2) FCS 中での試験
次に、人工膝関節が装着された生体内の環境を再現するために、FCS をもちいた検討をおこなった。

① 蛍光顕微鏡観察

未処理の純 Ti 表面には点在する細菌が観察されたが、MPC で処理された表面では、PMB30 処理、PMPC 処理ともに、菌がほとんど観察されなかった（図 4）。

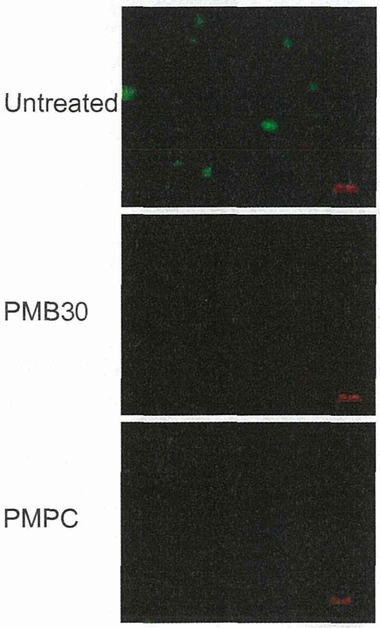

図 4. FCS 中で黄色ブドウ球菌と接触させた純 Ti 表面の蛍光顕微鏡観察像（200 倍）

② 走査型電子顕微鏡観察

未処理の純 Ti 表面には、凝集した細菌塊が観察された。一方、MPC 処理を施した純 Ti 表面には、PMB30 処理、PMPC 処理どちら場合でも、ほとんど菌が観察されなかった（図 5）。

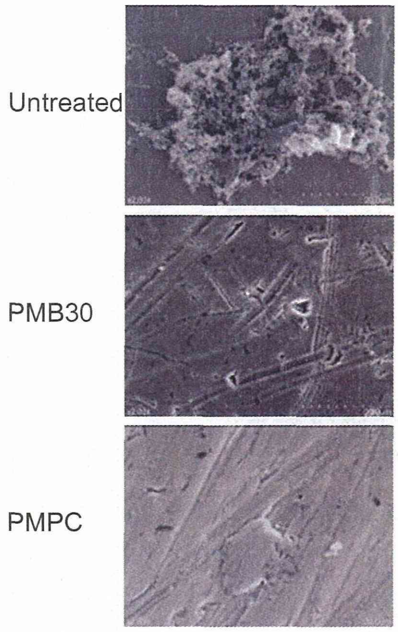

図 5. FCS 中で黄色ブドウ球菌と接触させた純 Ti 表面の走査型電子顕微鏡観察像（2000 倍）

③ 付着生菌数

PBS 中の場合と同様に、純 Ti 表面に PMB30 処理および PMPC 処理を施すことにより、菌の付着が約 99%減少した（図 6）。

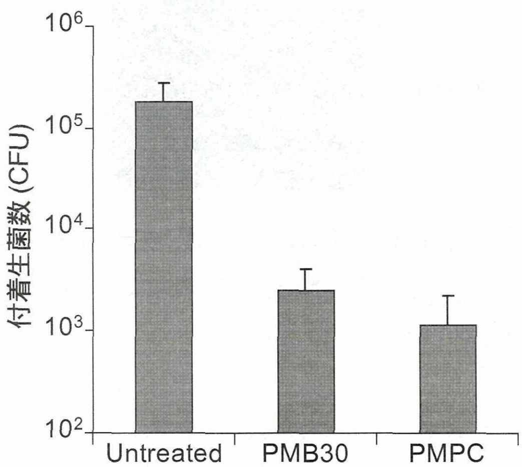

図 6. FCS 中における純 Ti 表面への黄色ブドウ球菌の付着生菌数

2. Co-Cr-Mo 合金表面への細菌付着抑制効果の検討

人工関節のステム部分の材料として純Tiとともに使用される、Co-Cr-Mo合金について、表面のMPC処理による細菌付着抑制効果を、PBS中とFCS中でそれぞれ検討した。

1) PBS中での試験

① 蛍光顕微鏡観察

未処理表面では菌が全表面に均一に付着していたのに対し、PMB30処理およびPMPC処理を施すことによって、菌の付着が顕著に抑制されていた（図7）。

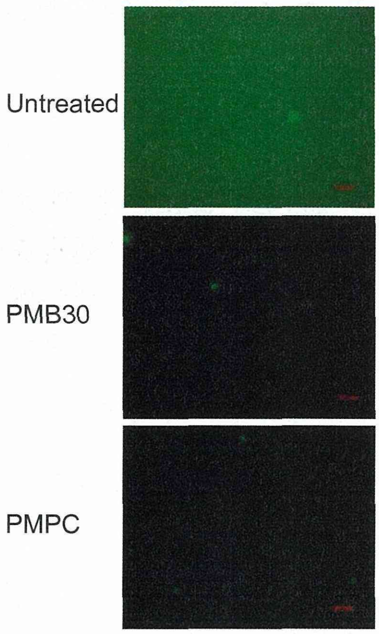

図7. PBS中で黄色ブドウ球菌と接触させたCo-Cr-Mo合金表面の蛍光顕微鏡観察像（200倍）

② 走査型電子顕微鏡観察

純Tiの場合と同様に、MPC処理によって菌の付着が顕著に抑制された（図8）。

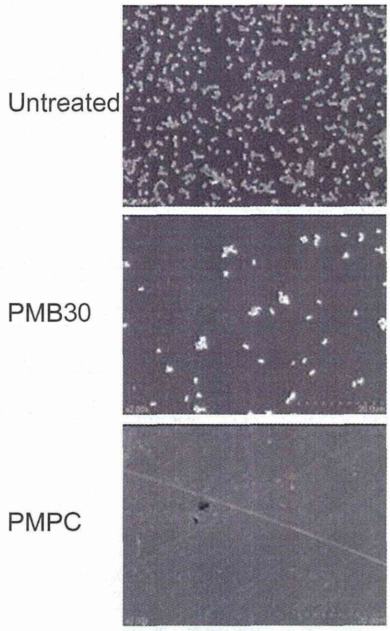

図8. PBS中で黄色ブドウ球菌と接触させたCo-Cr-Mo合金表面の走査型電子顕微鏡観察像（2000倍）

③ 付着生菌数

純Tiの場合と同様に、PMB30処理およびPMPC処理を施すことにより、菌の付着が約99%減少した（図9）。

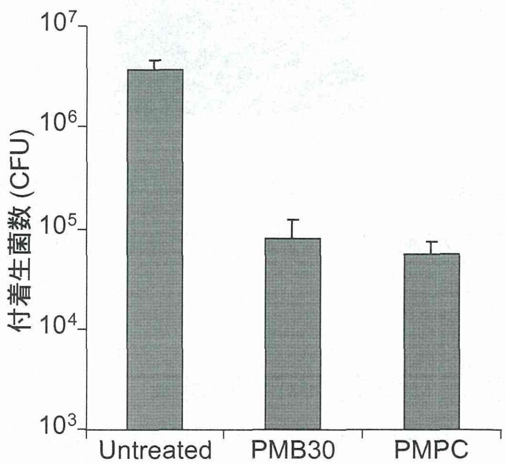

図9. PBS中におけるCo-Cr-Mo合金表面への黄色ブドウ球菌の付着生菌数

2) FCS中での試験

① 蛍光顕微鏡観察

純 Ti の場合と同様に、未処理表面で観察された細菌が、MPC 処理表面ではほとんど観察されなかった（図 10）。

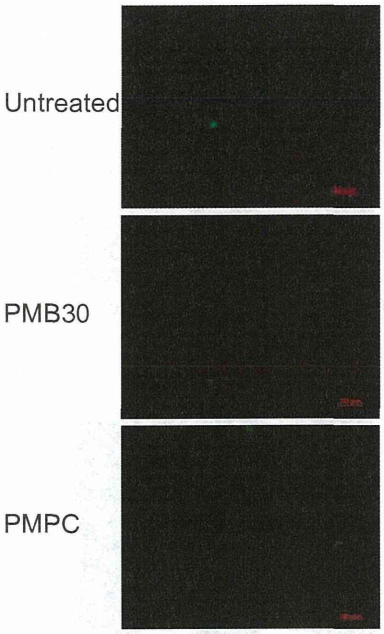

図 10. FCS 中で黄色ブドウ球菌と接触させた Co-Cr-Mo 合金表面の蛍光顕微鏡観察像（200 倍）

② 走査型電子顕微鏡観察

純 Ti の場合と同様に、PMPC 処理によって菌の付着が顕著に抑制された（図 11）。

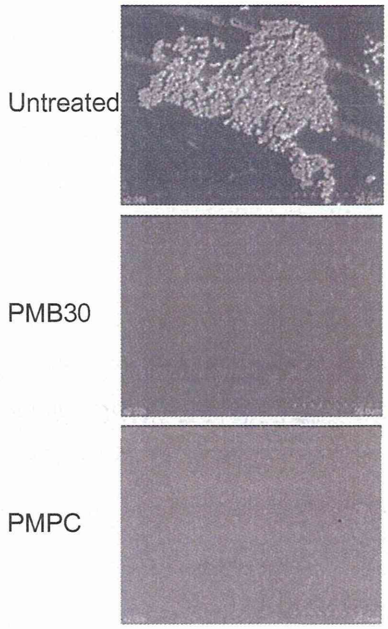

図 11. FCS 中で黄色ブドウ球菌と接触させた Co-Cr-Mo 合金表面の走査型電子顕微鏡観察像（2000 倍）

③ 付着生菌数

純 Ti の場合と同様に、Co-Cr-Mo 合金表面に PMB30 処理および PMPC 処理を施すことにより、菌の付着が約 99%減少した（図 12）。

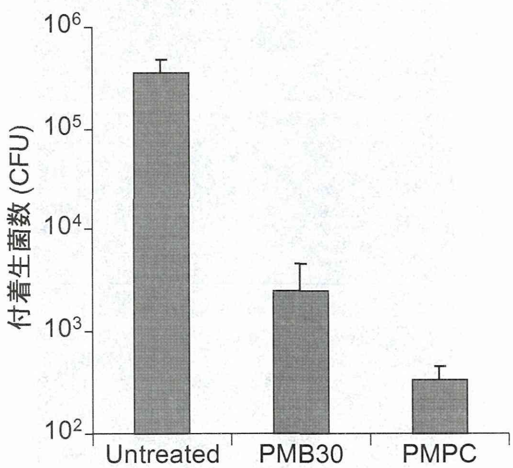

図 12. FCS 中における Co-Cr-Mo 合金表面への黄色ブドウ球菌の付着生菌数

3. バイオフィルム形成抑制効果の検討

MPC 処理による純 Ti 表面および Co-Cr-Mo 合金表面への菌の付着抑制効果はきわめて大きなものであるが、わずかに付着した菌が、バイオフィルムを形成する可能性がある。そこで、MPC 処理のバイオフィルム形成抑制効果について検討した。MPC 処理による黄色ブドウ球菌の付着抑制効果は、金属材料で差がなかったので、純 Ti でのみ検討をおこなった。

① 蛍光顕微鏡観察

未処理の場合には純 Ti 試験片表面が多数の菌体とバイオフィルムに覆われていたが、MPC で処理された表面では、PMB30 処理、PMPC 処理ともに、菌体とバイオフィルムがほとんど観察されなかった（図 13）。

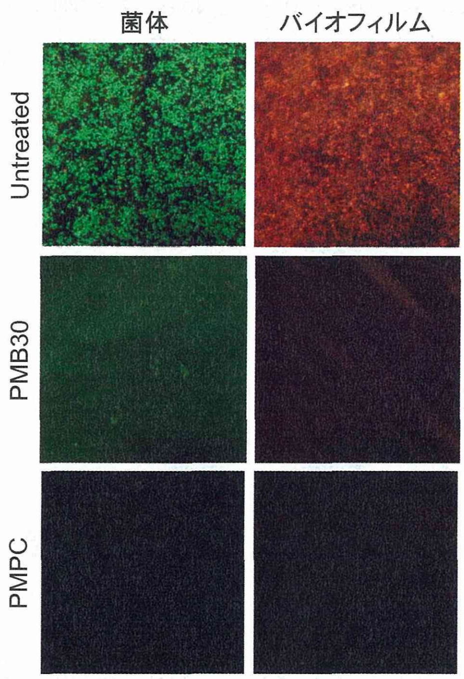

図 13. 純 Ti 表面におけるバイオフィルム形成（200 倍）

② 付着生菌数

純 Ti 表面に PM30 処理および PMPC 処理を施すことで、付着菌数が 99%減少した（図 14）。一方、浮遊菌数は、MPC 処理の有無で差がなかった。

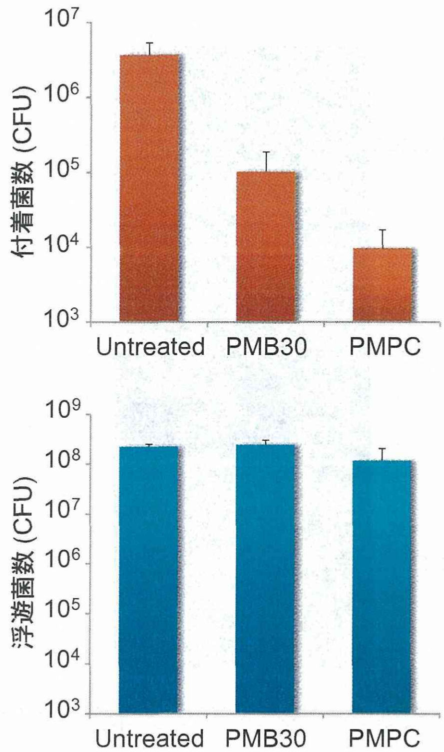

図 14. 純チタン表面のバイオフィルム中の生菌数（上）および未付着浮遊菌の生菌数（下）

4. 遺伝子発現の定量的評価

純 Ti 表面で形成されたバイオフィルム中の菌と、未付着の浮遊菌とで遺伝子発現を定量的に比較し、バイオフィルムで発現が上昇している遺伝子を探索した。その結果、純 Ti 表面のバイオフィルムでは、物質輸送、細胞壁、鉄イオン結合、代謝、などに関与する遺伝子の発現が、浮遊菌に比べて亢進していることがわかった（図 15）。

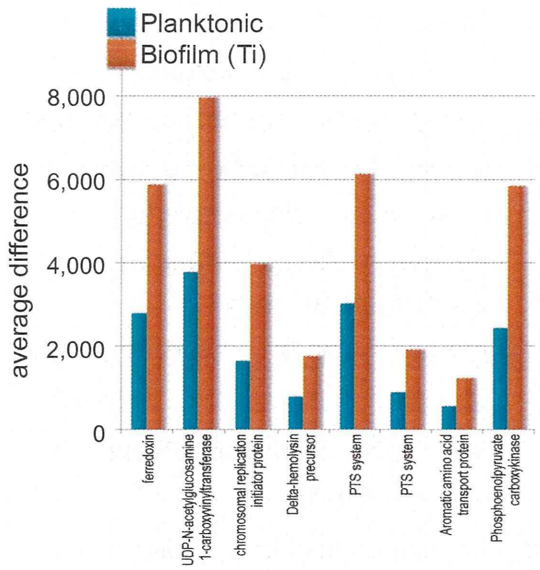

図 15. 未処理 Ti 表面のバイオフィルムで発現が誘導される遺伝子

D. 考察

純 Ti と Co-Cr-Mo 合金どちらの場合でも、MPC 処理によって菌の付着が著しく抑制されることがわかった。また、未処理純 Ti 表面でバイオフィルムがしっかりと形成される条件でも、MPC 処理によりバイオフィルム形成が劇的に抑制された。ただ、PMB30 処理表面では PMPC 処理表面の 10 倍程度の菌が付着していた。これは、長時間の培養による、コーティングの剥がれによるものであると考えられる。人工膝関節が生体内に長期間留置されることを考えると、抗感染性の観点からは、耐久性に優れた PMPC 処理が適していると考えられる。

MPC 処理の有無で浮遊菌数に差が認められないことから、MPC 処理による試験片表面の付着菌の減少は、菌の殺滅によるものではなく、表面への菌の付着そのものが阻害されたことによるものであるといえる。

さらに、純 Ti 表面のバイオフィルム形成に関連すると予想される遺伝子を同定することができた。MPC 処理により付着が阻害されると、これらバイオフィルム関連遺伝子の発現が誘導されないため、バイオフィルム形成がおこらないことが示唆された。

E. 結論

MPC 処理は、細菌付着の“下地”となる蛋白質吸着を抑制することにより、細菌の付着とそれに続くバイオフィルム形成を劇的に抑制する。人口膝関節材料の表面に MPC 処理を施すことで、効果的に抗感染性を付与することが期待できる。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況
　特になし。

別添5

研究成果の刊行に関する一覧表レイアウト

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kyomoto M, Moro T, Yamane S, Hashimoto M, Takatori Y, Ishihara K	Effect of UV-irradiation intensity on graft polymerization of 2-methacryloyloxyethyl phosphorylcholine on orthopedic bearing substrate.	***J Biomed Mater Res A***			in press
Murakami T Yarimitsu S, Nakashima K, Yamaguchi T, Sawae Y, Sakai N, Suzuki A	Superior lubricity in articular cartilage and artificial hydrogel cartilage.	***Proc IMechE Part J: J Engineering Tribology***			in press
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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: 00A:000–000, 2013.

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):

$$q=V_2/V_3 \quad (1)$$

(b) Cross-link density:

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

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

$$M_c=\ 1/\overline{M}c=Vv* \quad (3)$$

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

$$XLD=M_0/\overline{M}c \quad (4)$$

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. Load-soak controls ($n = 2$) were used to compensate for

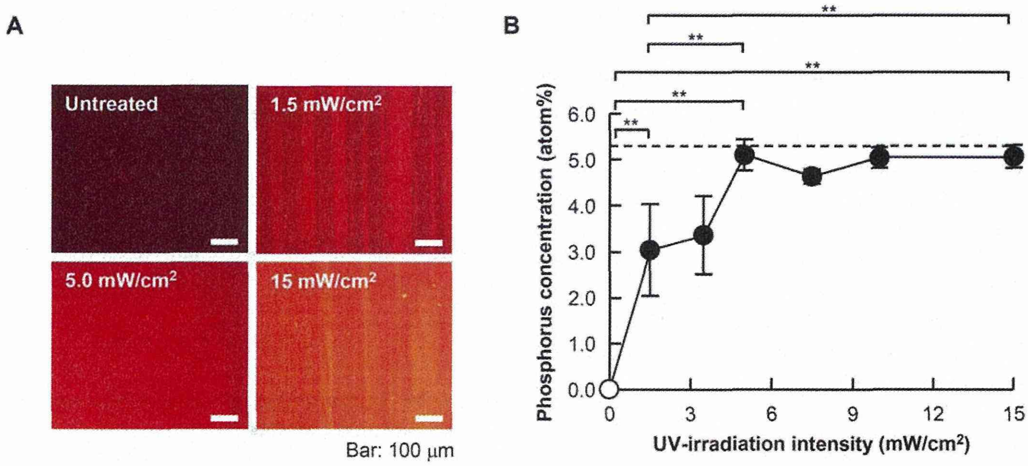

FIGURE 1. (A) Fluorescence microscopy images of rhodamine-stained samples and (B) phosphorus concentrations on PMPC-grafted CLPE surfaces obtained with various UV-irradiation intensities, as calculated using XPS. Open mark indicates untreated CLPE. Data are expressed as mean $\pm$ standard deviation. ** 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.]

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

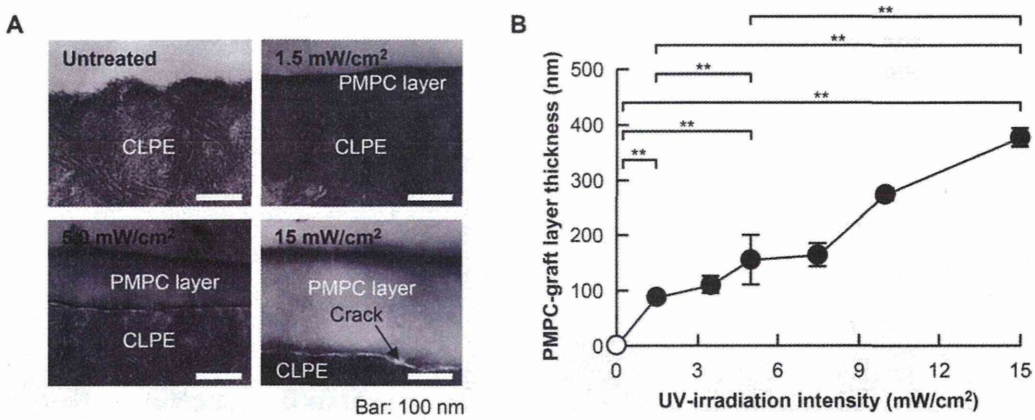

FIGURE 2. (A) Cross-sectional TEM images and (B) PMPC-graft layer thickness of PMPC-grafted CLPE obtained with various UV-irradiation intensities. Open symbol indicates untreated CLPE. Data are expressed as mean ± standard deviation. ** indicates $p < 0.01$.

interface of the PMPC layer and CLPE substrate when a thick polymer layer was formed.

The UV-irradiation intensity affected the hydration and friction kinetics of the PMPC graft layer. The static water contact angle of the untreated CLPE was ~90°, and decreased noticeably with an increase in the UV-irradiation intensity [Fig. 3(A)]. The lowest contact angle observed 30°, which was measured on the samples that was treated at 5.0 mW/cm^2. The angle then increased slightly at higher irradiation intensity. The dynamic coefficients of friction of PMPC-grafted CLPE decreased markedly with an increase in the UV-irradiation intensity, with the surface produced at 3.5–7.5 mW/cm^2 exhibiting an ~85% reduction compared with the untreated CLPE surface [Fig. 3(B)]. However, above 10 mW/cm^2, the values increased slightly. As shown in Figure 4, the dynamic coefficients of friction of the PMPC-grafted CLPE samples obtained using UV-irradiation intensities of 1.5 and 5.0 mW/cm^2 did not differ greatly between loadings, regardless of whether they were gamma-ray sterilized or not. Interestingly, for the nonsterilized PMPC-grafted CLPE obtained with a UV-irradiation intensity of 15 mW/cm^2, the dynamic coefficient of friction in the case of 9.8 N loading was twice as high as that in the case of 0.98 N loading; however, there was no significant difference for the gamma-ray sterilized sample ($p > 0.05$).

Some physical and mechanical properties of PMPC-grafted CLPE as a function of the UV-irradiation intensity are summarized in Figures 5–7. The swelling ratio was almost constant up to an intensity of 10 mW/cm^2, and then decreased slightly above this value [Fig. 5(A)]. The trend in cross-link density also underwent a change at 10 mW/cm^2, gradually increasing up to 0.87 mol % at this point, and then decreasing sharply [Fig. 5(B)]. The ultimate tensile strength and elongation of the untreated CLPE sample differed slightly to the values obtained for the PMPC-grafted CLPE obtained using UV-irradiation intensities of 5.0 and 15 mW/cm^2 [Fig. 6(A,B)]. In contrast, the hardness and impact strength remained almost the same ($p > 0.05$), and appeared to be independent of the UV-irradiation intensity [Fig. 6(C,D)]. The tensile, hardness, and impact resistance properties of all untreated CLPE and PMPC-grafted CLPE samples met the requirements of ASTM F648. The small punch peak strength and work to failure of PMPC-grafted CLPE gradually decreased slightly with UV-irradiation intensity (Fig. 7).

The characteristics of the PMPC-grafted surface affected the durability of the CLPE liners. During the hip simulator

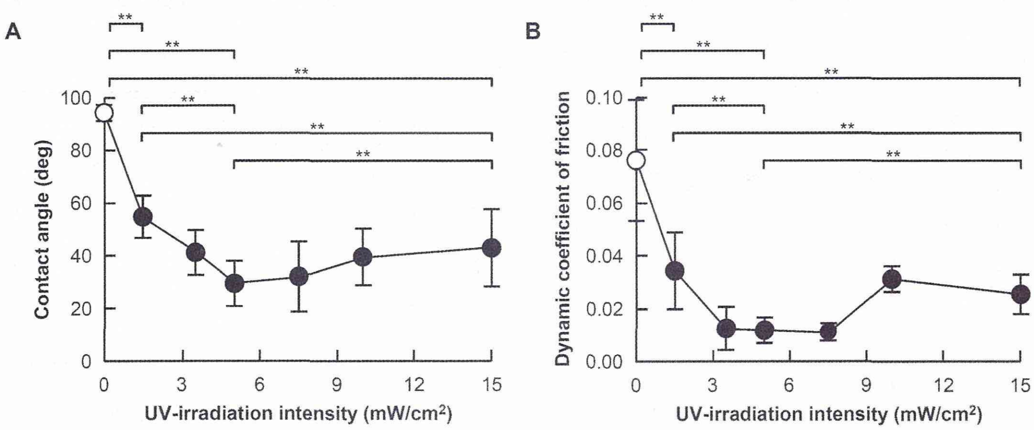

FIGURE 3. (A) Static water contact angle and (B) dynamic coefficient of friction of PMPC-grafted CLPE as a function of the UV-irradiation intensity. Open symbols indicate untreated CLPE. Data are expressed as mean ± standard deviation. ** indicates $p < 0.01$.

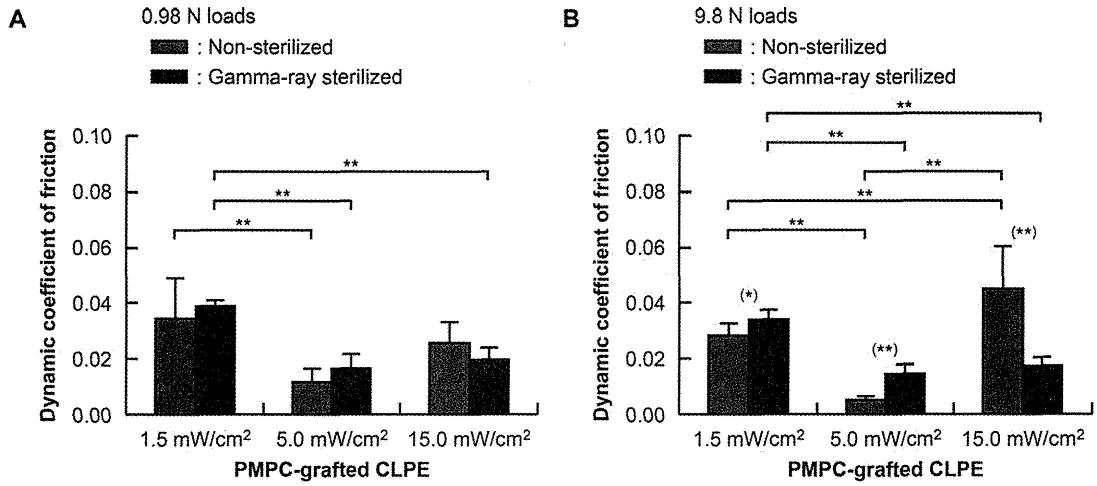

FIGURE 4. Dynamic coefficients of friction of PMPC-grafted CLPE in the ball-on-plate friction test with (A) 0.98 N and (B) 9.8 N loads. Data are expressed as mean ± standard deviation. (*) and (**): *t*-Test, significant differences ($p < 0.05$ and $p < 0.01$, respectively) as a comparison between non-sterilized and gamma-ray sterilized groups, and **: one-factor ANOVA and post-hoc test, significant difference ($p < 0.01$) as comparison between the three groups of the PMPC-grafted CLPE.

wear test, the PMPC-grafted CLPE liner was observed to undergo significantly less gravimetric wear than the untreated CLPE liners [Fig. 8(A)]. Furthermore, there was a slight and gradual increase in weight of the untreated and PMPC-grafted CLPE liners during the testing period, which was partially attributed to greater fluid (e.g., water, proteins, and lipids) absorption by the tested liners than was allowed for by the load-soak controls. As noted earlier, correction using the load-soak control is not perfect because only the tested liners were continuously moved and loaded. Remarkably, extremely small and barely observable wear particles were produced by the PMPC-grafted CLPE liners after 5.0×10^6 cycles ($4.5–5.0 \times 10^6$ cycles) of the hip simulator test [Fig. 8(B)]. The wear particles of the untreated CLPE liners, and the small quantity produced by the PMPC-grafted CLPE, consisted of only sub-micrometer-sized granules. The PMPC grafting did not affect the morphologies of the CLPE wear particles. 3D coordinate measurements of the PMPC-grafted CLPE liners revealed barely detectable volumetric wear, in contrast to the substantial wear detected for the untreated CLPE liners [Fig. 9(A)]. The volumetric wear images in Figure 9(A) are in agreement with the gravimetric wear data shown in Figure 8(A). In the confocal laser scanning microscope images in Figure 9(B), the surface of the untreated CLPE liner against the Co–Cr–Mo alloy femoral head appears smooth. In contrast, the PMPC-grafted CLPE liners exhibit a different morphology; with the machining marks still evident in the bearing surface. There were no differences among the surface morphologies of the three groups of the PMPC-grafted CLPE produced using different UV-irradiation intensities.

DISCUSSION

In this study, we investigated the effects of varying the UV-irradiation intensity on graft polymerization of MPC. The results provide preliminary evidence that the UV-irradiation intensity affected the extent of PMPC grafting and the underlying CLPE substrate. They also demonstrate that the hydrophilic layer increased lubrication to levels that match articular cartilage, and when grafted onto the acetabular liner surface of a THA prosthesis, caused high wear

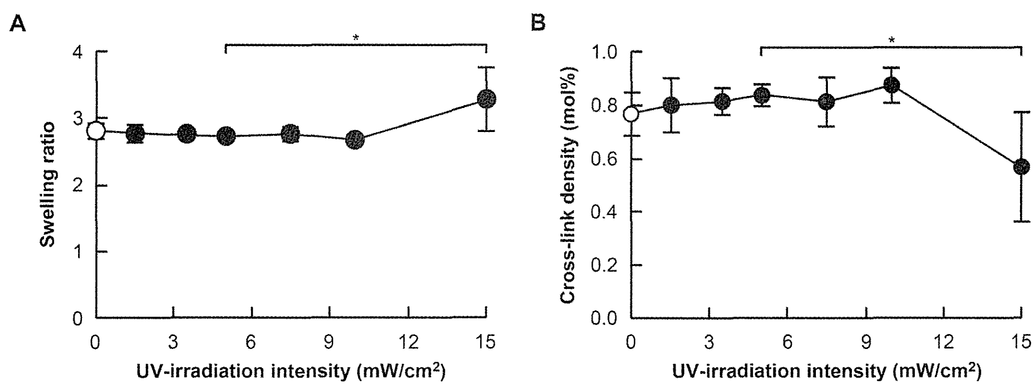

FIGURE 5. (A) Swelling ratio and (B) cross-link density of PMPC-grafted CLPE as a function of UV-irradiation intensity. Open symbols indicate untreated CLPE. Data are expressed as mean ± standard deviation. * indicates $p < 0.05$.

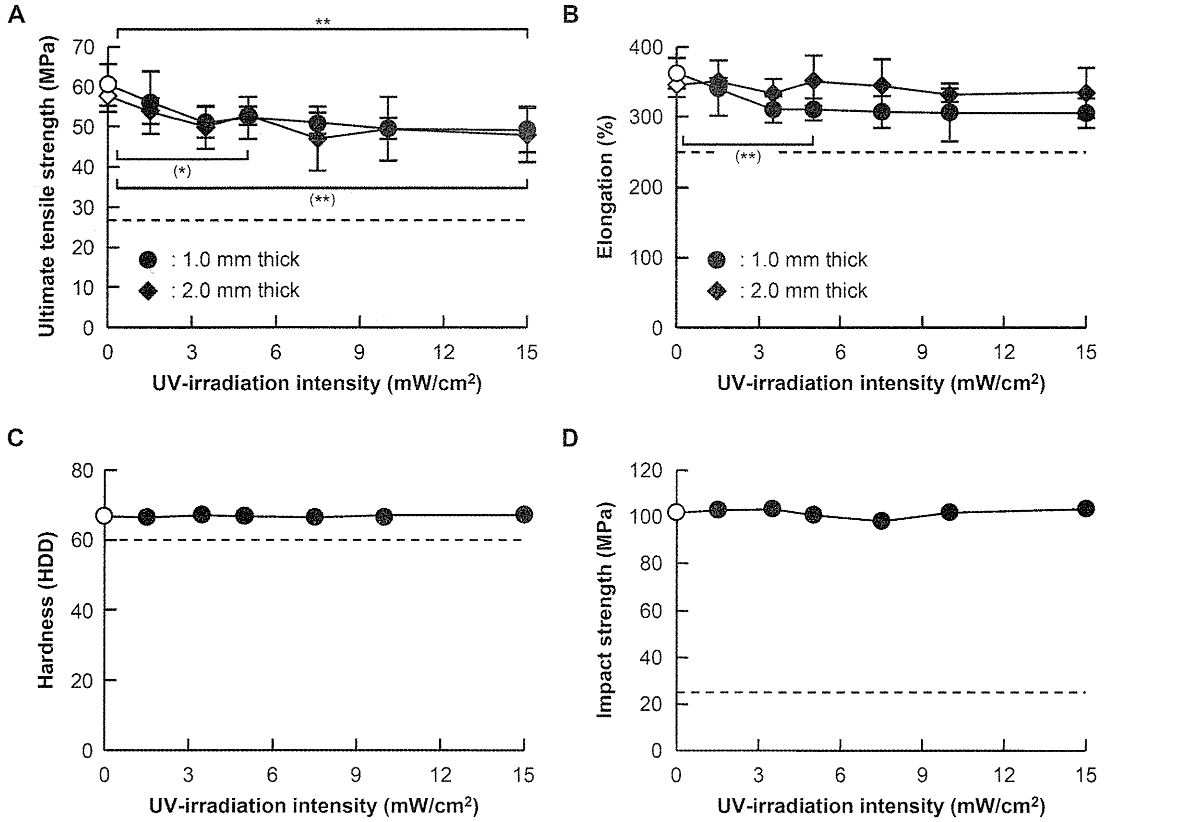

FIGURE 6. Mechanical properties of PMPC-grafted CLPE as a function of UV-irradiation intensity. (A) Ultimate tensile strength; (*) and (**): one-factor ANOVA and post-hoc test, significant difference ($p < 0.05$ and $p < 0.01$, respectively) as compared with the ultimate tensile strength of 1.0 mm thick test specimens, and **: significant difference ($p < 0.01$) of 2.0 mm thick test specimens. (B) Elongation; (**): one-factor ANOVA and post-hoc test, significant difference ($p < 0.01$) as compared to the elongation of 1.0 mm thick test specimens. (C) Hardness and (D) impact strength. Open symbols indicate untreated CLPE. Data are expressed as mean ± standard deviation. Broken lines indicate lower limits of ASTM requirements.

resistance. This suggests the grafting of PMPC may be a promising approach for extending the longevity of THA.

Despite these promising results, our study has a number of limitations. First, *in vitro* findings do not always translate to a clinical success. However, we conducted multicenter clinical trials of PMPC-grafted CLPE liners between 2007 and 2009 in Japan.[22] Based on other related evidence and these clinical trials, the Japanese government (Ministry of

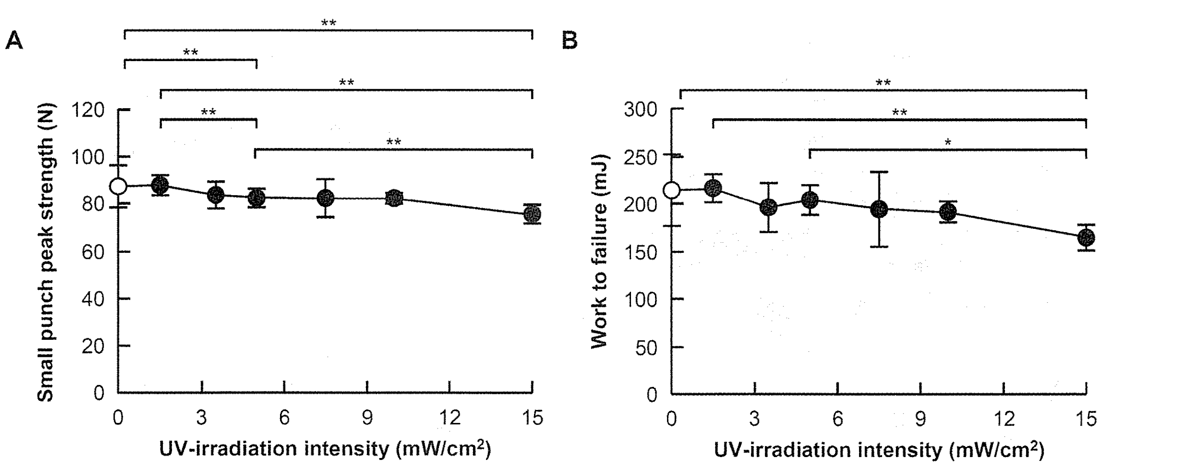

FIGURE 7. Small punch test properties of PMPC-grafted CLPE as a function of UV-irradiation intensity. (A) Small punch peak strength; **: one-factor ANOVA and post-hoc test, significant difference ($p < 0.01$) as compared with the peak strength in four groups of untreated and PMPC-grafted CLPE. (B) Work to failure; * and **: one-factor ANOVA and post-hoc test, significant difference ($p < 0.05$ and $p < 0.01$, respectively) as compared with the work to failure in four groups of untreated and PMPC-grafted CLPE. Open symbols indicate untreated CLPE. Data are expressed as mean ± standard deviation.

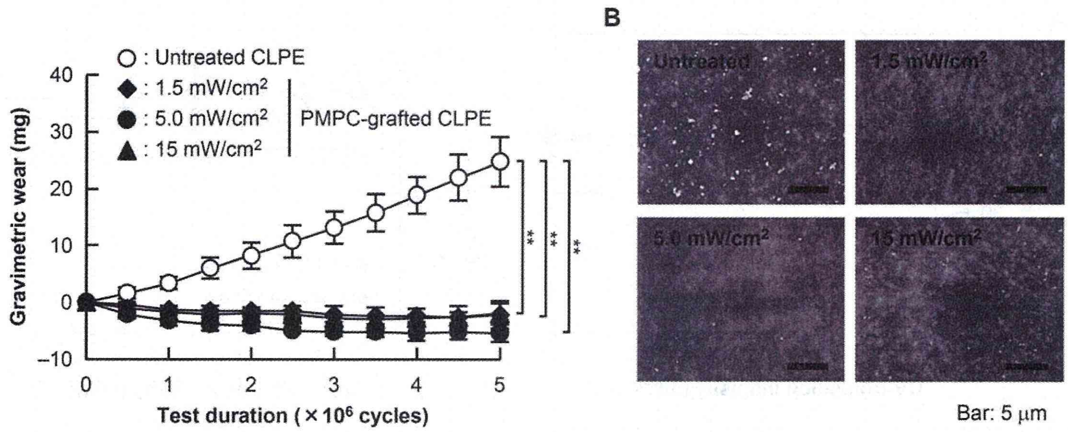

FIGURE 8. A: Time course of the gravimetric wear of the PMPC-grafted CLPE liners obtained using various UV-irradiation intensities. Data are expressed as mean ± standard deviation. **: one-factor ANOVA and post-hoc test, significant difference ($p < 0.01$) as compared with the gravimetric wear after the test in four groups of untreated and PMPC-grafted CLPE. B: SEM images of wear particles from PMPC-grafted CLPE isolated from lubricants of the hip simulator wear test.

Health, Labor, and Welfare, Japan) approved the clinical use of PMPC-grafted CLPE acetabular liners (Aquala® liner; KYOCERA Medical Corp.) in artificial hip joints in April 2011. We observed neither osteolysis nor a need for revision surgery up to 6 years of follow-up. Second, we used a confined period for the hip simulator wear test. Although experiencing 5.0×10^6 cycles in the hip simulator is comparable to 5 years of physical walking, the duration may not be sufficiently long for young active patients. We are now running the hip simulator for longer, and thus far, have confirmed almost no wear on the PMPC-grafted CLPE liners after 1.5×10^7 cycles.[29] Third, we did not entirely capture the range of loading and motion conditions of the *in vivo* environment in terms of the variety of positions, the magnitude of loading, or the daily routine; however, in accordance with ISO 14242-3, we believe that these results can provide a good indication of wear performance. Fourth, the procedure for the isolation of wear particles in this study was not able to capture the contribution of wear particles with a diameter of less than 0.1 μm, as previously reported.[30] 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. Fifth, 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 CLPE liner. Although aseptic loosening is one of the most common reasons for late 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

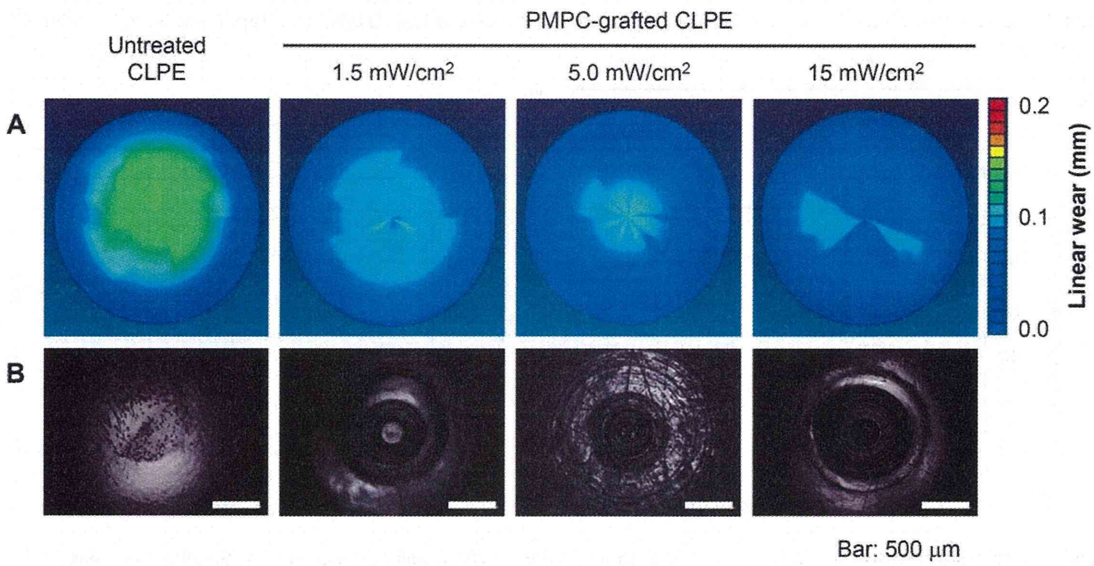

FIGURE 9. (A) 3D coordinate measurement images and (B) confocal laser scanning microscopy images of the PMPC-grafted CLPE liners obtained using various UV-irradiation intensities after 5.0×10^6 cycles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

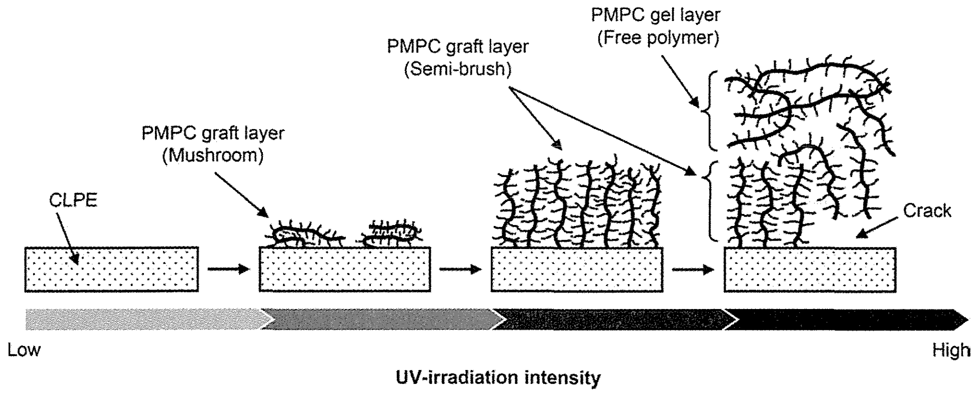

FIGURE 10. Schematic illustration of the PMPC-grafted CLPE surface obtained with various UV-irradiation intensities.

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 simulation. We are now running the hip simulator test with larger Co–Cr–Mo alloy and zirconia toughened alumina ceramic femoral heads and thin acetabular liners.

Effects on extent of PMPC grafting

It is important to be able to control the graft layer on CLPE surfaces in order to optimize lubrication and resistance to wear. In Figures 1 and 2, when the MPC concentration and UV-irradiation time were fixed (0.50 mol/L and 90 min), the extent of PMPC grafting on the CLPE surface increased with the UV-irradiation intensity, and then became almost constant at 5.0 atom% over 5.0 mW/cm^2. It is well known that the amount of photoinduced radicals depends on the photo-irradiation intensity[31]; therefore, the extent of grafting appeared to be successfully controlled by varying the quantity of radicals produced during the radical polymerization process. Figure 10 shows a schematic that illustrates how the PMPC grafting is suggested to vary with UV-irradiation intensity during the polymerization.[32] The PMPC graft layer thickness linearly increased with UV-irradiation intensity above 7.5 mW/cm^2, reaching ~380 nm at 15 mW/cm^2. However, as shown in Figure 2(A), the sample produced using 15 mW/cm^2 UV displayed the formation of a crack at the interface between the PMPC layer and CLPE substrate. When the PMPC layer has a semi-brush-like structure (Fig. 10), the layer thickness may correlate with the molecular weight of the grafted PMPC. It is generally known that the reaction rate of radical polymerization is extremely high; therefore, the length (molecular weight) of the graft chains can be assumed to be controlled by the monomer concentration. However, the MPC concentration and UV-irradiation time were fixed in the present study,[27,33] and the graft polymerization reaction with free radicals was photoinduced by UV irradiation using benzophenone as a radical initiator. A certain amount of UV irradiation energy can directly produce free radicals from the methacrylic acid group of the MPC unit in the monomer solution. When the UV-irradiation intensity is high, graft polymerization can occur between the radicals on the CLPE surface and the MPC monomer, in addition to homopolymerization of MPC. The free radicals not only facilitate direct grafting of MPC to CLPE, thereby forming C–C covalent bonds between the PMPC and the CLPE substrate, but also induce homopolymerization of MPC, forming free polymer in solution. Moreover, the diffusion of the monomer might be disrupted in a solution with high homopolymer concentration because of high viscosity. When the monomer and initiator initially attached to the CLPE surface were subjected to UV irradiation, radicals would have freely formed on the CLPE surface in the early stage but not in the late stage of polymerization, probably because the increased polymer radicals and/or free homopolymer chains blocked the diffusion of the radicals to the CLPE surface. Therefore, it is supposed that areas of unmodified CLPE would remain below the PMPC gel (free polymer) layer, which would leave a gap (or crack) at the interface between PMPC and CLPE substrate. In summary, it is assumed that when the UV-irradiation intensity is low (<7.5 mW/cm^2), the rate of MPC graft polymerization is higher than that of MPC homopolymerization. In contrast, when the UV-irradiation intensity is high (>10 mW/cm^2), the rate of homopolymerization might be higher than that of graft polymerization. Moreover, while the rate of MPC graft polymerization increases with the UV-irradiation intensity, the entire polymerization system begins to show gelation, with the formation of PMPC gel layer (on the PMPC graft layer) at UV-irradiation intensities above 10 mW/cm^2, decreasing the grafting efficiency. Therefore, in order to obtain a stable PMPC grafted layer without gelation of PMPC, the UV-irradiation intensity should be carefully controlled.

The water wettabilities of the PMPC-grafted CLPE surfaces were found to be considerably greater than that of the untreated CLPE surfaces [Fig. 3(A)]. This is because of the presence of a nanometer-scale PMPC graft layer resulting from the polymerization of the highly hydrophilic MPC monomer. It can be observed in Figure 3(B) that the dynamic coefficients of friction of the PMPC-grafted CLPE surfaces were significantly lower than those of the untreated CLPE surface. This was attributed to the significant increase in hydrophilicity evident from the reduction in the static water contact angles of the PMPC-grafted surfaces. The fabrication of the PMPC gel layer clearly influenced the friction response, with the dynamic coefficient of friction for the PMPC-grafted CLPE obtained at 15 mW/cm^2 being less than half of the value for the untreated material at 0.98 N loading. In contrast, at 9.8 N loading, the dynamic coefficient of friction for the 15 mW/cm^2 sample was significantly higher than for those treated at 1.5 and 5.0 mW/cm^2 (Fig. 4). It was previously reported that the dynamic coefficients of friction of MPC polymer coated CLPE prepared by physical adsorption or weak chemical bonding, increased to the level of the untreated CLPE at loads above 1.96 N.[24] It was therefore assumed that these particular surface modification layers became dislodged from the surface at high loading, and were therefore ineffective. In the present study, it is suggested that the PMPC gel layer on the PMPC-grafted CLPE obtained with a UV-irradiation intensity of 15 mW/cm^2 was removed from the bearing surface, resulting in an increase in friction. On the other hand, interestingly, this same sample, but gamma-ray sterilized, expressed high lubricity regardless of loading. In our previous study, we reported that the higher energy radiation used for gamma-ray sterilization induced cross-links not only within the PMPC graft layer, but also between the PMPC graft layer and the CLPE substrate.[34] It was similarly reported that when a high energy beam was irradiated onto a polymer with a grafted layer, strong bonds were formed between the grafted layer and polymer substrate.[35] Moreover, Lewis et al. reported that the force required to remove a coating with cross-linking was greater than that without.[36] Generally, when a high energy gamma-ray beam is irradiated on a polymer, free radicals are formed by the scission of molecular chains.[37] This is followed by the re-termination and cross-linking of the molecules. Hence, it was speculated that in the present study, a higher degree of cross-linking, and perhaps adhesion of PMPC graft and gel layers to the substrate, was induced by the gamma-ray irradiation in comparison to the non-sterilized PMPC-grafted CLPE. This would result in a much stronger and stable PMPC graft layer on the bearing surface.

Effects on CLPE substrate

The tested physical and mechanical properties of CLPE were altered slightly by the PMPC grafting, as shown in Figures 5–7. Most previous studies have assumed that photoinduced polymerization is a surface restricted phenomenon.[26,38] However, in reality, the changes (i.e., cross-linking and chain scission) of the polymer structure under UV radiation can result in changes to the bulk physical and mechanical properties (swelling ratio, cross-link density, and tensile- and small punch-tests properties) of thin test specimens, such as those with a thickness of 0.5–2.0 mm that were used in this study. In the case of the photoinduced cross-linking and scission of the CLPE substrate, one aspect that has been evaluated is the relationship between initiation and the depth of UV penetration. Shyichuk et al. reported that the photoinduced cross-linking and scission of (low-density) PE was observed in the surface region of PE in the range 0–1.5 mm, after UV-irradiation with an intensity of 0.2 mW/cm^2 for over 3 weeks.[26] Hence, it was thought that the observed changes in physical and mechanical properties would be the result of a complex combination of cross-linking and scission effects in a surface restricted region. In particular, it is assumed that when the UV-irradiation intensity is low ($<$7.5 mW/cm^2), the rate of cross-linking is higher than that of chain scission. In contrast, when the UV-irradiation intensity is high ($>$10 mW/cm^2), the rate of scission might be higher than that of cross-linking. However, it should be noted that these phenomena would also be combined with the effects of other polymerization conditions, such as temperature, dissolved oxygen concentration of monomer solution, and photoinitiator concentration. 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*. As mentioned above, 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 in thin acetabular liners of PMPC-grafted CLPE by the possible impingements must therefore be monitored. From the data gathered to date, we have observed neither mechanical fracture nor complications in the clinical use of the PMPC-grafted CLPE liner for a minimum of 4 years and a maximum of 6 years of follow-up.

The production of wear particles in THA is recognized as the main factor behind the initiation of periprosthetic osteolysis and aseptic loosening.[4,5] The inflammatory 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 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 this study, the collected wear particles from the PMPC-grafted CLPE liners were on the scale of sub-micrometers, regardless of the PMPC grafting and its UV-irradiation intensity. Considering the results of the wear particle analysis, we expect the biological response of the PMPC-grafted CLPE liners *in vivo* to be comparable with those of other conventional untreated CLPE.[39] However, attention must be paid to the abnormal wear particles (sub-micrometer-size and number) in the PMPC-grafted CLPE liner, formed by possible scission reactions of the CLPE substrate. The remarkably fewer wear particles isolated from the lubricants used for the PMPC-grafted CLPE liners compared with those from the lubricants used for the untreated liners may help predict whether the abnormal wear will occur.

High wear resistance of PMPC-grafted CLPE liners

In the hip simulator wear test of the present study, the observed significant improvements in the water wettabilities and frictional properties of the PMPC-grafted CLPE liners resulted in substantial improvements in their wear resistances. The high friction of 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 surfaces were considerably greater. Fluid film lubrication (or hydration lubrication) of the PMPC-grafted surface was therefore provided by the hydrated layer. The fluid film-forming ability of a 10 nm thick PMPC layer would be equivalent to that of a micrometer-order-thick PMPC layer because the outermost layer is responsible for this property. The hip simulator wear test confirmed that the wear resistance was almost same in each of the three groups of PMPC-grafted CLPE with different PMPC graft layer thicknesses, with the 90 nm thick PMPC graft layer formed at a UV-irradiation intensity of 1.5 mW/cm^2 providing similar wear resistance to the 380 nm thick PMPC graft layer at a UV-irradiation intensity of 15 mW/cm^2. In our previous study, it was found that even a 10 nm thick PMPC graft layer exhibited improved wear resistance.[27] It was therefore speculated that the improved wear resistance was independent of PMPC graft layer thickness. Additionally, the retention of the improved wear resistance of the cross-linked PMPC gel layer combined with PMPC graft layer and/or the substrate (PMPC-grafted CLPE obtained with a UV-irradiation intensity of 15 mW/cm^2) is very interesting. As mentioned above, the cross-links produced by the extra energy of the gamma-rays was effective even for multidirectional high-loading of the hip simulator.

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 activity.[13] The PMPC structure attracts water in a similar way to the extracellular matrix molecules present in cartilage, providing a lubricating layer on the surface that they are attached to.[15] This study therefore demonstrates the advantages that can be gained from investigating and subsequently mimicking natural structures and systems.

CONCLUSIONS

In this study, we confirmed that the UV-irradiation intensity affected the extent of PMPC grafting, along with cross-linking and scission reactions of the CLPE substrate. The extent of PMPC grafting on the surface of the CLPE gradually increased with increasing UV-irradiation intensity up to 7.5 mW/cm^2, and then remained constant above this value. It was found that in order to obtain a stable PMPC grafted layer without gelation of PMPC, the UV-irradiation intensity needed to be carefully controlled. When the CLPE surface under the grafted polymer was exposed to UV-irradiation, some of the physical and mechanical properties of the CLPE were altered slightly due to cross-linking and scission effects in the surface region. The hydrophilic PMPC layer grafted onto the CLPE surface significantly increased lubrication to levels that match articular cartilage. 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.

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