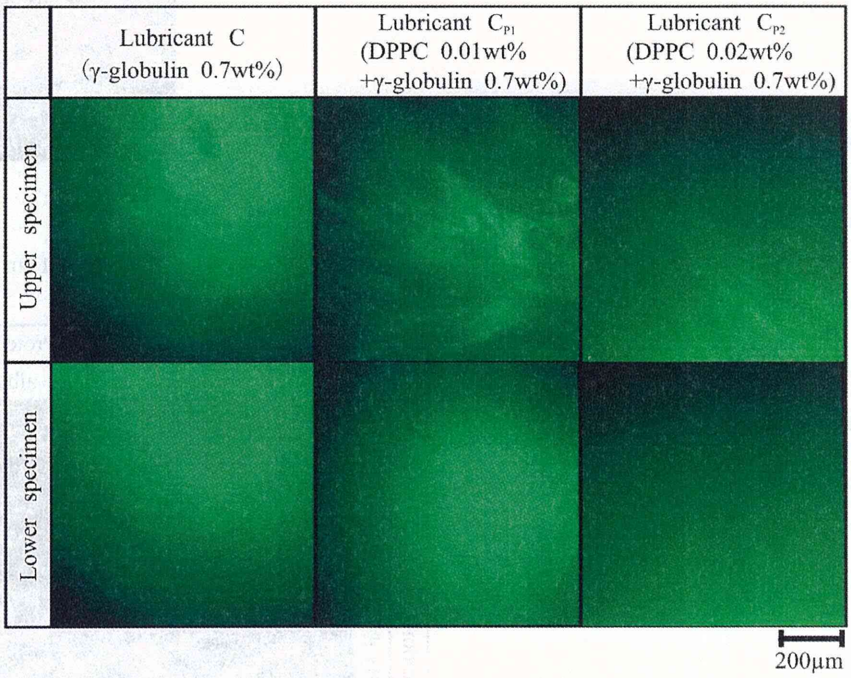

Fig.5 Fluorescent images of γ-globulin film on PVA hydrogel

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.

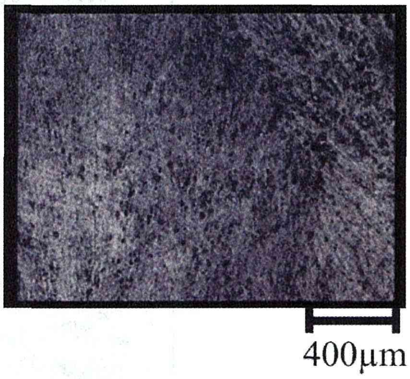

Fig.6 Intact surface of PVA hydrogel

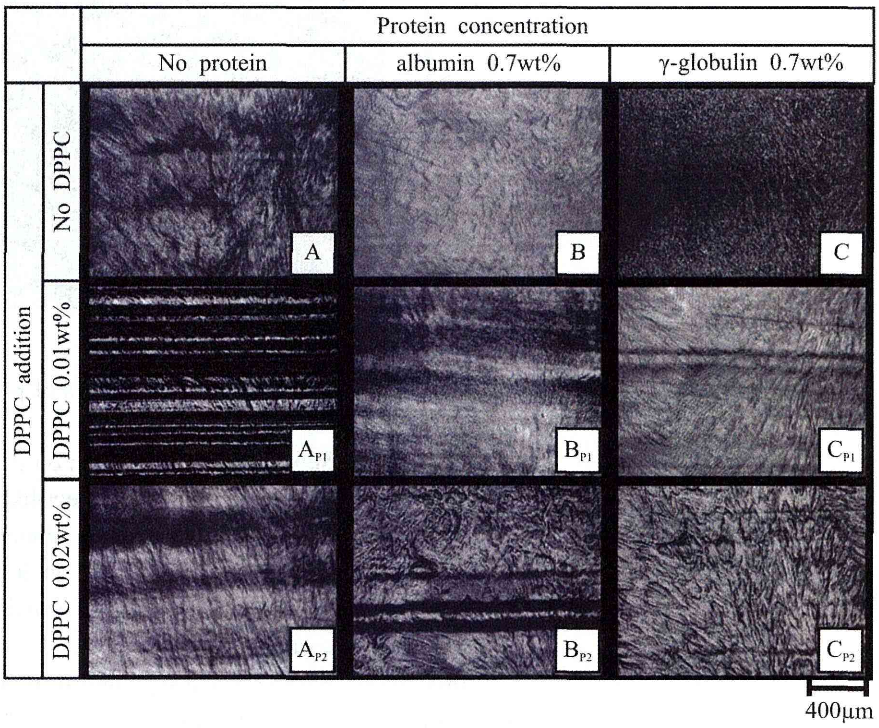

Fig.7 Worn surface of PVA hydrogel as lower specimens

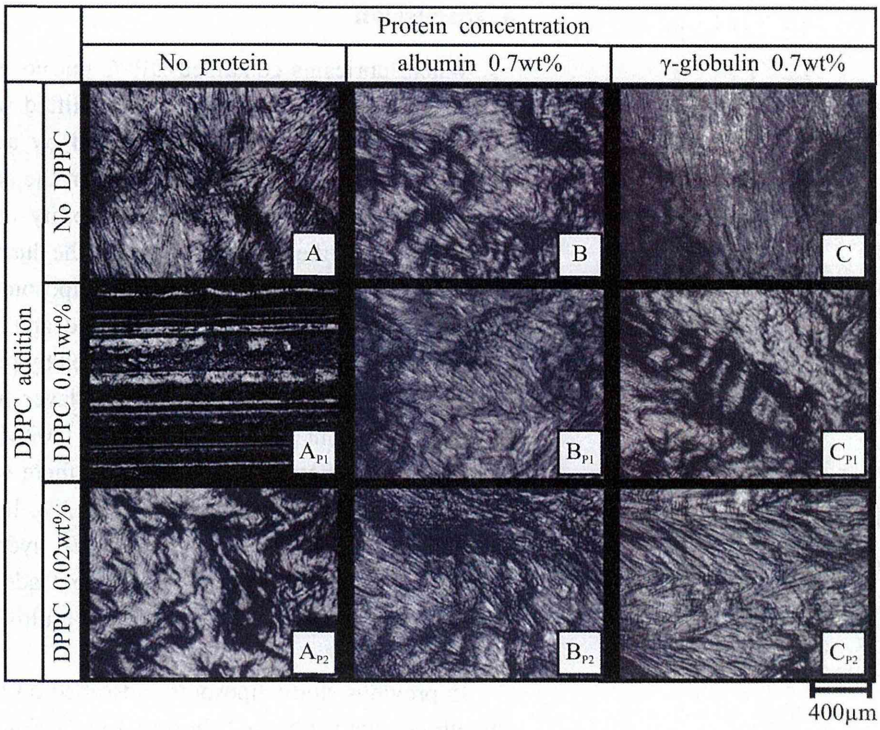

Fig.8 Worn surface of PVA hydrogel as upper specimens

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.

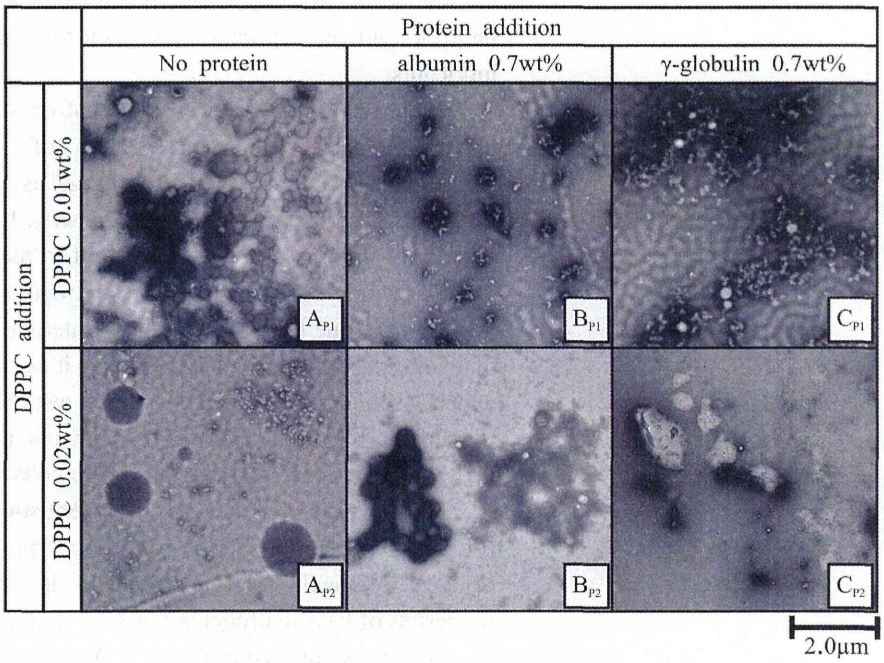

Fig.9 TEM images of lubricant components

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.

Acknowledgements

This research was supported by Grant-in-Aid for Specially Promoted Research of JSPS (23000011).

The authors would like to make a most cordial acknowledgment to Mr. T. Kanemaru in Kyushu University for his technical guidance on TEM observation and Mr. S. Hashimoto in Kyushu University for his effort in this research activity.

References

(1) Willert, H.G., Semlitsch M. Reactions of the artificial capsule to wear products of artificial joint prostheses. J Biomed Mater Res., Vol.11, No.2 (1977), pp.157-164.

(2) Ingham, E., Fisher, J., Biological reactions to wear debris in total joint replacement. Proc Inst Mech Eng Part H, Vol.214, No.1 (2000), pp.21-37

(3) McKellop, H., Shen, F.W., Lu, B., Campbell P, Salovey R. Development of an extremely wear-resistant ultra high molecular weight polyethylene for total hip replacements. J. Orthop. Res., Vol.17, No.2 (1999), pp.157-167.

(4) Oonishi, H., Kadoya, Y., Masuda, S., Gamma-irradiated cross-linked polyethylene in total hip replacements–analysis of retrieved sockets after long-term implantation, J. Biomed. Mater. Res., Vol.58, No.2 (2001), pp.167-171.

(5) Moro, T., Takatori, Y., Ishihara, K., Konno, T., Takigawa, Y., Matsushita, T., Chung, U.I., Nakamura, K., Kawaguchi, H., Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis, Nat. Mater. Vol.3, No.11 (2004), pp.829-836.

(6) Jin, Z. M., Dowson, D., Fisher, J., Analysis of fluid film lubrication in artificial hip joint replacements with surfaces of high elastic modulus, Proc. Instn. Mech. Engrs. Part H, Engineering in Medicine, Vol.211, No.3 (1997), pp.247-256.

(7) Ikeuchi, K., Ohashi, M., Tomita, N., Oka, M., The lubrication in ceramic-on-ceramic prostheses, Jpn. J. Clin. Biomech., Vol.16 (1995), pp.381-383. (in Japanese)

(8) Sasada, T., Takahashi, M., Watanabe, M., Mabuchi, K., Tsukamoto, Y., and Nanbu, M., Friction behavior of a total hip prosthesis containing artificial articular cartilage, J. Jpn. Soc. Biomat., Vol.3, No.3 (1985), pp.151-157. (in Japanese)

(9) Unsworth, A., Pearcy, M.J., White, E.F.T., White, G., Frictional properties of artificial hip joints, Engng. Med. Vol.17, No.3 (1988), pp.101-104.

(10) Auger, D.D., Dowson, D., Fisher, J., Cushion form bearing for total knee joint

replacement Part 1:design, friction and lubrication, Proc. Inst. Mech. Engrs., Vol.209 (1995), pp.73-81.

(11) Oka, M., Noguchi, T., Kumar, P., Ikeuchi, K., Yamamuro, T., Hyon, S.H., Ikada, Y., Development of an artificial articular cartilage, Clin. Biomater., Vol.6 (1990), pp.361-381.

(12) Murakami, T., Ohtsuki, N., Higaki, H., The adaptive multimode lubrication in knee prostheses with compliant layer during walking motion, Thin Films in Tribology, (1993), pp.673-682.

(13) Murakami, T., Sawae, Y., Nakashima, K., Fisher, J., Tribological behaviour of artificial cartilage in thin film lubrication. Thinning Films and Tribological Interfaces, (2000), pp.317-327.

(14) Mabuchi, K., Tsukamoto, Y., Yamamoto, M., Ueno, M., Sasada, T., Nambu, M., Lubrication property and mechanical durability of the joint prostheses containing cartilage-like polyvinyl alcohol gel, J. Soc. Orthop. Biomech., Vol.8 (1986), pp.101-105. (in Japanese)

(15) Shintani, H., Oka, M., Cha, W.I., Hyon, S.H., Ikada, Y., Ikeuchi, K., Sakaguchi, K., Hojo, H., Nakamura, T., The wear-resistant properties of PVA-hydrogel for artificial articular cartilage, J. Jpn. Soc. Biomat., Vol.12, No.3 (1994), pp.127-134. (in Japanese)

(16) Saikko, V., Ahlroos, T., Phospholipids as boundary lubricants in wear tests of prosthetic joint materials, Wear, Vol.207, Issues.1-2 (1997), pp.86-91.

(17) Sawae, Y., Murakami, T., Chen, J., Effect of synovia constituents on friction and wear of ultra-high molecular weight polyethylene sliding against prosthetic joint materials, Wear, Vol.216 (1998), pp.213-219.

(18) Namiki, O., Toyoshima, H., Morisaki, N., Therapeutic effect of intra-articular injection of high molecular weight hyaluronic acid on osteoarthritis of the knee, Int. J. Clin. Pharmacol. Ther Toxicol., Vol. 20, Issue. 11 (1982), pp.501-507.

(19) Kawano, T., Miura, H., Mawatari, T., Moro-Oka, T., Nakanishi, Y., Higaki, H., Iwamoto, Y., Mechanical effects of the intraarticular administration of high molecular weight hyaluronic acid plus phospholipid on synovial joint lubrication and prevention of articular cartilage degeneration in experimental osteoarthritis, Arthritis Rheum., Vol.48, Issue 7 (2003), pp.1923-1929.

(20) Hua, Z. K., Su, S. H., Zhang J. H., Tribological study on new therapeutic bionic lubricants, Tribol. Lett., Vol.28, Issue 1 (2007), pp.51-58.

(21) Nakashima, K., Murakami, T., Sawae, Y., Evaluation of wear property of PVA hydrogel as artificial cartilage and effect of protein film on wear-resistant property, Trans. JSME Ser. C, Vol.70, No.697 (2004), pp.2780-2787. (in Japanese).

(22) Nakashima, K., Sawae, Y., Murakami, T., Study on wear reduction mechanisms of artificial cartilage by synergistic protein boundary film formation, JSME Int. J. Ser C, Vol.48, No.4 (2005), pp.555-561.

(23) Yarimitsu, S., Nakashima, K., Sawae, Y., Murakami, T., Study on the mechanisms of wear reduction of artificial cartilage through in situ observation on forming protein boundary film, Tribology Online, Vol.2, No.4 (2007), pp.114-119.

(24) Yarimitsu, S., Nakashima, K., Sawae, Y. and Murakami, T., Influences of synovia constituents on frictional behavior of artificial cartilage material and formation of boundary lubricating film, J. Jpn. Soc. Tribol., Vol.55, No.7 (2010), pp.489-498. (in Japanese)

(25) Rabinowitz, J. L., Gregg, J. R., Nixon, J. E., Lipid composition of the tissues of human knee joints. II. Synovial fluid in trauma, Clin. Orthop. Relat. Res., Vol. 190 (1984), pp.292-298.

(26) Mazzucco, D., Scott, R., Spector M., Composition of joint fluid in patients undergoing total knee replacement and revision arthroplasty:correlation with flow properties, Biomaterials, Vol.25, Issue 18 (2004), pp. 4433-4445.

(27) Delecrin, J., Oka, M., Takahashi, A., Yamamuro, T., Nakamura T., Changes in joint fluid after total arthroplasty, Clin. Orthop. Relat. Res., Vol.307 (1994), pp240-249.

(28) Hills, B.A., Oligolamellar lubrication of joints by surface active phospholipids, J. Rheum., vol.16, No.1 (1989), pp.82-91.

(29) Pawlak, Z., Pao, R., Bayraktar, E., Kaldonski, T., Oloyede, A., Lamellar lubrication in vivo and vitro: Friction testing of hexagonal boron nitride, Biosystems, Vol.94, Iss.3 (2008), pp.202-208.

(30) Hernandez-Caselles, T., Villalain, J., Gomez- Fernandez, J.C., Influence of liposome charge and composition on their interaction with human blood serum proteins, Mol. Cell. Biochem., vol.120, No.2 (1993), pp.119-126.

(31) Dimitrova, M. N., Matsumura, H., Protein-induced leakage and membrane destabilization of phosphatidylcholine and phpsphatidylserine liposomes, Colloids and Surfaces B: Biointerfaces, Vol. 8 (1997), pp. 287-294.

(32) Sakai, N., Hagihara, Y., Furusawa, T., Hosoda, N., Sawae, Y., Murakami, T., Analysis of biphasic lubrication of articular cartilage loaded by cylindrical indenter, Tribol Intern., Vol.46, Iss.1 (2012), pp.225-236.

(33) Murakami, T., Yarimitsu, S., Nakashima, K., Sawae, Y., Sakai, N., Adaptive multimode lubrication mechanism in articular cartilage and its application to artificial cartilage, Proc. International Tribology Conference, Hiroshima 2011, USB memory (2011), G4-02.

JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 31 (2014) 100–106

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Research Paper

Grafting of poly(2-methacryloyloxyethyl phosphorylcholine) on polyethylene liner in artificial hip joints reduces production of wear particles

Toru Moro[a,b,*], *Masayuki Kyomoto*[a,c,d], *Kazuhiko Ishihara*[c], *Kenichi Saiga*[a,c,d], *Masami Hashimoto*[e], *Sakae Tanaka*[b], *Hideya Ito*[b], *Takeyuki Tanaka*[b], *Hirofumi Oshima*[b], *Hiroshi Kawaguchi*[b], *Yoshio Takatori*[a,b]

[a]*Division of Science for Joint Reconstruction, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*

[b]*Sensory & Motor System Medicine, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*

[c]*Department of Materials Engineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*

[d]*Research Department, KYOCERA Medical Corporation, 3-3-31 Miyahara, Yodogawa-ku, Osaka 532-0003, Japan*

[e]*Materials Research and Development Laboratory, Japan Fine Ceramics Center, 2-4-1 Mutsuno, Atsuta-ku, Nagoya 456-8587, Japan*

ARTICLE INFO

Article history:
Received 20 April 2012
Received in revised form
7 March 2013
Accepted 18 March 2013
Available online 17 April 2013

Keywords:
Artificial hip joint
Total hip arthroplasty
Periprosthetic osteolysis
Aseptic loosening
Polyethylene
Surface modification

ABSTRACT

Despite improvements in the techniques, materials, and fixation of total hip arthroplasty, periprosthetic osteolysis, a complication that arises from this clinical procedure and causes aseptic loosening, is considered to be a major clinical problem associated with total hip arthroplasty. With the objective of reducing the production of wear particles and eliminating periprosthetic osteolysis, we prepared a novel hip polyethylene (PE) liner whose surface graft was made of a biocompatible phospholipid polymer—poly(2-methacryloyloxyethyl phosphorylcholine (MPC)). This study investigated the wear resistance of the poly(MPC)-grafted cross-linked PE (CLPE; MPC-CLPE) liner during 15×10^6 cycles of loading in a hip joint simulator. The gravimetric analysis showed that the wear of the acetabular liner was dramatically suppressed in the MPC-CLPE liner, as compared to that in the non-treated CLPE liner. Analyses of the MPC-CLPE liner surface revealed that it suffered from no or very little wear even after the simulator test, whereas the CLPE liners suffered from substantial wears. The scanning electron microscope (SEM) analysis of the wear particles isolated from the lubricants showed that poly(MPC) grafting dramatically decreased the total number, area, and volume of the wear particles. However, there was no significant difference in the particle size distributions, and, in particular, from the SEM image, it was observed that particles with diameters less than 0.50 μm were present in the range of the highest frequency. In addition, there were no significant differences in the particle size descriptors and particle shape descriptors.

---

[*] Corresponding author at: Division of Science for Joint Reconstruction, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: +81 3 3815 5411x30474; fax: +81 3 3818 4082.
E-mail address: moro-ort@h.u-tokyo.ac.jp (T. Moro).

http://dx.doi.org/10.1016/j.jmbbm.2013.03.011

The results obtained in this study show that poly(MPC) grafting markedly reduces the production of wear particles from CLPE liners, without affecting the size of the particles. These results suggest that poly(MPC) grafting is a promising technique for increasing the longevity of artificial hip joints.

1. Introduction

Sir John Charnley introduced the use of polyethylene (PE) components in total hip arthroplasty (THA) in the 1960s, and since then, these components have been extensively used for 50 years (Charnley, 1961). However, aseptic loosening resulting from periprosthetic osteolysis—which is a clinical complication arising from THA—is the prevalent cause of revision surgery (Bozic et al., 2009). Previous studies have revealed that PE particles generated from liners play a major etiological role in periprosthetic osteolysis. Macrophage phagocytosis of the PE particles is followed by the secretion of prostaglandin E2 (PGE2) and cytokines, which induce the receptor activator of the NF-κB ligand (RANKL) expression, consequently resulting in osteoclastogenesis and bone resorption (Harris, 2004; Jacobs et al., 2001). Further, periprosthetic osteolysis is closely related to the rate of PE wear and the characteristics of the wear particles (Catelas and Jacobs, 2010). Hence, various attempts have been made to improve the wear resistance of PE liners, such as enhancing the cross-linking of PE (CLPE) (Callaghan et al., 2008).

In the previous studies, we introduced a nanometer-scaled poly(2-methacryloyloxyethyl phosphorylcholine (MPC)) grafting layer on the surface of CLPE liners. We found that such type of grafting dramatically decreased the wear of the liner surface (Moro et al., 2009, 2006). In the present study, we investigated the effect of poly(MPC) grafting on the production of wear particles, using a hip wear simulator up to 15×10^6 cycles.

2. Materials and methods

2.1. *Poly(MPC) grafting*

Nanometer-scaled grafting (100–150 nm in thickness) of the poly (MPC) onto the PE liner surface was carried out by a photoinduced polymerization technique. The CLPE liners (K-MAX® CLQC; KYOCERA Medical Corp., Osaka, Japan) were immersed in an acetone solution containing 10 mg/mL of benzophenone for 30 s and then dried at room temperature to remove the acetone. Then, MPC (NOF Corp., Tokyo, Japan) (Ishihara et al., 1990) was dissolved in degassed pure water to obtain a 0.50 mol/L MPC aqueous solution, and the benzophenone-coated CLPE liners were immersed in this solution. Photoinduced graft polymerization was carried out on the CLPE liner surface using ultraviolet irradiation (UVL-400HA ultra-high-pressure mercury lamp; Riko-Kagaku Sangyo Co., Ltd., Funabashi, Japan) with an intensity of 5.0 mW/cm^2 at 60 °C for 90 min; subsequently, a filter (Model D-35; Toshiba Corp., Tokyo, Japan) was used to restrict the passage of ultraviolet light to wavelengths of 350 ± 50 nm. After the poly (MPC)-grafted CLPE (MPC-CLPE) liners were polymerized, they were washed with pure water and ethanol and dried at room temperature. These specimens were then sterilized by 25-kGy gamma rays under N_2 gas (Kyomoto et al., 2008).

2.2. *Hip joint simulator*

A 12-station hip simulator (MTS Systems Corp., Eden Prairie, MN) with CLPE and MPC-CLPE liners, each with inner and outer diameters of 26 and 52 mm, respectively, was used for the hip simulator wear test performed according to the ISO Standard 14242-3. A Co–Cr alloy femoral head with a diameter of 26 mm (K-MAX® HH-02; KYOCERA Medical Corp.) was used as the femoral component. A biaxial rocking motion was applied to the head/cup interface via an offset bearing assembly with an inclined angle of +23°. Both the loading and motion were synchronized at 1 Hz. According to the double-peaked Paul-type physiologic hip load, the applied peak loads were 1793 and 2744 N (Paul, 1967). Bovine calf serum (25 vol%) diluted in distilled water was used as a lubricant. Sodium azide (10 mg/L) and EDTA (20 mM) were added to prevent microbial contamination and to minimize the formation of calcium phosphate on the implant surface.

The simulator was run up to 15×10^6 cycles. The liners were cleaned and weighed on a microbalance (Sartorius Genius ME215S, Sartorius AG, Goettingen, Germany) at intervals of 0.5×10^6 cycles. The lubricant was collected and stored at −20 °C for further analysis. Wear was determined from the weight loss of each liner and corrected by cyclically loaded soak controls according to the ISO Standard 14242-2. The wear rates were determined by linear regression.

After complete loading, morphological changes in the liner surface were measured using a three-dimensional (3D) coordinate measuring machine (BHN-305, Mitsutoyo Corp., Kawasaki, Japan) and reconstructed using 3D modeling software (Imageware, Siemens PLM Software Inc., TX, USA). The liner surface was analyzed using a confocal scanning laser microscope (OLS1200, Olympus, Tokyo, Japan), as previously reported.

The wear particles were isolated from the bovine serum solution. For isolating the wear particles from the lubricant, the lubricant was incubated with 5.0 mol/L of NaOH solution for 3 h at 65 °C after it was tested, in order to digest adhesive proteins that were degraded and precipitated. To avoid artifacts, contaminating proteins were removed by extraction with sugar solution (1.20 g/cm^3 and 1.05 g/cm^3) and isopropyl alcohol solutions (0.98 and 0.90 g/cm^3). After the lubricant was centrifuged at 25,500 rpm for 3 h at 5 °C, the particles were collected, subjected to sequential filtrations (minimum pore size of 0.1 μm) (Fisher et al., 2004; Tipper et al., 2006), and subsequently dried. The filter was then sputter coated with gold palladium and digitally imaged on a field emission scanning electron microscope (JSM-633OF, JEOL Datum Co., Ltd, Tokyo, Japan). An image-processing program (Scion image, Scion Corp., Frederick, MD) based on the

NIH image software was used to measure the total number, area, and volume of the wear particles per 10^6 cycles (Campbell et al., 1996; Dean et al., 1999). Two size descriptors, namely, the equivalent circle diameter (ECD) and the diameter (D), and two shape descriptors, namely, the aspect ratio (AR) and roundness (R), were used to define each wear particle, according to ASTM F1877-98. Each parameter is defined as follows. ECD is defined as the diameter of a circle with an area that is equivalent to that of one wear particle. Diameter is defined using the maximum dimensions determined by the SEM analysis. Aspect ratio is defined as the ratio of the major diameter to the minor diameter. It should be noted that the major diameter is the longest straight line that can be drawn between any two points on the outline. On the other hand, the minor diameter is the longest line that is perpendicular to the major diameter. Roundness is a measure of how closely a wear particle resembles a circle; its values range from 0 to 1, with a perfect circle having a roundness value of 1.

2.3. *Statistical analysis*

The significance of differences was determined by the student's *t*-test. All statistical analyses were performed using add-in software (Statcel 2; OMS publishing Inc, Tokorozawa, Japan) on a computerized worksheet (Microsoft Excel® 2003; Microsoft Corp, Redmond, WA).

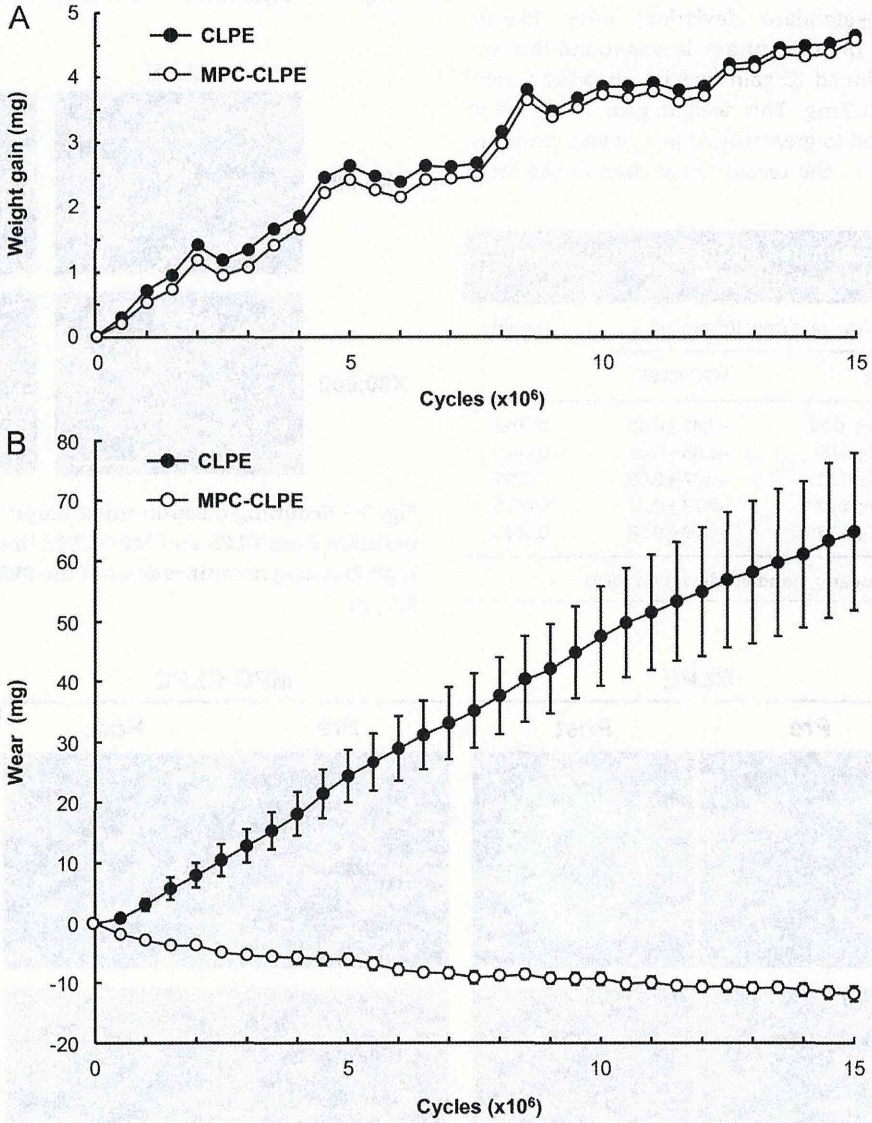

Fig. 1 – Wear amounts of cross-linked PE liners with or without MPC grafting in the THA simulator. (A) Load-soak controls. Fluid absorption of the liners that were axially loaded cyclically to the acetabular liners with the same pressure as the THA simulator, but without rotational motion. Data are expressed as means (symbols) for 2 inserts/group. (B) Time course of wear amount in the THA simulator during 15×10^6 cycles of rotational motion and axial loading against Co–Cr alloy femoral heads. The wear amount was estimated from the weight loss of the inserts after correction by the average weight gain in the respective load-soak controls (weight loss in the THA simulator+average of weight gain in the load-soak control). Data are expressed as means (symbols)±standard deviation (SD) for 4 liners/group.