Table 1
Mechanical properties of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE).

Properties	Untreated CLPE (PE)	PMPC-grafted CLPE (PMPC-PE)	PMPC-grafted HD-CLPE(VE) (PMPC-VE)	Statistical analysis groups		
				PE vs PMPC-PE	PE vs PMPC-VE	PMPC-PE vs PMPC-VE
Yield strength (MPa)	22.6 (0.3)[a]	23.0 (0.3)	24.6 (0.2)	<0.05	<0.01	<0.01
Ultimate strength (MPa)	54.4 (3.8)	52.8 (2.1)	55.3 (3.9)	N.S.[b]	N.S.	N.S.
Elongation (%)	346.0 (18.1)	351.6 (35.6)	317.0 (16.2)	N.S.	<0.05	<0.05
Impact strength (kJ/m^2)	101.4 (5.6)	100.5 (0.8)	93.0 (1.1)	N.S.	<0.01	<0.01
Hardness (shore D)	66.8 (0.4)	66.8 (0.3)	66.6 (0.8)	N.S.	N.S.	N.S.
Creep deformation (%)	0.73 (0.07)	0.72 (0.06)	0.68 (0.06)	N.S.	N.S.	N.S.

[a] The standard deviations are shown in parentheses.
[b] N.S. indicates no statistical difference.

The vitamin E blending was found to affect the oxidation stability of CLPE by scavenging free radicals. As shown in Fig. 6A–B, all CLPE samples before gamma-ray sterilization contained slightly detectable free radicals that had not been completely eliminated. Multiple peaks corresponding to the alkyl or allyl radicals were observed in all samples after gamma-ray sterilization (Fig. 6C) [27]. The small detectable peaks corresponding to the tocopheroxyl radical (α-TO•) were observed only in the PMPC-grafted HD-CLPE(VE) samples. The residual free-radical concentration after gamma-ray sterilization did not differ significantly between all groups examined in this study (Fig. 6D). Interestingly, the oxidative-induction time of the PMPC-grafted HD-CLPE(VE) samples with residual free radicals was much longer than that of the untreated CLPE and PMPC-grafted CLPE samples (Fig. 7A). As a consequence, the oxidation index of the PMPC-grafted HD-CLPE(VE) sample that was subjected to accelerated aging was much lower (almost zero) than that of the untreated CLPE and PMPC-grafted CLPE samples (Fig. 7B). There was no significant difference in both oxidative-induction time and oxidation index between the untreated CLPE and PMPC-grafted CLPE samples, regardless of residual free-radical concentration.

4. Discussion

In this study, we demonstrated the fabrication of a highly hydrophilic and lubricious nanometer-scale modified surface by PMPC grafting onto the surface of an antioxidative CLPE substrate with vitamin E blending; we also investigated the effects of PMPC grafting and vitamin E blending on the wear resistance, oxidative stability, and mechanical properties of liners for artificial hip joints. The results provide preliminary evidence that the surface and substrate modifications affected the extent of the wear resistance, oxidative stability, and the underlying mechanical properties of the acetabular liner. This suggests that the PMPC-grafted surface and vitamin E-blended substrate may be a promising approach to extending the longevity of THA artificial joints.

To ensure the *in vivo* long-term wear resistance and oxidative stability of the acetabular liners, we used the photoinduced-radical graft polymerization technique to create strong bonding between the CLPE substrate and the PMPC graft chain [10,19], and we used antioxidant vitamin E blending to scavenge the residual free radicals. It has been known that the presence of vitamin E in the PE substrate, which prevents oxidation degradation, reduces the cross-linking efficiency because of the establishment of competition between the vitamin E itself and the alkyl or allyl radicals in the reaction with the radicals produced by gamma-ray irradiation [24]. In general, increased cross-linking in the CLPE with an increase in gamma-ray irradiation dose degrades its mechanical properties, producing a trade-off between wear resistance and mechanical properties. It is desirable to reduce wear while maintaining the mechanical properties necessary for proper *in vivo* function. Therefore, we optimized the gamma-ray irradiation dose for sufficiently effective cross-links to simultaneously obtain wear resistance and mechanical properties; the

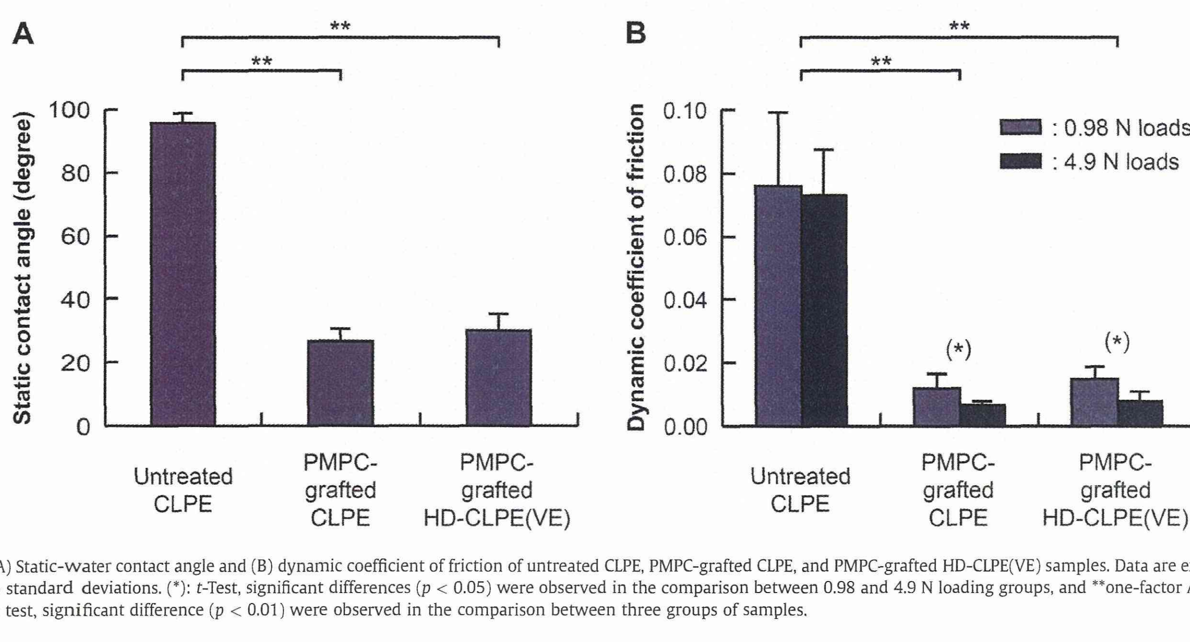

Fig. 3. (A) Static-water contact angle and (B) dynamic coefficient of friction of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) samples. Data are expressed as means ± standard deviations. (*): *t*-Test, significant differences ($p < 0.05$) were observed in the comparison between 0.98 and 4.9 N loading groups, and **one-factor ANOVA and post-hoc test, significant difference ($p < 0.01$) were observed in the comparison between three groups of samples.

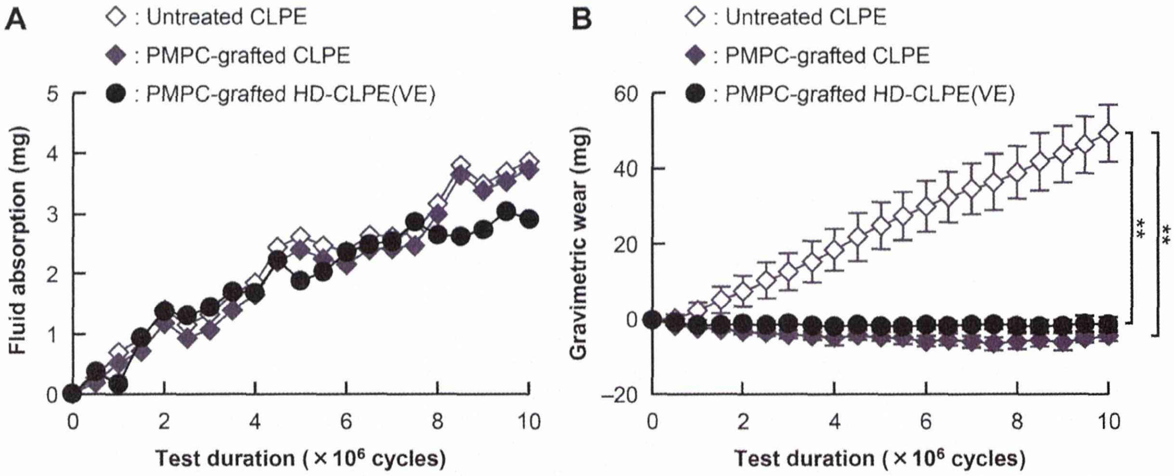

Fig. 4. Time course of (A) fluid absorption and (B) gravimetric wear of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) liners during the hip-simulator wear test. Data are expressed as means ± standard deviations. **One-factor ANOVA and post-hoc test, significant differences ($p < 0.01$) were observed in the comparison of gravimetric wear after the test for all three groups of liners.

HD-CLPE(VE) was irradiated with a high-dose of 100-kGy gamma-rays in this study.

At the same time, there was concern that the presence of vitamin E on the CLPE surface reduced the PMPC-grafting efficiency because of the establishment of competition between the vitamin E and the semi-benzopinacol radicals in the benzophenone as a photoinitiator, in the reaction with photoinduced radicals. In Figs. 1 and 2, the results indicate that PMPC was successfully grafted on the CLPE and HD-CLPE(VE) surfaces independent of the presence of vitamin E [8,17,19,20]. The photoinduced-radical graft polymerization proceeded only on the surfaces of the CLPE and HD-CLPE(VE) substrates [16]. Therefore, surface cleanliness without fouling that involved an antioxidant was critical for the polymerization. The HD-CLPE(VE) of this study was fully washed with aqueous polysorbate-surfactant solutions and ethanol to completely remove vitamin E from the surface.

The bearing surfaces of a natural synovial joint are covered with a specialized type of hyaline cartilage, called articular cartilage, which protects the joint interface from mechanical wear and facilitates smooth motion of the joint during daily activities [20,28]. Articular cartilage consists of chondrocytes surrounded by extracellular matrix macromolecules (e.g., proteoglycans, glycosaminoglycans, and collagens) and surface active phospholipids (e.g., phosphatidylcholine derivatives). Owing to the charge on these molecules, they can trap water to maintain the water–fluid and electrolyte balance within the articular cartilage tissue, making it highly hydrophilic and providing an effective boundary lubricant [20,29]. The fluid thin-film lubrication achieved by the presence of

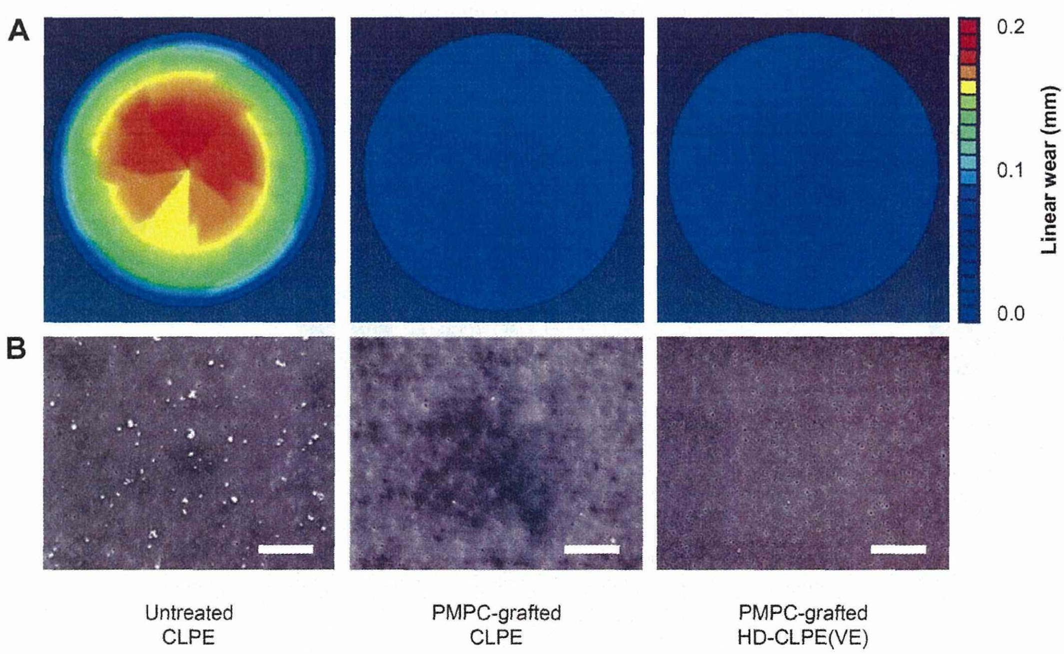

Fig. 5. Analyses of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) liners after the hip-simulator wear test. (A) Three-dimensional coordinate measurements of various liners and (B) SEM images of wear particles isolated from lubricants of the hip-simulator wear test. The scale bar corresponds to 5 μm.

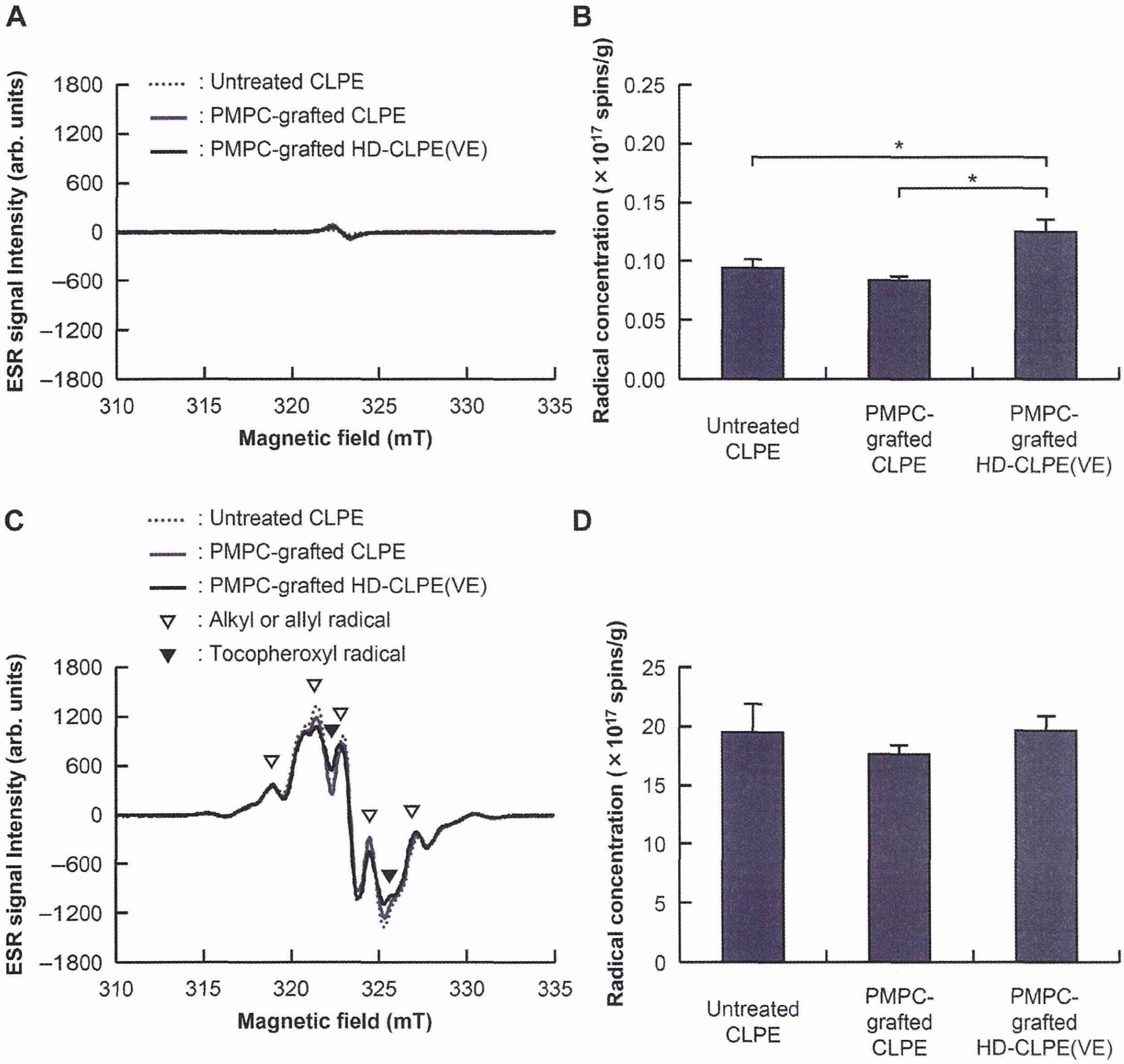

Fig. 6. Analyses of residual free radical of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) samples. (A) ESR spectra and (B) residual free radical concentration before gamma-ray sterilization. (C) ESR spectra and (D) residual free radical concentration after gamma-ray sterilization. Data are expressed as means ± standard deviations. *One-factor ANOVA and post-hoc test, significant differences ($p < 0.05$) were observed in the comparison between all three groups of samples.

this hydrated layer is essential for the smooth motion of natural synovial joints.

The water wettabilities of the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) surfaces were found to be considerably greater than that of the untreated CLPE surfaces (Fig. 3A). This is caused by the presence of a nanometer-scale grafted PMPC layer that resulted from the polymerization of the highly hydrophilic MPC monomer. The fabrication of the PMPC gel layer clearly influenced the friction response; it can be observed in Fig. 3B that the dynamic coefficients of friction of the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) surfaces were significantly lower than those of the untreated CLPE surface. This is attributed to the significant increase in hydrophilicity evident from the reduction in the static-water contact angles on the PMPC-grafted surfaces [17]. Additionally, the improvement in the dynamic coefficients of friction of the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) surfaces in the case of 4.9 N loading compared with those in the case of 0.98 N loading is very interesting. These results show that the PMPC-grafted layer did not follow Amonton's law of $F = \mu N$. The contact stress of approximately 49 MPa at a load of 4.9 N, which was roughly calculated by the Hertzian theory, is higher than the tensile yield strength of the untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) (approximately 23–25 MPa in Table 1). The elastic CLPE and HD-CLPE(VE) substrates were slightly deformed by the loads, and the low friction coefficients may have been necessary to build a wider thin film of water over the larger contact area of the concave surfaces [19]. These results may also imply that the lubrication of PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) was dominated by the hydration-lubrication mechanism [30]. Fortunately, the PMPC-grafted CLPE showed an extremely low and stable dynamic coefficient of friction in the case of large loads. The surface modification layer obtained by PMPC grafting was combined with the HD-CLPE(VE) substrate by strong C—C covalent bonding. Therefore, the obtained results support the motivation regarding PMPC grafting on the HD-CLPE(VE) surface. Additionally, it is thought that a sufficient number of strong bonds

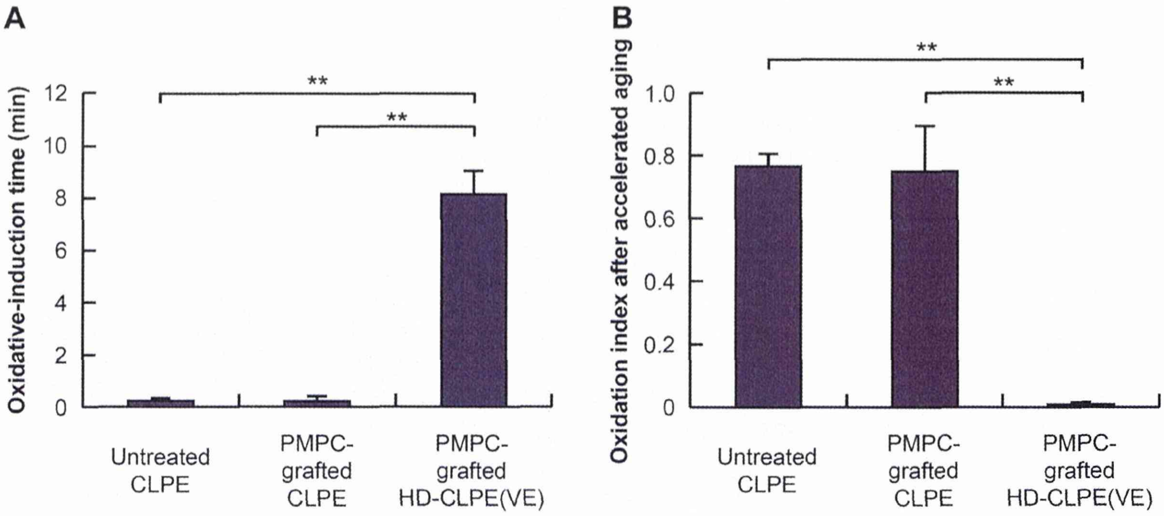

Fig. 7. Analyses of oxidative stability of untreated CLPE, PMPC-grafted CLPE, and PMPC-grafted HD-CLPE(VE) samples. (A) Oxidative-induction time and (B) oxidation index after accelerated aging. Data are expressed as means ± standard deviations. **One-factor ANOVA and post-hoc test, significant differences ($p < 0.01$) were observed in the comparison between all three groups of samples.

between the surface modification layer and the HD-CLPE(VE) surface are essential for the long-term retention of benefits of the grafted PMPC layer in artificial joints under variable and multidirectional loads [10].

In the hip-simulator wear test of the present study, the significant improvements observed in the water wettabilities and frictional properties of the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) liners resulted in substantial improvements in their wear resistances (Fig. 4B). The high friction of the untreated CLPE surfaces is one of their main disadvantages because it results in greater wear and possible seizure of bearing couples. The higher frictional properties of untreated CLPE surfaces were found to affect the wear properties, as determined by the hip-simulator wear test. In contrast, as noted earlier, the water wettabilities of the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) surfaces were considerably greater. Fluid-film lubrication (or hydration lubrication) of the PMPC-grafted surface was therefore provided by the hydrated layer. The obtained results confirm that orthopedic bearings using PMPC are able to mimic the natural articular cartilage that protects the joint interface from mechanical wear and facilitates smooth movement of the joints during daily activities [28,31]. The PMPC structure attracts water in a way that is similar to that of the extracellular matrix molecules present in cartilage, providing a lubricating layer on the surface to which they are attached [29]. The hydrophilic PMPC layer grafted onto the CLPE surface significantly increased lubrication to levels that match articular cartilage [32,33]. By mimicking the properties of the extracellular matrix of cartilage, the high wear resistance of the native tissue could be replicated by the use of an artificial polymer. Learning from and mimicking natural structures and systems have been shown to be a highly successful and advantageous approach to producing artificial tissues and implants. In particular, this study has demonstrated that investigating and subsequently mimicking natural structures and systems; the strategy of investigating and then reproducing the natural bearing surfaces in artificial joints to mimic the role of cartilage has great potential.

The production of wear particles in THA is recognized as the main factor behind the initiation of periprosthetic osteolysis and aseptic loosening [2,3]. The inflammatory cellular response to particles is thought to be dependent upon factors such as particle number, size, shape, surface area, and material chemistry [34]. If nanometer-scale particles are produced *in vivo*, it would be important to determine their biological activity relative to that of the micrometer-scale particles. In the wear particle analysis carried out in the present study, the wear particles collected from the PMPC-grafted CLPE and PMPC-grafted HD-CLPE(VE) liners were on the scale of sub-micrometers, regardless of PMPC grafting and vitamin E blending (Fig. 5B). Based on these results, we expect the *in vivo* biological response of the PMPC-grafted CLPE liners to be comparable with that of conventional, untreated CLPE [5]. Compared to particles generated by the lubricants used for the untreated liners, the remarkably smaller number of wear particles isolated from the lubricants used for the PMPC-grafted liners may help predict whether abnormal wear would occur. Moreover, several previous studies reported that vitamin E and wear particles containing vitamin E can possibly reduce the inflammatory cellular responses [35]. However, owing to the relatively short history of these materials, continuous attention must be paid to the sub-micrometer-size and number of abnormal wear particles in the PMPC-grafted CLPE and HD-CLPE(VE) liners, respectively.

Oxidation is primarily caused by the residual free radicals that are trapped in the crystalline phase of PE after gamma-ray irradiation. Therefore, post-irradiation thermal treatment has been used to reduce or eliminate those residual free radicals. One thermal-treatment process is annealing at a temperature below the melting point of PE (approximately 138 °C). In this process, the crystalline phase is partially melted, and the free radicals are reduced but not completely eliminated. As shown in Fig. 6A–B, all CLPE samples obtained in this study with annealing at 120 °C in N_2 gas contained slightly detectable free radicals. Another thermal-treatment process is melting at a temperature above 150 °C. Using this process, the number of residual free radicals is reduced until they are completely eliminated. On the other hand, the crystallinity is reduced after post-irradiation melting owing to the hindrance created by the newly formed cross-linking; the resulting mechanical properties including fatigue are reduced [36]. The retention of the bulk properties of the substrates is extremely important in clinical applications because the biomaterials used as implants act not only as surface-functional materials, but also as structural materials *in vivo*. For example, dislocation is the biggest short-term problem associated with THA [3]. A thin acetabular liner against a large femoral head not only allows for an increased head/neck ratio, which is directly related to the range of motion prior to impingement of the trunnion on the liner, but also increases the jump distance. Hence, the use of implants with such dimensions is becoming more common in order to improve the stability of the bearing surface. Mechanical fracture attributed to scission of the PE molecular backbone owing to oxidation degradation in thin acetabular liners by the possible impingements must therefore be monitored. Several previous studies reported that the mechanical fracture was caused by neck impingements in the CLPE liners that were thermally treated via melting [37]. Among the data gathered to date for over 12,000 clinical applications of PMPC-grafted CLPE liner (Aquala® liner; KYOCERA Medical Corporation) that were thermally treated via annealing, we have observed neither mechanical fracture nor complication during follow-up assessment periods that spanned a minimum of 5 years and a maximum of 7 years. Therefore, we think that annealing as the post-irradiation thermal treatment has an advantage from the view point of mechanical properties. In reality, as shown in Table 1, all CLPE samples in this study maintained high level of mechanical properties as *in vivo* structural materials. Furthermore, the mechanical properties of the PMPC-grafted HD-CLPE(VE) samples remained almost unchanged even after vitamin E blending and PMPC grafting. This indicates that the diffusion of vitamin E during blending proceeded only in the amorphous phase of PE, primarily around the PE grain boundary [38], and photoinduced-radical graft polymerization occurred only on the surface of the substrates, whereas the properties of the substrates remained unchanged.

Moreover, it was recently reported that *in vivo* oxidation occurred, not only for the CLPE liner obtained after annealing, but also for the CLPE liner that was treated via melting [22]. In fact, the clinical impact of this oxidation degradation remains unclear. Although its clinical significance is still the subject of scientific debate, *in vivo* oxidation is regarded as undesirable. It is thought that the stabilization of the residual free radicals with an antioxidant such as vitamin E is necessary as an additional or alternative process. In Figs. 6 and 7, the PMPC-grafted HD-CLPE(VE) samples exhibited extremely high oxidative stability even though the amount of residual free radicals was at a detectable level. Despite the high-dose gamma-ray irradiation for cross-linking and further UV irradiation for PMPC grafting, the CLPE substrate modified by vitamin E blending maintained high resistance to oxidation. Indeed, vitamin E is an extremely efficient radical scavenger. The PMPC-grafted HD-CLPE(VE) samples contained 0.1 mass% of vitamin E; it was thought that the concentration of vitamin E was sufficient to obtain high oxidative stability even after cross-linking and PMPC grafting.

Despite these promising results, our study has a number of limitations. First, *in vitro* findings do not always translate to clinical success. We conducted clinical trials of PMPC-grafted CLPE liners at multiple medical centers between 2007 and 2009 in Japan [9]. Based on other related evidence and these clinical trials, the Japanese government (Ministry of Health, Labor, and Welfare, Japan) approved the clinical use of PMPC-grafted CLPE acetabular liners in artificial hip joints in April 2011. We observed neither osteolysis nor a need for revision surgery during follow-up periods of up to 7 years for the clinical trials. Second, we did not completely capture the range of loading and motion conditions of the *in vivo* environment in terms of the variety of positions during the hip-simulator wear test, the magnitude of loading, or the subjects' daily routine; however, in accordance with ISO 14242-3, we believe that these results can provide a good indication of wear performance. Third, as previously reported [34], the procedure for the isolation of wear particles in this study could not capture wear particles with a diameter below 0.1 μm. The cellular response to particles is thought to be dependent upon factors such as particle number, size, shape, surface area, and material chemistry. If nanometer-scale particles are generated *in vivo*, it will be important to determine their biological activity in relation to that of micrometer-scaled particles. Fourth, the wear performance we report is only valid for this specific combination of Co–Cr–Mo alloy femoral head with a diameter of 26 mm and PMPC-grafted HD-CLPE(VE) liner. Although aseptic loosening is one of the most common reasons for late-term revision surgery, dislocation is the biggest short-term problem [3]. A large femoral head not only allows for an increased head/neck ratio, which is directly related to the range of motion prior to impingement of the trunnion on the liner, but also increases the jump distance. Hence, larger femoral heads have recently come into more frequent use to improve the stability of the bearing surface. We believe that this drawback is partially offset by the long duration of the simulation. We are now running the hip-simulator wear tests using larger (i.e., 32–44 mm) Co–Cr–Mo alloy and zirconia-toughened alumina ceramic femoral heads and thin acetabular liners.

5. Conclusions

We have demonstrated that the PMPC grafting layer was successfully fabricated on the surface of an antioxidative vitamin E-blended CLPE substrate. The PMPC-grafted HD-CLPE(VE) provided high wear resistance, oxidative stability, and mechanical properties simultaneously. Since MPC is a highly hydrophilic compound, the water wettability and lubricity of the PMPC-grafted CLPE and HD-CLPE(VE) surfaces were greater than those of the untreated CLPE surface because of the formation of a PMPC grafting layer and its hydration, which can serve as an extremely efficient lubricant. It was also observed that the PMPC grafting significantly contributed to wear reduction. Despite the high-dose gamma-ray irradiation for cross-linking and further UV irradiation for PMPC grafting, the substrate modified by vitamin E blending maintained high oxidative stability because vitamin E is an extremely efficient radical scavenger. Furthermore, the results clearly showed that the mechanical properties of the substrate were minimally changed, if at all, even after PMPC grafting or vitamin E blending, or both PMPC grafting and vitamin E blending.

Acknowledgments

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References

[1] Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. J Bone Joint Surg Am 2005;87:1487–97.
[2] Harris WH. The problem is osteolysis. Clin Orthop Relat Res 1995;311:46–53.
[3] Bozic KJ, Kurtz SM, Lau E, Ong K, Vail TP, Berry DJ. The epidemiology of revision total hip arthroplasty in the United States. J Bone Joint Surg Am 2009;91(1):128–33.
[4] Jacobs JJ, Roebuck KA, Archibeck M, Hallab NJ, Glant TT. Osteolysis: basic science. Clin Orthop Relat Res 2001;393:71–7.
[5] Moro T, Takatori Y, Ishihara K, Konno T, Takigawa Y, Matsushita T, et al. Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis. Nat Mater 2004;3:829–37.

[6] Moro T, Takatori Y, Ishihara K, Nakamura K, Kawaguchi H. 2006 Frank Stinchfield Award: grafting of biocompatible polymer for longevity of artificial hip joints. Clin Orthop Relat Res 2006;453:58–63.
[7] Moro T, Kawaguchi H, Ishihara K, Kyomoto M, Karita T, Ito H, et al. Wear resistance of artificial hip joints with poly(2-methacryloyloxyethyl phosphorylcholine) grafted polyethylene: comparisons with the effect of polyethylene cross-linking and ceramic femoral heads. Biomaterials 2009;30(16): 2995–3001.
[8] Kyomoto M, Moro T, Takatori Y, Kawaguchi H, Ishihara K. Cartilage-mimicking, high-density brush structure improves wear resistance of crosslinked polyethylene: a pilot study. Clin Orthop Relat Res 2011;469(8): 2327–36.
[9] Takatori Y, Moro T, Kamogawa M, Oda H, Morimoto S, Umeyama T, et al. Poly(2-methacryloyloxyethyl phosphorylcholine)-grafted highly cross-linked polyethylene liner in primary total hip replacement: one-year results of a prospective cohort study. J Artif Organs 2013;16(2):170–5.
[10] Moro T, Takatori Y, Kyomoto M, Ishihara K, Hashimoto M, Ito H, et al. Long-term hip simulator testing of the artificial hip joint bearing surface grafted with biocompatible phospholipid polymer. J Orthop Res 2014;32(3):369–76.
[11] Iwasaki Y, Ishihara K. Cell membrane-inspired phospholipid polymers for developing medical devices with excellent biointerfaces. Sci Technol Adv Mater 2012;13:064101 [p. 14].
[12] Kuiper KK, Nordrehaug JE. Early mobilization after protamine reversal of heparin following implantation of phosphorylcholine-coated stents in totally occluded coronary arteries. Am J Cardiol 2000;85:698–702.
[13] Selan L, Palma S, Scoarughi GL, Papa R, Veeh R, Di Clemente D, et al. Phosphorylcholine impairs susceptibility to biofilm formation of hydrogel contact lenses. Am J Ophthalmol 2009;147:134–9.
[14] Snyder TA, Tsukui H, Kihara S, Akimoto T, Litwak KN, Kameneva MV, et al. Preclinical biocompatibility assessment of the EVAHEART ventricular assist device: coating comparison and platelet activation. J Biomed Mater Res A 2007;81(1):85–92.
[15] Kyomoto M, Moro T, Konno T, Takadama H, Yamawaki N, Kawaguchi H, et al. Enhanced wear resistance of modified cross-linked polyethylene by grafting with poly(2-methacryloyloxyethyl phosphorylcholine). J Biomed Mater Res A 2007;82(1):10–7.
[16] Kyomoto M, Moro T, Konno T, Takadama H, Kawaguchi H, Takatori Y, et al. Effects of photo-induced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on physical properties of cross-linked polyethylene in artificial hip joints. J Mater Sci Mater Med 2007;18:1809–15.
[17] Kyomoto M, Moro T, Miyaji F, Hashimoto M, Kawaguchi H, Takatori Y, et al. Effect of 2-methacryloyloxyethyl phosphorylcholine concentration on photo-induced graft polymerization of polyethylene in reducing the wear of orthopaedic bearing surface. J Biomed Mater Res A 2008;86(2):439–47.
[18] Kyomoto M, Moro T, Miyaji F, Konno T, Hashimoto M, Kawaguchi H, et al. Enhanced wear resistance of orthopaedic bearing due to the cross-linking of poly(MPC) graft chains induced by gamma-ray irradiation. J Biomed Mater Res B Appl Biomater 2008;84:320–7.
[19] Kyomoto M, Moro T, Miyaji F, Hashimoto M, Kawaguchi H, Takatori Y, et al. Effects of mobility/immobility of surface modification by 2-methacryloyloxyethyl phosphorylcholine polymer on the durability of polyethylene for artificial joints. J Biomed Mater Res A 2009;90(2):362–71.
[20] Kyomoto M, Moro T, Saiga K, Hashimoto M, Ito H, Kawaguchi H, et al. Biomimetic hydration lubrication with various polyelectrolyte layers on cross-linked polyethylene orthopedic bearing materials. Biomaterials 2012;33(18): 4451–9.
[21] Currier BH, Currier JH, Mayor MB, Lyford KA, Collier JP, Van Citters DW. Evaluation of oxidation and fatigue damage of retrieved crossfire polyethylene acetabular cups. J Bone Joint Surg Am 2007;89(9):2023–9.
[22] MacDonald D, Sakona A, Ianuzzi A, Rimnac CM, Kurtz SM. Do first-generation highly crosslinked polyethylenes oxidize in vivo? Clin Orthop Relat Res 2011;469(8):2278–85.
[23] Bracco P, Oral E. Vitamin E-stabilized UHMWPE for total joint implants: a review. Clin Orthop Relat Res 2011;469(8):2286–93.
[24] Oral E, Godleski Beckos C, Malhi AS, Muratoglu OK. The effects of high dose irradiation on the cross-linking of vitamin E-blended ultrahigh molecular weight polyethylene. Biomaterials 2008;29(26):3557–60.
[25] Ishihara K, Ueda T, Nakabayashi N. Preparation of phospholipid polymers and their properties as polymer hydrogel membranes. Polym J 1990;22(5): 355–60.
[26] Kurtz SM, Muratoglu OK, Buchanan F, Currier B, Gsell R, Greer K, et al. Interlaboratory reproducibility of standard accelerated aging methods for oxidation of UHMWPE. Biomaterials 2001;22(13):1731–7.
[27] Ridley MD, Jahan MS. Effects of packaging environments on free radicals in gamma-irradiated UHMWPE resin powder blend with vitamin E. J Biomed Mater Res A 2009;88(4):1097–103.
[28] Kirk TB, Wilson AS, Stachowiak GW. The morphology and composition of the superficial zone of mammalian articular cartilage. J Orthop Rheumatol 1993;6: 21–8.
[29] Goldberg R, Schroeder A, Silbert G, Turjeman K, Barenholz Y, Klein J. Boundary lubricants with exceptionally low friction coefficients based on 2D close-packed phosphatidylcholine liposomes. Adv Mater 2011;23:3517–21.
[30] Ishikawa Y, Hiratsuka K, Sasada T. Role of water in the lubrication of hydrogel. Wear 2006;261:500–4.
[31] Bhosale AM, Richardson JB. Articular cartilage: structure, injuries and review of management. Br Med Bull 2008;87:77–95.
[32] Raviv U, Glasson S, Kampf N, Gohy JF, Jérôme R, Klein J. Lubrication by charged polymers. Nature 2003;425:163–5.
[33] Chen M, Briscoe WH, Armes SP, Klein J. Lubrication at physiological pressures by polyzwitterionic brushes. Science 2009;323(5922):1698–701.
[34] Tipper JL, Galvin AL, Williams S, McEwen HM, Stone MH, Ingham E, et al. Isolation and characterization of UHMWPE wear particles down to ten nanometers in size from in vitro hip and knee joint simulators. J Biomed Mater Res A 2006;78(3):473–80.
[35] Bladen CL, Teramura S, Russell SL, Fujiwara K, Fisher J, Ingham E, et al. Analysis of wear, wear particles, and reduced inflammatory potential of vitamin E ultrahigh-molecular-weight polyethylene for use in total joint replacement. J Biomed Mater Res B Appl Biomater 2013;101(3):458–66.
[36] Oral E, Malhi AS, Muratoglu OK. Mechanisms of decrease in fatigue crack propagation resistance in irradiated and melted UHMWPE. Biomaterials 2006;27(6):917–25.
[37] Ast MP, John TK, Labbisiere A, Robador N, Valle AG. Fractures of a single design of highly cross-linked polyethylene acetabular liners: an analysis of voluntary reports to the United States Food and Drug Administration. J Arthroplasty; in press.
[38] Shibata N, Tomita N. The anti-oxidative properties of alpha-tocopherol in gamma-irradiated UHMWPE with respect to fatigue and oxidation resistance. Biomaterials 2005;26(29):5755–62.

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Effect of UV-irradiation intensity on graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on orthopedic bearing substrate

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Abstract: Photoinduced grafting of 2-methacryloyloxyethyl phosphorylcholine (MPC) onto cross-linked polyethylene (CLPE) was investigated for its ability to reduce the wear of orthopedic bearings. We investigated the effect of UV-irradiation intensity on the extent of poly(MPC) (PMPC) grafting, and found that it increased with increasing intensity up to 7.5 mW/cm^2, and the remained fairly constant. It was found to be extremely important to carefully control the UV intensity, as at higher values, a PMPC gel formed via homopolymerization of the MPC, resulting in the formation of cracks at the interface of the PMPC layer and the CLPE substrate. When the CLPE was exposed to UV-irradiation during the graft polymerization process, some of its physical and mechanical properties were slightly changed due to cross-linking and scission effects in the surface region; however, the results of all of the tests exceed the lower limits of the ASTM standards. Modification of the CLPE surface with the hydrophilic PMPC layer increased lubrication to levels that match articular cartilage. The highly hydrated thin PMPC films mimicked the native cartilage extracellular matrix that covers synovial joint surface, acting as an extremely efficient lubricant, and providing high-wear resistance. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 3012–3023, 2014.

Key Words: joint replacement, polyethylene, phosphorylcholine, graft polymerization, photoirradiation

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INTRODUCTION

Total hip arthroplasty (THA) has consistently been one of the most successful joint surgeries to date. Owing to the aging global population, the number of primary and revised THAs increases significantly year on year.[1] However, the incidence of osteolysis greatly limits the duration and clinical outcome of this type of surgery.[2,3] Osteolysis is triggered by a host inflammatory response to wear particles produced at the bearing interface of the artificial joint. A typical device consists of cross-linked polyethylene (CLPE) acetabular liner and a cobalt–chromium–molybdenum (Co–Cr–Mo) alloy femoral head, particles of which undergo phagocytosis by macrophages and induce the secretion of bone resorptive cytokines.[4,5] Efforts to reduce the number of these particles and increase the longevity of artificial hip joints have focused on a number of bearing alternatives and improvements to the currently used materials.[6–11] The use of a hard-on-hard THA, such as a metal-on-metal bearing, has been proposed to reduce the wear. However, this has raised new concerns regarding adverse local and systemic effects of metal ion release and electrochemical corrosion, which could cause serious problems such as local soft tissue reactions and pseudotumor formation.[12]

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The bearing surfaces of a natural synovial joint are covered with a specialized type of hyaline cartilage, termed articular cartilage, which protects the joint interface from mechanical wear and facilitates a smooth motion of joints during daily activity.[13,14] Articular cartilage consists of chondrocytes surrounded by extracellular matrix macromolecules (e.g., proteoglycans, glycosaminoglycans, and collagens) and surface active phospholipids (e.g., phosphatidylcholine derivatives). Owing to the charge on these molecules, they can trap water to maintain the water–fluid and electrolyte balance within the articular cartilage tissue, making it highly hydrophilic and providing an effective boundary lubricant.[14,15] The fluid thin-film lubrication achieved by the presence of this hydrated layer is essential for the smooth motion of natural synovial joints. Learning from and mimicking nature has been shown to be a highly successful approach to producing artificial tissues and implants. Therefore, the strategy of investigating and then reproducing the natural bearing surfaces in artificial joints in order to mimic the role of cartilage has great potential.

In this study, we produced nanometer-scale hydrophilic layers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) on the CLPE surface of an artificial hip joint, with the aim of reducing wear and avoiding bone resorption. Modification of the bearing surfaces of an artificial joint with a hydrophilic layer should increase lubrication to levels that match articular cartilage under physiological conditions. MPC is commonly used to synthesize highly hydrophilic and antibiofouling polymer biomaterials.[16–22] Polymers based on this structure have great potential in the fields of biomedical science and bioengineering because they possess beneficial properties such as excellent antibiofouling ability and low friction. Thus, several medical devices, including intravascular stents,[19] soft contact lenses,[20] artificial hearts,[21] and artificial hip joints,[22] have been developed from MPC polymers and subsequently clinically applied. The biomedical efficacy and safety of MPC polymers are therefore well established. In this study, the nanometer-scale surface modification was accomplished using a photoinduced (i.e., ultraviolet (UV) irradiation) radical polymerization technique[23] similar to the "grafting from" method. This approach has an advantage in that it facilitates the synthesis of both semi-dilute and high-density polymer brushes.[24] This is in contrast to photoinitiated cross-linking and scission reactions of polyolefins, which are similarly used.[25,26] When polyolefins are exposed to UV-irradiation under the radical graft polymerization processing, the effect would be a result of complicated combination of different processes.

In the present study, we investigated the effect of different intensities of UV-irradiation on the extent of photopolymerization of MPC to form a poly(MPC) (PMPC) layer on a CLPE substrate. Such investigations are of great importance in the design of life-long artificial joints, and for obtaining better understanding of their lubrication and wear mechanisms. Here, we evaluated whether UV-irradiation intensity would affect the extent of the PMPC grafting and the properties of the CLPE substrate. In addition, we assessed the potential of the PMPC-graft and/or its layer characteristics for improving the durability of artificial hip joints.

MATERIALS AND METHODS

Graft polymerization with different UV-irradiation intensities

A compression-molded polyethylene (PE; GUR1020 resin; Quadrant PHS Deutschland GmbH, Vreden, Germany) bar stock was irradiated with a 50 kGy dose of gamma rays in a N_2 gas atmosphere, and annealed at 120°C for 7.5 h in N_2 gas in order to facilitate cross-linking. The resulting CLPE specimens were then machined from this bar stock after cooling.

The CLPE specimens were immersed in acetone (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 10 mg/mL benzophenone (Wako Pure Chemical Industries) for 30 s, and then dried in the dark at room temperature in order to remove the acetone. MPC was industrially synthesized using the method reported by Ishihara et al. and supplied by NOF Corp. (Tokyo, Japan).[16] The MPC was dissolved in degassed pure water to a concentration of 0.5 mol/L. Subsequently, the benzophenone-coated CLPE specimens were immersed in the MPC aqueous solutions. Photoinduced graft polymerization was carried out on the CLPE surface using UV irradiation (UVL-400HA ultra-high pressure mercury lamp; Riko-Kagaku Sangyo, Funabashi, Japan) with an intensity of 1.5–15 mW/cm^2 at 60°C for 90 min; a filter (model D-35; Toshiba, Tokyo, Japan) was used to restrict the passage of UV light to a wavelengths of 350 ± 50 nm. After the polymerization, the PMPC-grafted CLPE specimens were removed, washed with pure water and ethanol, and dried at room temperature.

Surface analyses

The PMPC-grafted CLPE samples obtained using the range of UV-irradiation intensities were stained using an aqueous solution of 200 ppm (mass) rhodamine 6G (Wako Pure Chemical Industries) because it rapidly associates with the MPC polymer, which is structurally highly similar to lipids.[27] The PMPC-grafted CLPE samples were immersed in the rhodamine 6G solution for 30 s and then washed twice with distilled water for 30 s, and dried. All the samples were examined and imaged using fluorescence microscopy (Axioskop 2 Plus; Carl Zeiss AG, Oberkochen, Germany). Pseudo-color images were obtained using a charge-coupled device (CCD) camera (VB-7010; Keyence, Osaka, Japan) and imaging software (VH analyzer 2.51; Keyence Co.). Lenses with a $\times 10$ magnification and an appropriate exposure time ($\sim$0.1 s) were employed to obtain clear images of the samples.

The surface phosphorus concentration of the PMPC-grafted CLPE samples were analyzed using X-ray photoelectron spectroscopy (XPS) using an AXIS-HSi165 spectrometer (Kratos/Shimadzu Co., Kyoto, Japan) equipped with a 15 kV Mg-Kα radiation source at the anode. The take-off angle of the photoelectrons was maintained at 90°, and the P 2p peak was used for phosphorus quantification. Six specimens of each of the PMPC-grafted CLPE samples were prepared, and each sample was scanned five times.

Cross-sectional observations by transmission electron microscopy

Cross-sections of each of the PMPC-grafted CLPE samples were observed using transmission electron microscopy (TEM). The specimens were embedded in epoxy resin, stained with ruthenium oxide vapor at room temperature, and finally sliced into ultra-thin films (approximately 100 nm thick) using a Leica Ultra Cut UC microtome (Leica Microsystems, Wetzlar, Germany). A JEM-1010 electron microscope (JEOL, Tokyo, Japan) was used for the TEM observations at an acceleration voltage of 100 kV. The thickness of the PMPC layer was determined by averaging 10 points on each cross-sectional TEM image.

Wettability and friction tests

Static-water contact angles were measured on each of the PMPC-grafted CLPE samples by employing the sessile drop method using an optical bench-type contact angle goniometer (Model DM300; Kyowa Interface Science, Saitama, Japan). Drops of purified water (1 μL) were deposited on the PMPC-grafted CLPE surfaces, and the contact angles were directly measured after 60 s using a microscope. Fifteen areas were evaluated for each sample, and average values were calculated.

Unidirectional friction tests were performed using a ball-on-plate machine (Tribostation 32; Shinto Scientific, Tokyo, Japan). Six samples of PMPC-grafted CLPE for each irradiation intensities were evaluated. Each specimen was either left non-sterilized or was sterilized by 25 kGy gamma-rays in N_2 gas. A 9 mm diameter pin made from Co–Cr–Mo alloy was also prepared. The surface roughness (R_a) of the pin was <0.01, which was comparable with that of currently used femoral head products. The friction test was performed for each specimen at room temperature using a load of 0.98 or 9.8 N (contact stress roughly calculated by Hertzian theory was ~29 or 62 MPa, respectively), a sliding distance of 25 mm, and a frequency of 1 Hz. A maximum of 100 cycles were carried out, and pure water was used for lubrication. The mean dynamic coefficients of friction were determined by averaging the values of five data points taken from the 96–100 cycles.

Evaluation of physical properties

The swelling ratio and cross-link density of the PMPC-grafted CLPE substrates obtained with various UV-irradiation intensities were evaluated according to previously reported methods.[28] Each of the PMPC-grafted CLPE specimens (23 × 23 × 1 mm) was divided into three sample pieces. The specimens were weighed (approximately 0.5 g, V_1), allowed to swell for 72 h in *p*-xylene containing 0.5 mass% 2-*t*-butyl-4-methylphenol at 130°C, and then reweighed (V_2). The samples were then immersed in acetone, dried at 60°C under vacuum, and weighed again (V_3). The swelling ratio was determined from the weight gain and densities of the PE and xylene, and the physical properties were calculated as follows:

(a) Swelling ratio (q):

$$q = V_2/V_3 \tag{1}$$

(b) Cross-link density:

$$v^{*} = In(1 - q^{1}) + q^{1} + \chi q^{2}/V_1\left(q^{-2/3} - 0.5q^{-1}\right) \tag{2}$$

where v^* is the network chain density, $V_1 = 136$ mL/mol, and $\chi = 0.37$ (for PE)

$$M_c = 1/\overline{M}c = Vv* \tag{3}$$

where M_c is the molecular weight between cross-links, and $V = 1$/specimen density.

$$XLD = M_0/\overline{M}c \tag{4}$$

where XLD is the cross-link density, and $M_0 = 14$ (PE)

Mechanical tests

The mechanical properties of the PMPC-grafted CLPE substrates were evaluated using a series of tests. Tensile testing was performed according to ASTM D638 using type IV tensile bar specimens of 1.0 and 2.0 mm in thickness, and a crosshead speed of 50.8 mm/min. Each of the PMPC-grafted CLPE specimens was divided into ten sample pieces, with each evaluated individually. Shore hardness (D) was measured according to the ASTM D2240 test method, with five samples tested for each UV intensity. A double-notched (notch depth = 4.57 ± 0.08 mm) Izod impact test was performed to ASTM F648 standard, with six samples tested for each UV intensity. A small punch test was performed according to ASTM F2183, using a disk specimen of diameter 6.4 mm and thickness 0.5 mm, and a crosshead speed of 0.5 mm/min. Ten sample pieces were evaluated for each UV intensity.

Hip simulator wear test

A 12-station hip simulator (MTS Systems Corp., Eden Prairie, MN) using untreated CLPE and the PMPC-grafted CLPE liners with an inner and outer diameter of 26 and 52 mm, respectively, was used for the wear test according to ISO 14242-3. PMPC-grafted CLPE liners were obtained using UV-irradiation intensities of 1.5, 5.0, and 15 mW/cm^2 and subsequently subject to hip simulator wear test. Three samples of each of the untreated CLPE and the PMPC-grafted CLPE liners were prepared. A Co–Cr–Mo alloy ball 26 mm in diameter (K-MAX® HH-02; KYOCERA Medical Corp., Osaka, Japan) was used as the femoral head. A mixture of 25 vol % bovine serum, 20 mmol/L ethylene diamine tetraacetic acid (EDTA), and 0.1 mass % sodium azide was used as the lubricant. The lubricant was replaced every 5.0 × 10^5 cycles. Gait cycles were applied to simulate a physiological loading curve (Paul-type) with double peaks at 1793 and 2744 N, and a multidirectional (biaxial and orbital) motion of 1 Hz frequency. Gravimetric wear was determined by weighing the liners at intervals of 5.0 × 10^5 cycles.

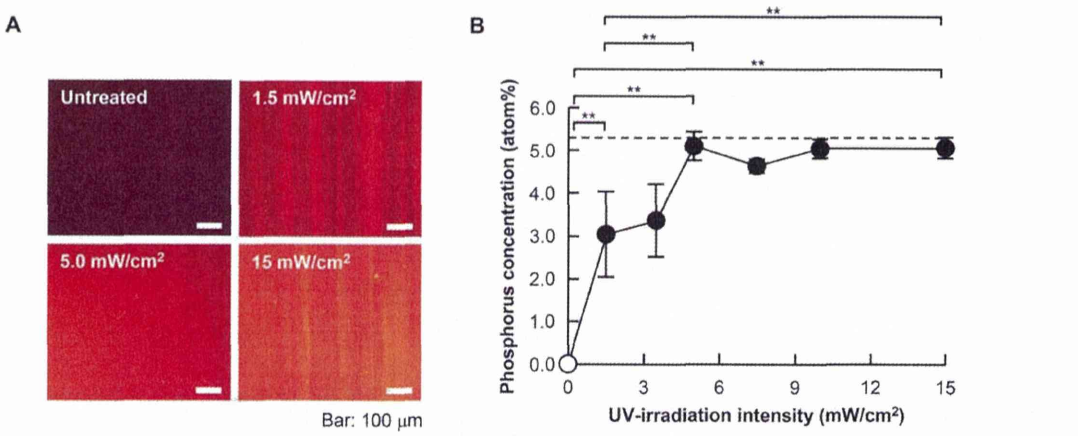

FIGURE 1. (A) Fluorescence microscopy images of rhodamine-stained samples and (B) phosphorus concentrations on PMPC-grafted CLPE surfaces obtained with various UV-irradiation intensities, as calculated using XPS. Open mark indicates untreated CLPE. Data are expressed as mean ± standard deviation. ** indicates $p < 0.01$. Broken lines indicate the theoretical elemental composition (5.3 atom%) of PMPC. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Load-soak controls ($n = 2$) were used to compensate for fluid absorption by the specimens, according to ISO 14242-2. Testing was continued for a total of 5.0×10^6 cycles. Because the gravimetric method was used, the weight loss of each of the tested liners was corrected by subtracting the weight gain due to the load-soak control. However, this correction was not considered to be perfect because only the tested liners were continuously moved and subjected to the load.

The wear particles were isolated from the bovine serum solution, which was then used as a lubricant in the hip joint simulator wear test. To isolate the wear particles, the lubricant was incubated in a 5 mol/L sodium hydroxide solution for 3 h at 65°C to digest adhesive proteins that were degraded and precipitated. In order to avoid artifacts, the contaminating proteins were removed by extraction with solutions of several densities: sugar solution, 1.20 g/cm^3 and 1.05 g/cm^3; and isopropyl alcohol solution, 0.98 g/cm^3 and 0.90 g/cm^3. This was followed by centrifugation at 2.55×10^4 rpm for 3 h at 5°C (himac CP 70MX; Hitachi Koki, Tokyo, Japan). The collected solution was sequentially filtered through a 0.1-μm membrane filter, and the membrane was observed under an FE-SEM (JSM-6330F; JEOL DATUM, Tokyo, Japan) at an acceleration voltage of 20 kV after gold deposition.

In addition, after 5.0×10^6 cycles of the hip simulator wear test, the volumetric wear of the liners was evaluated using a three-dimensional (3D) coordinate measurement machine (BHN-305; Mitutoyo Corp., Kawasaki, Japan). The structures were then reconstructed using 3D modeling software (Imageware; Siemens PLM Software Inc., Plano, TX). To evaluate the wear conditions, the features of the bearing surfaces of the liners were observed using a confocal laser scanning microscope (OLS1200; Olympus Corp., Tokyo, Japan).

Statistical analyses

The mean values of the three or four groups (untreated CLPE and PMPC-grafted CLPE obtained with UV-irradiation intensities of 1.5, 5.0, and 15 mW/cm^2) were compared by one-factor analysis of variance (ANOVA), and the significance of differences of the all comparable properties were determined by post-hoc testing using the Bonferroni method. The dynamic coefficients of friction (ball-on-plate friction test) of PMPC-grafted CLPE with and without gamma-ray sterilization were evaluated using a Student's *t*-test. All the statistical analyses were performed using an add-on (Statcel 2; OMS Publishing, Tokorozawa, Japan) to Microsoft Excel® 2003 (Microsoft Corp., Redmond, WA).

RESULTS

The UV-irradiation intensity was found to affect the extent of PMPC grafting, including the surface phosphorous concentration and the graft layer thickness. As can be seen from the images of rhodamine-treated surface in Figure 1(A), at all irradiation intensities, a PMPC graft layer was formed on the CLPE substrate. The brightness of the uniform fluorescent staining can be seen to increase with UV-irradiation intensity, indicating an increase in the amount of PMPC present. The multiple lines that can be observed on the fluorescence microscopic images are machining marks from cutting of the CLPE bar stock. The phosphorous concentrations of PMPC-grafted CLPE surface, as measured using XPS, increased with the UV-irradiation intensity, and became almost constant at 5.0 atom% over 5.0 mW/cm^2 [Fig. 1(B)]. These values were almost equal to the theoretical elemental composition (5.3 atom%) of PMPC, indicating that the PMPC graft layer fully covered the CLPE substrate. For the samples prepared with UV-irradiation intensities of 1.5 and 5.0 mW/cm^2, a PMPC graft layer 80–150 nm thick can be clearly observed on the surface of the CLPE substrate in the cross-sectional TEM images shown in Figure 2(A). The PMPC-graft layer thickness linearly increased with UV-irradiation intensity, achieving a layer ~380 nm thick at 15 mW/cm^2 [Fig. 2(B)]. However, as can be seen in the TEM image in Figure 2(A) a crack was observed at the