以上の結果から、被検体から得られた抽出液は本試験条件下において、細胞毒性を示さないものと考えられた。

③ MPC ポリマー処理 UHMWPE の培養細胞を用いた染色体異常試験

被検体を培地で抽出した抽出液について、短時間処理法及び連続処理法のいずれの場合も 25～100%の濃度で試験を行ったところ、構造異常及び数的異常の出現頻度はいずれも 5%未満であった(図 5, 6)。

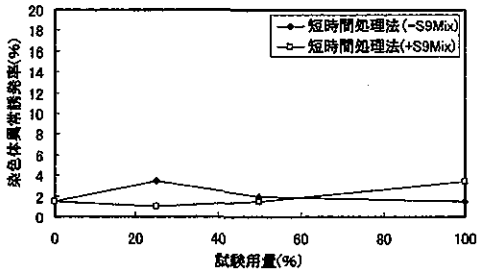

図 5 試験液濃度による染色体構造異常誘発率（短時間処理法）

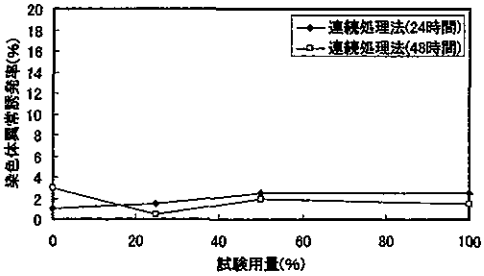

図 6 試験液濃度による染色体構造異常誘発率（連続処理法）

染色体異常試験と同時に実施した細胞増殖抑制試験においては、細胞増殖の抑制は認められなかった(図 7, 8)。

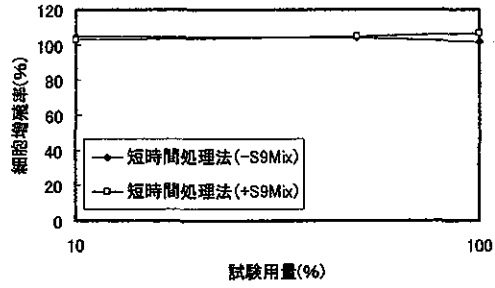

図 7 試験液濃度による細胞増殖率（短時間処理法）

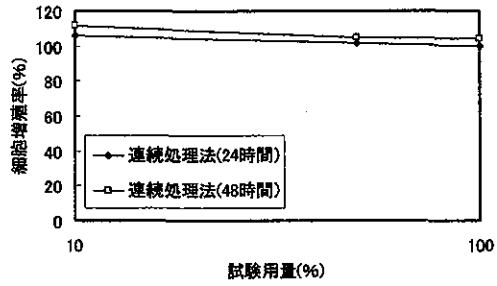

図 8 試験液濃度による細胞増殖率（連続処理法）

従って、本試験条件下における被検体の染色体異常誘発性は、陰性と結論した。

④ MPC ポリマー処理 UHMWPE の感作性試験

図 9～11 に、適用後 24 時間の試験群、陰性対照群及び陽性対照群を示す。

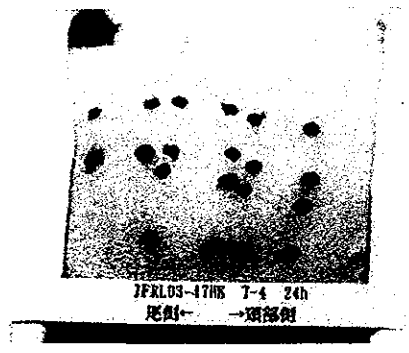

図 9 適用後 24 時間の試験群

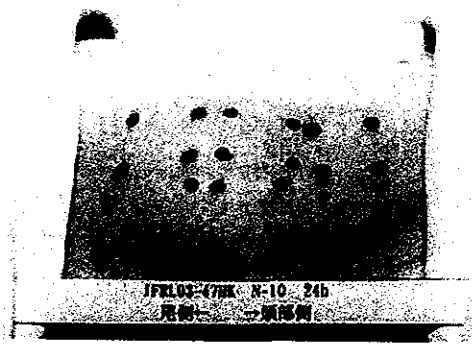

図 10　適用後 24 時間の陰性対照群

図 11　適用後 24 時間の陽性対照群

適用後、24、48 及び 72 時間の各観察時間において、いずれの適用部位においても皮膚反応は認められず、陽性率はいずれも 0%であった。

以上より、被検体は本試験条件下において皮膚感作性を有さないものと考えられた。

⑤ MPC ポリマー処理 UHMWPE のラットにおける骨内埋植試験

図 12～23 に、肉眼的外観観察写真を示す。

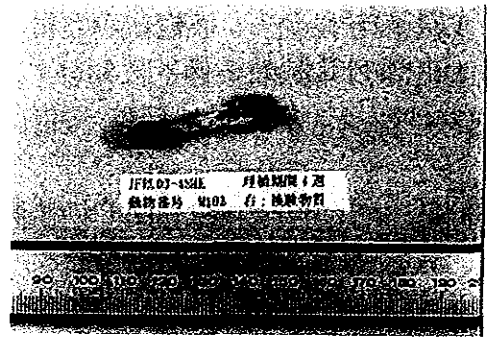

図 12　埋植 4 週(被検体, 雄)

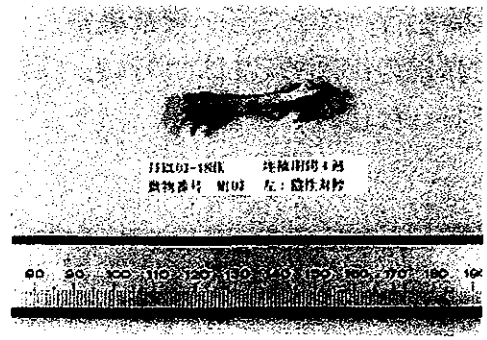

図 13　埋植 4 週(陰性対照試料, 雄)

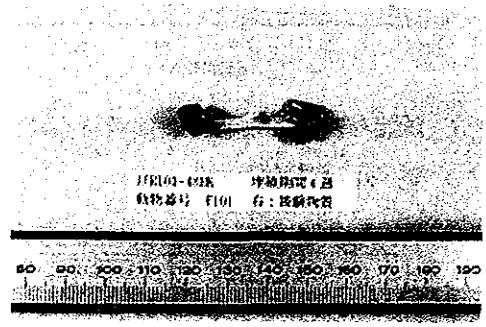

図 14　埋植 4 週(被検体, 雌)

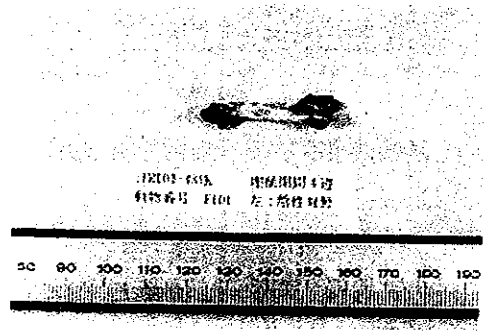

図 15　埋植 4 週(陰性対照試料, 雌)

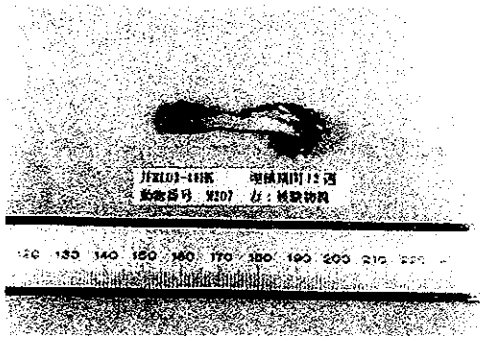

図 16　埋植 12 週(被検体, 雄)

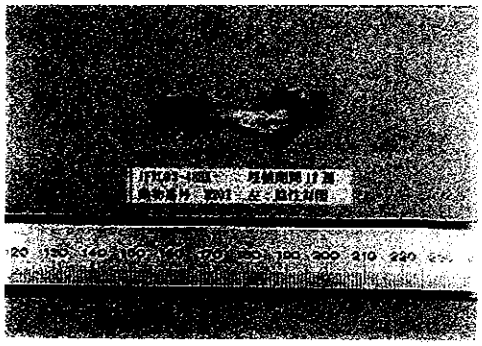

図 17　埋植 12 週(陰性対照試料，雄)

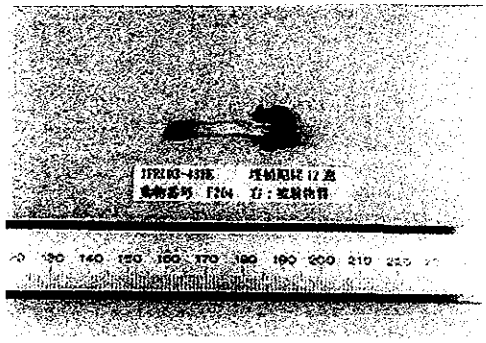

図 18　埋植 12 週(被検体, 雌)

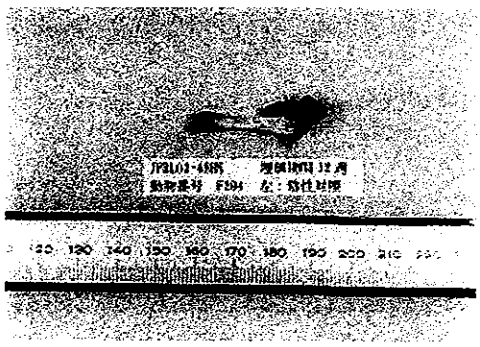

図 19　埋植 12 週(陰性対照試料, 雌)

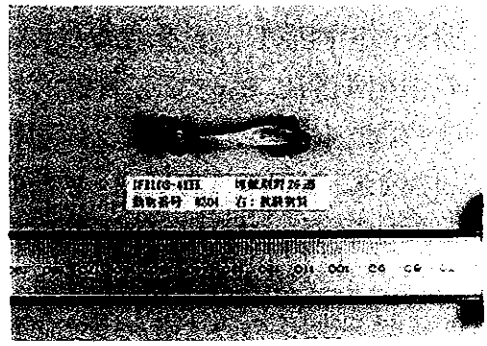

図 20　埋植 26 週(被検体，雄)

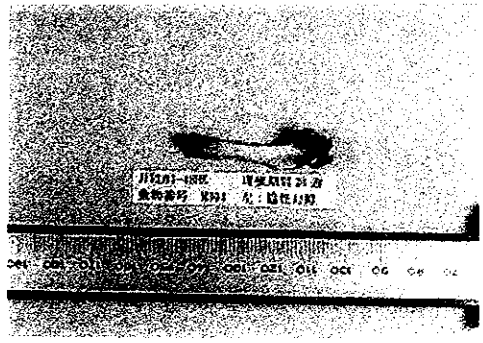

図 21　埋植 26 週(陰性対照試料，雄)

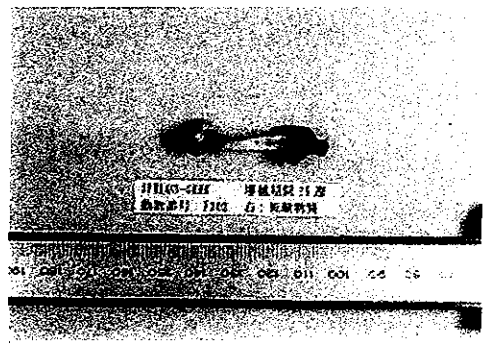

図 22　埋植 26 週(被検体，雌)

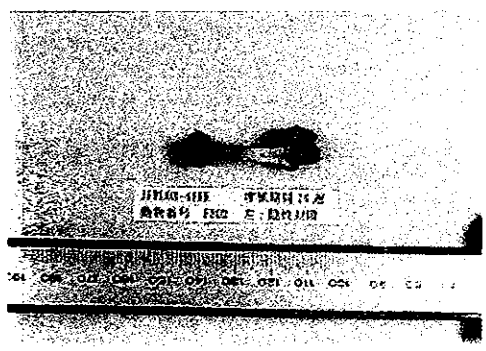

図 23　埋植 26 週(陰性対照試料，雌)

被検体を埋植した大腿骨では、いずれの埋植期間においても肉眼的に異常は見られなかった。また、病理組織学的検査においても明らかな炎症反応は認められず、埋植部位周囲に新生骨の形成及び繊維性被膜の増殖が部分的に見られるのみであった（図 24～35）。

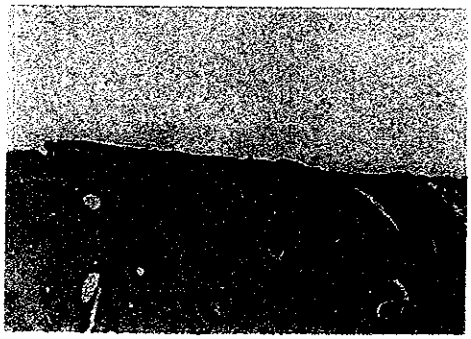

図 24　埋植 4 週大腿骨（被検体，雄）

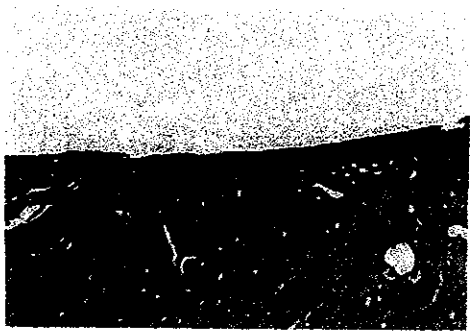

図 25　埋植 4 週大腿骨（陰性対照試料，雄）

図 26　埋植 4 週大腿骨（被検体，雌）

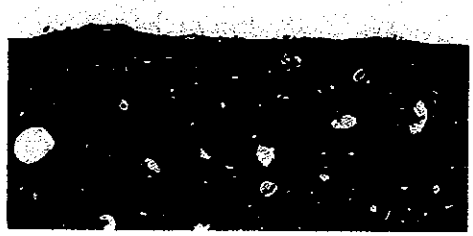

図 27　埋植 4 週大腿骨（陰性対照試料，雌）

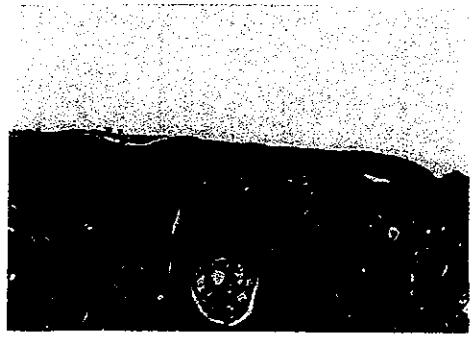

図 28　埋植 12 週大腿骨（被検体，雄）

図 29　埋植 12 週大腿骨（陰性対照試料，雄）

図 30　埋植 12 週大腿骨（被検体，雌）

図 31　埋植 12 週大腿骨(陰性対照試料，雌)

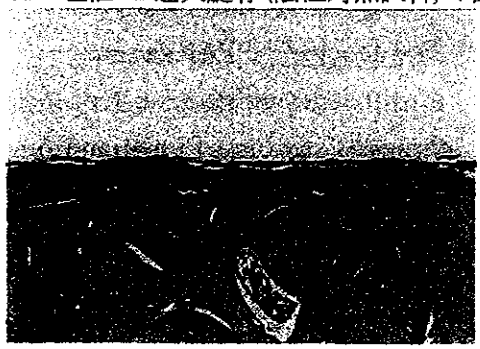

図 32　埋植 26 週大腿骨(被検体，雄)

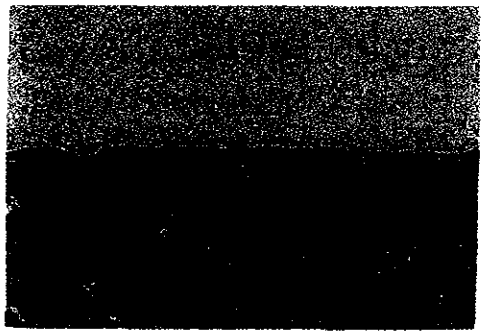

図 33　埋植 26 週大腿骨(陰性対照試料，　雄)

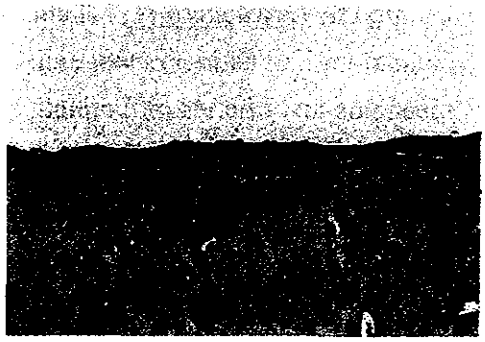

図 34　埋植 26 週大腿骨(被検体，雌)

図 35　埋植 26 週大腿骨(陰性対照試料，雌)

この所見は、陰性対照試料を埋植した大腿骨の組織反応と同様であり、その程度も同等であった。

以上から、被検体をラット大腿骨内へ埋植した場合の反応は、新生骨の形成及び繊維性被膜の増殖が主体であり、炎症や組織の変性を引き起こすことはないと考えられた。

D. 考察

MPC ポリマーでナノ表面処理した UHMWPE の基礎的な生物学的安全性を調べる目的で、MPC ポリマー処理 UHMWPE の細菌を用いる復帰突然変異試験、培養細胞を用いたコロニー形成阻害試験、染色体異常試験、感作性試験及びラットにおける骨内埋植試験を行った。いずれの試験結果も陰性であり、MPC ポリマーでナノ表面処理したことによる生物学的安全性への影響はないといえる。

E. 結論

MPC ポリマーでナノ表面処理した UHMWPE は、著しい低摩耗を実現する人工股関節摺動部材であり、またその生物学的安全性も確認できたことから、将来的に安全な長寿命型人工股関節となることが期待できる。

F. 健康危険情報
特になし。

G. 研究発表
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2.学会発表

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H. 知的財産権の出願・登録状況

特になし。

Ⅲ. 研究成果の刊行物

雑誌

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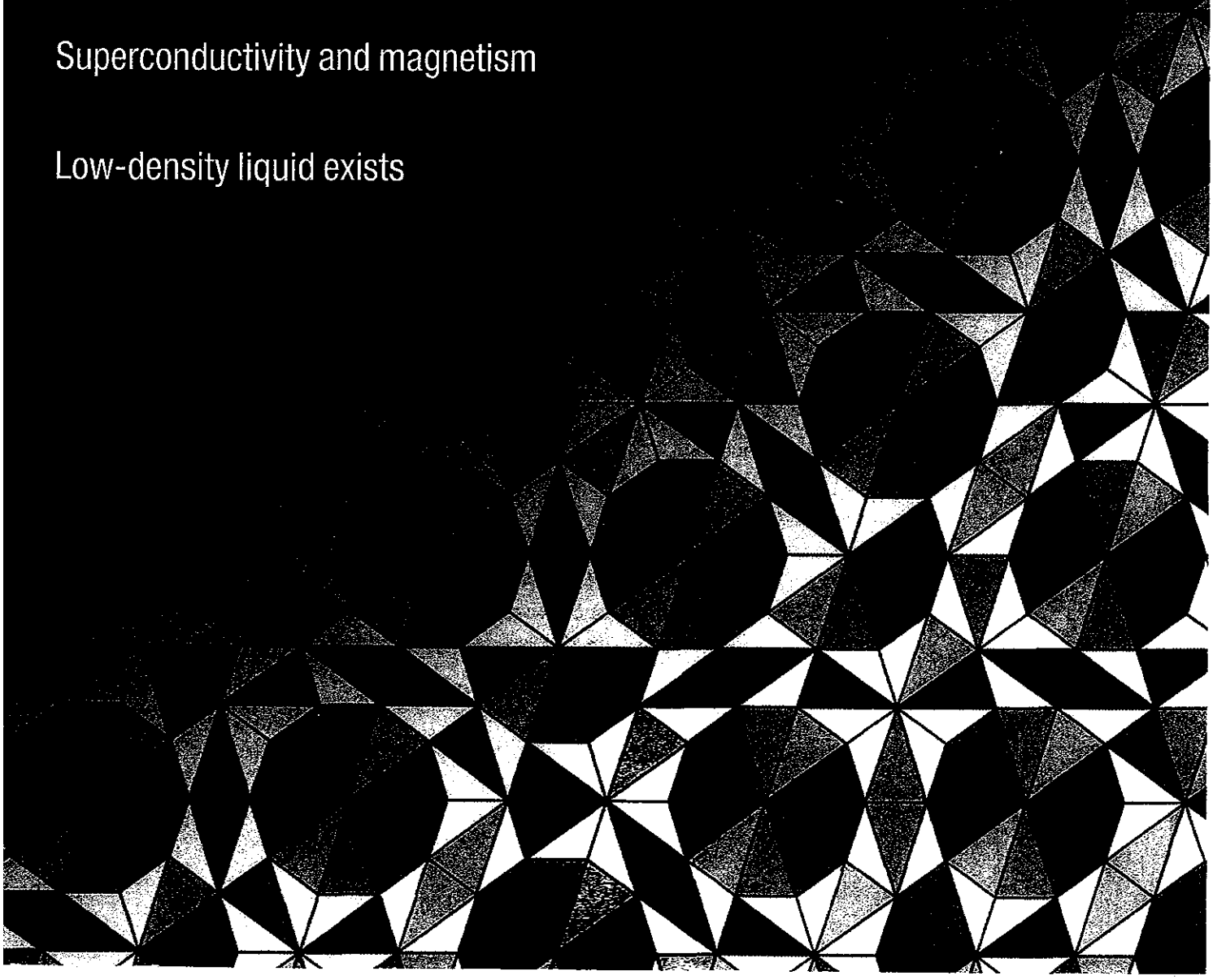
Cluster packing of quasicrystals

Graft reduces wear

Superconductivity and magnetism

Low-density liquid exists

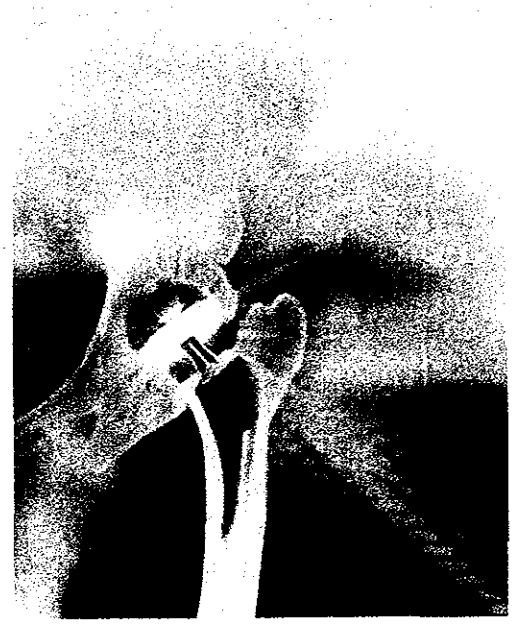


nature materials

COVER STORY

CHEMISTRY IMPROVES WEAR IN ARTIFICIAL JOINTS

Friction of metallic hip prostheses against polyethylene-lined cups in artificial joints causes bone loss through an inflammatory response to wear particles. It is a serious problem with dire consequences that ultimately can undermine improvements in implant design and surgical techniques. H. Kawaguchi and colleagues have now found a way to overcome this problem through a chemical modification of the polyethylene surface. Through a photoinduced polymerization technique they grafted a biocompatible phospholipid polymer, 2-methacryloyloxyethyl phosphorylcholine, to the polyethylene surface. This has a surprisingly positive effect on the amount of wear. Moreover, biological experiments suggest that the bone-resorption response will be avoided for wear particles from the phospholipid-grafted surface.



Grafting of a biocompatible phospholipid polymer on the articulating surface of polyethylene artificial joints drastically reduces wear and may prevent periprosthetic bone loss.

Article

Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis

TORU MORO, YOSHIO TAKATORI, KAZUHIKO ISHIHARA, TOMOHIRO KONNO, YORINOBU TAKIGAWA, TOMIHARU MATSUSHITA, UNG-IL CHUNG, KOZO NAKAMURA & HIROSHI KAWAGUCHI

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Surface grafting of artificial joints with a biocompatible polymer for preventing periprosthetic osteolysis

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Periprosthetic osteolysis—bone loss in the vicinity of a prosthesis—is the most serious problem limiting the longevity of artificial joints. It is caused by bone-resorptive responses to wear particles originating from the articulating surface. This study investigated the effects of graft polymerization of our original biocompatible phospholipid polymer 2-methacryloyloxyethyl phosphorylcholine (MPC) onto the polyethylene surface. Mechanical studies using a hip-joint simulator revealed that the MPC grafting markedly decreased the friction and the amount of wear. Osteoclastic bone resorption induced by subperiosteal injection of particles onto mouse calvariae was abolished by the MPC grafting on particles. MPC-grafted particles were shown to be biologically inert by culture systems with respect to phagocytosis and resorptive cytokine secretion by macrophages, subsequent expression of receptor activator of NF-κB ligand in osteoblasts, and osteoclastogenesis from bone marrow cells. From the mechanical and biological advantages, we believe that our approach will make a major improvement in artificial joints by preventing periprosthetic osteolysis.

Total joint replacement is the most significant advance in the treatment of osteoarthritis, rheumatoid arthritis and other arthritic diseases affecting major joints of the upper and lower extremities[1]. Despite improvements in implant design and surgical techniques, periprosthetic osteolysis causing aseptic loosening of artificial joints remains the most serious problem limiting their survival and clinical success[2].

Pathogenesis of the periprosthetic osteolysis is known to be a consequence of the host inflammatory response to wear particles originating from the prosthetic devices[1,2]. Many clinical and animal studies have shown that the most abundant and bone-resorptive particle within the periprosthetic tissues is polyethylene (PE) generated from the interface between the PE and metal components[3–5]. A key role has generally been attributed to the phagocytosis of the PE particles by macrophages, followed by secretion of prostaglandin E_2 (PGE_2) and the cytokines tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6 (ref. 6). These bone-resorptive factors induce the expression of a receptor activator of NF-kB ligand, the key member-associated molecule for osteoclastogenesis, in osteoblasts, consequently resulting in osteoclastic bone resorption[2,7,8]. Hence, reducing the production of wear particles and bone-resorptive responses may lead to the elimination of periprosthetic osteolysis. Based on this hypothesis, we prepared a novel hip PE component grafted with MPC onto its surface. The MPC polymer is our original biocompatible polymer whose side chain is composed of phosphorylcholine resembling phospholipids of biomembranes (Fig. 1a)[9]. The MPC grafting onto the surface of medical devices has already been shown to suppress biological reactions even when they are in contact with living organisms[10,11], and is now clinically used on the surfaces of intravascular stents, intravascular guide wires, soft contact lenses and the oxygenator (artificial lung) under the authorization of the Food and Drug Administration of the United States[12–14]. The present study investigated the mechanical and biological effects of the MPC grafting onto the surface of the PE component of artificial joints.

Grafting of the MPC onto the PE surface of hip acetabular liners was performed by a photoinduced polymerization technique, producing a covalent bond between the MPC and PE polymers (Fig. 1a)[15]. The stable grafting of MPC on the PE was confirmed using highly sensitive X-ray photoelectron spectroscopy (XPS; PHI5400MC, Perkin Elmer, USA) (Fig. 1b). The peaks in the carbon atom region (C_{1s}) at 286.5eV and 289 eV, indicating the ether

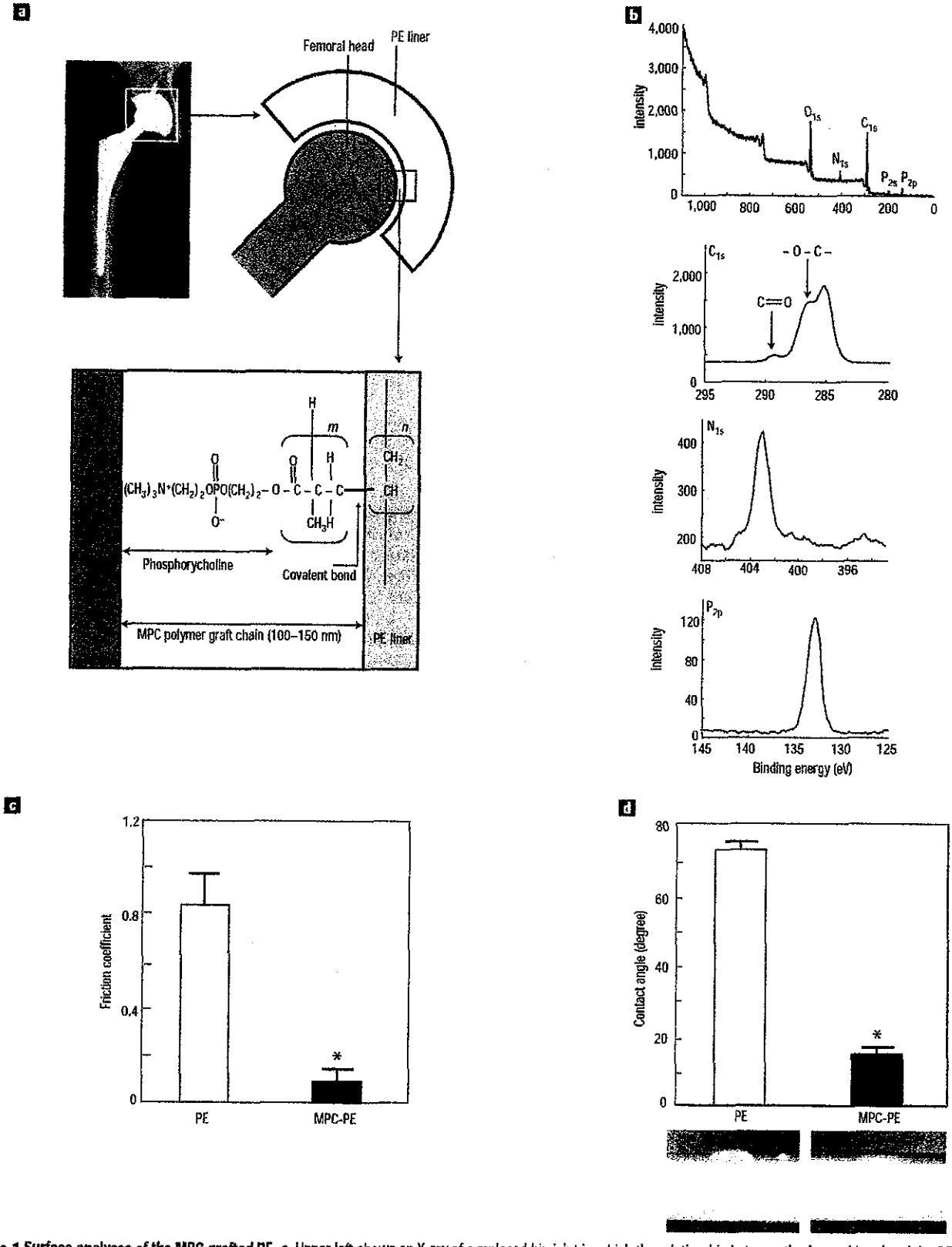

Figure 1 Surface analyses of the MPC grafted PE. **a**, Upper left shows an X-ray of a replaced hip joint in which the relationship between the femoral head and the PE liner is indicated at upper right. MPC is bound to the PE liner by the covalent bond with a photoinduced graft polymerization technique. **b**, XPS charts of the PE liner surface with the MPC grafting. The peaks in the carbon (C_{1s}), nitrogen (N_{1s}) and phosphorus (P_{2p}) atom regions are specific to the MPC, indicating successful grafting. **c**, Lubricity determined by the friction coefficient of PE plates with and without the MPC grafting (MPC-PE and PE, respectively). **d**, Hydrophilicity determined by the contact angle of a water drop with PE and MPC-PE plates. Representative pictures are shown below. Data are expressed as means (bars) ± s.e.m. (error bars) for 12 plates per group. * significant difference from PE; $P<0.01$.

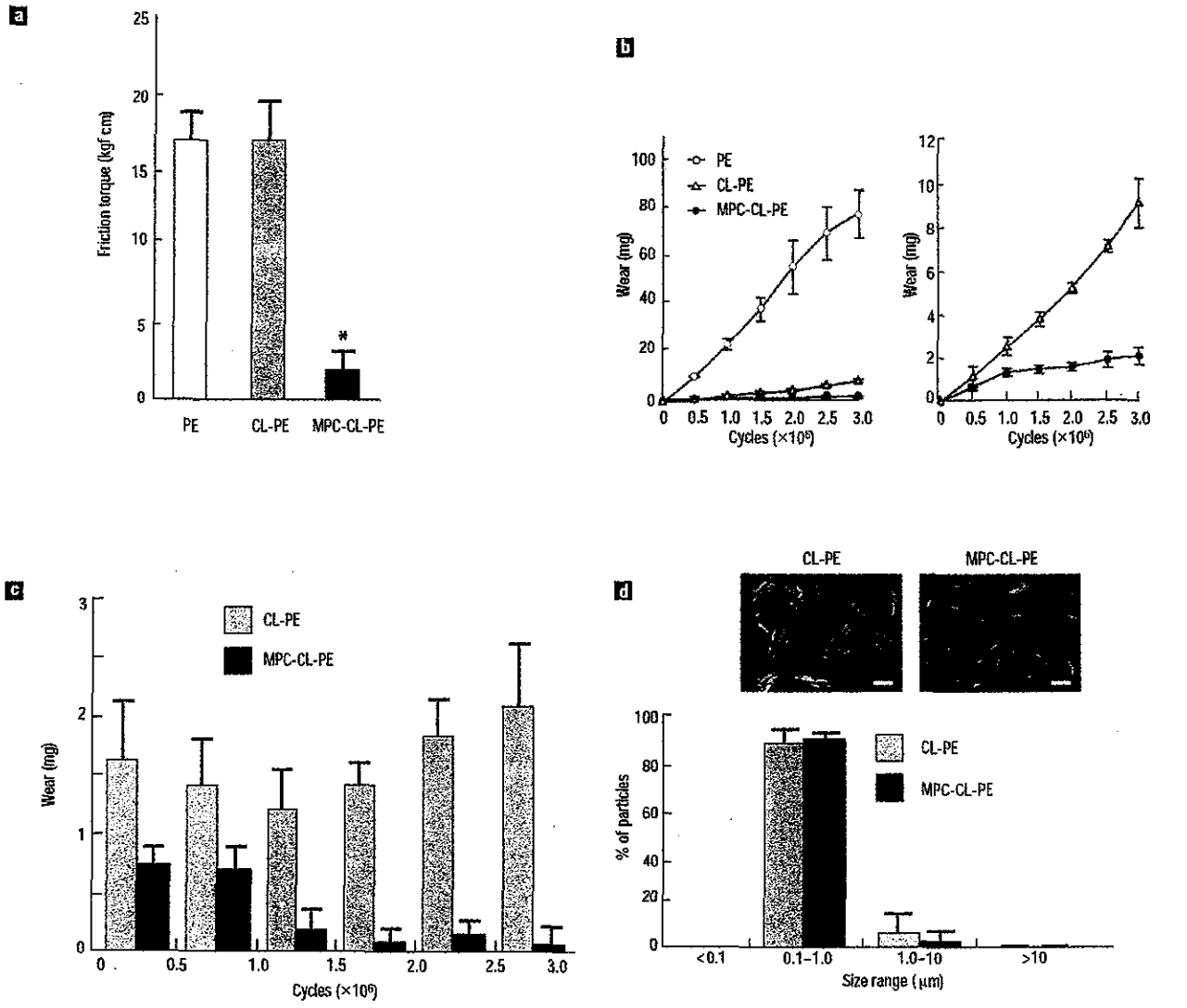

Figure 2 The friction torque and the wear amount in the hip-joint simulator with three kinds of PE liners. **a**, Friction torque of the three liners against the femoral heads measured before the loading test. **b**, Time course of the wear amount produced from the three liners during 3×10^6 cycles of loading. The CL-PE and MPC-CL-PE data are shown in an expanded scale on the right. **c**, The wear amount from the CL-PE and MPC-CL-PE liners for every 5×10^5 cycle intervals. **d**, Representative SEM images of the wear particles isolated from lubricants of the simulators with CL-PE and MPC-CL-PE liners. The graph below shows the distribution of particles in each size range. Data are expressed as means (symbols and bars) ± s.e.m. (error bars) for 10 liners per group. * significant difference from PE; $P<0.01$.

bond and the ester bond, respectively, and those in the nitrogen atom at 403 eV (N_{1s}) and phosphorus atom at 133 eV (P_{2p}) were specific to the phosphorylcholine group in the MPC unit.

To assess the lubricity and hydrophilicity, the MPC was grafted onto the PE plate (MPC-PE plate). The friction coefficient measured using a tensile test device and the contact angle of a water drop measured using the sessile drop method with a goniometer on the MPC-PE plate were about 1/7 and 1/5, respectively, of those on the non-grafted PE plate (Fig. 1c,d). These results indicate that the MPC grafting on PE greatly increases both lubricity and hydrophilicity.

Mechanical effects of the MPC grafting on the hip prosthesis were examined using a hip-joint wear simulator[16] under the conditions recommended by the International Organization for Standardization (ISO). We prepared crosslinked acetabular PE liners with photoinduced grafting of MPC onto their surface (MPC-CL-PE liner), and compared them with crosslinked PE liners without the MPC grafting (CL-PE liner) and non-crosslinked PE liners without the MPC grafting (PE liner). The friction torques of the three liners against the femoral head were compared before the loading test. There was no difference between PE and CL-PE liners; however, the MPC-CL-PE liner showed 80–90% lower torque than these two (Fig. 2a). Throughout the 3×10^6 cycles of gravimetric loading by the hip-joint simulator, the wear amount of the MPC-CL-PE liner was about 4 and 40 times less than those of the CL-PE and the PE liners, respectively (Fig. 2b). Clinically, the wear rate at the initial stage after a total hip replacement is thought to be well correlated with the incidence of periprosthetic osteolysis, because the wear particles may gain access to the articulation and accelerate the additional wear by a three-body mechanism[17]. In fact, the time-course analysis of the wear amount for every 5×10^5 cycle intervals

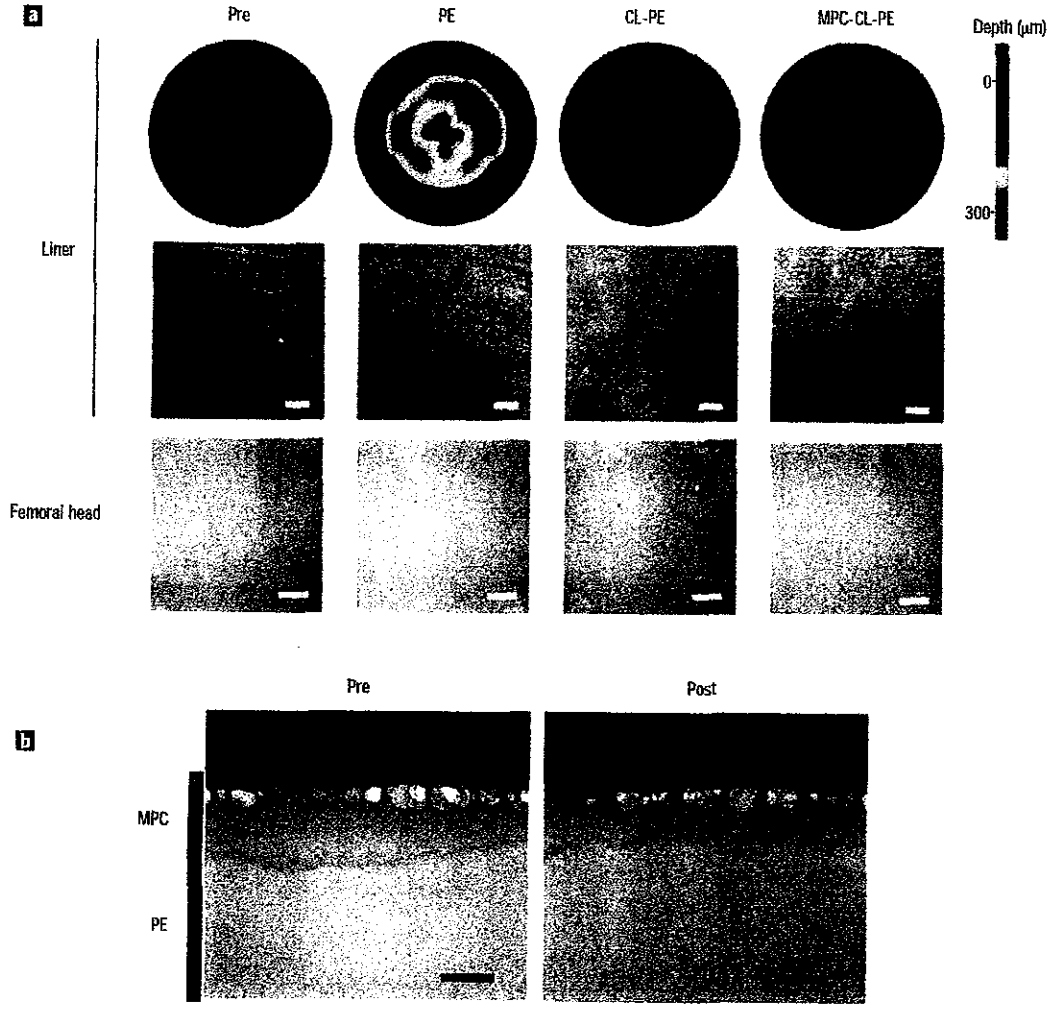

Figure 3 Optical findings of the surfaces of liners and corresponding femoral heads. **a**, Three-dimensional morphometric and SEM analyses of the liner surfaces (top and middle, respectively) and SEM analyses of the femoral head surfaces (bottom) before (Pre) and after 3×10^6 cycles of loading. Scale bars, 500 μm and 20 μm in middle and bottom, respectively. **b**, FE-TEM images of the thickness of MPC layer before (Pre) and after (Post) the loading. The bubbles on the surface were produced in the process of preparing the specimen. Scale bars, 100 nm.

revealed that the amount from the CL-PE liner was about twice as large as that from the MPC-CL-PE in the initial cycles, and somewhat increased in the later cycles (Fig. 2c). Contrarily, about 70% of the total wear amount was produced from the MPC-CL-PE liner in the initial 1×10^6 cycles, and decreased thereafter. In the last 5×10^5 cycles, the wear amount of MPC-CL-PE was less than 1/20 that of CL-PE. Although the present 3×10^6 cycles of 280 kgf (kilogram force) load is assumed equivalent to 3–10 years of physical walking, this result suggests that the mechanical effect of the MPC grafting will be maintained or somewhat more pronounced even after loading beyond 3×10^6 cycles. In fact, our preliminary simulator experiment with 1×10^7 cycles of loading revealed much stronger wear resistance by this grafting (data not shown). Scanning electron microscopy (SEM; JSM-5800LV, JEOL, Tokyo, Japan) analysis of the wear particles isolated from the lubricants revealed no significant difference of the particle size distribution between CL-PE and MPC-CL-PE liners, the great majority of which was 0.1–1.0 μm (Fig. 2d).

Optical examination of the liner surface using a three-dimensional morphometric analysis after 3×10^6 cycles of loading revealed that there was little or no wear in the MPC-CL-PE liner, whereas substantial wear was detected in the PE and CL-PE liners (Fig. 3a, top). The SEM analysis of the liner surface revealed that the original machine marks by the manufacturer's processing still remained on the MPC-CL-PE liner surface, which were completely obliterated in the two control liners (Fig. 3a, middle). Furthermore, the field emission transmission electron microscopy (FE-TEM) analysis showed that most of the liner surface was covered by the MPC polymer layer even after 3×10^6 cycles of loading (Fig. 3b). The XPS analysis also confirmed the remainder of the specific spectra of C_{1s}, P_{2p} and N_{1s} on the MPC-PE liner surface just as in Fig. 1b after the loading (data not shown). Contrarily, the SEM analysis of the femoral head showed no difference among the three groups (Fig. 3a, bottom). The femoral heads were free of visible scratches and the surface roughness expressed by the R_a values was not different before or after the loading in all groups (R_a = 0.05–0.06 μm), suggesting there was no abrasive contamination with metal particles from the heads in the hip-joint simulator.

With respect to the reduction of wear by the MPC grafting, we should consider the lubrication mechanism between the liners and

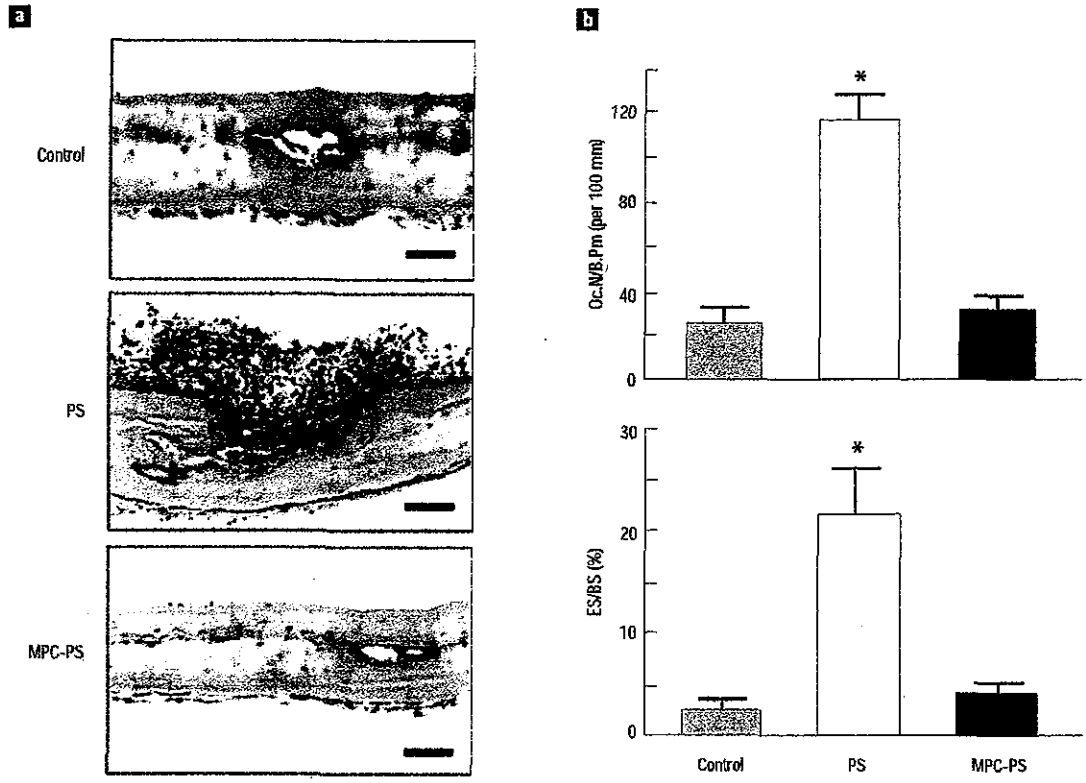

Figure 4 ***In vivo*** **bone resorption in mouse calvariae.** Resorption induced by a subperiosteal injection of an aliquot of PS particles with and without the MPC grafting (MPC-PS and PS, respectively) or an equal volume of solvent alone (control). **a**, Representative histological findings of the injected sites where osteoclasts were stained red with TRAP. Scale bars, 100 μm. **b**, Histomorphometric analyses of the injected sites: number of mature osteoclasts in 100 mm of bone perimeter (Oc.N/B.Pm; top) and percentage of eroded surfaces (ES) / bone surfaces (BS) (bottom). Data are expressed as means (bars) ± s.e.m. (error bars) for 8 calvariae per group. * significant difference from control; $P<0.01$.

metal heads of the hip-joint simulator. Although phospholipids themselves are known to work as effective boundary lubricants[18,19], recent studies of natural synovial joints have shown that fluid film lubrication by the intermediate hydrated layer is the predominant mechanism under physiological walking conditions[20]. Because the present study revealed that the MPC grafting onto the PE plate increased the hydrophilicity (Fig. 1d) and our previous study showed that the free-water fraction on the MPC polymer surface is kept at a higher level[21], the reduction of wear is likely to arise from the hydrated lubricating layer that is formed by the MPC grafting.

As PE particles are known to be most abundant and catabolic among wear particles in the periprosthetic tissues[3–5], alternative bearing surfaces have been proposed such as ceramic-on-ceramic and metal-on-metal articulations; however, these have their own potential disadvantages[22,23]. The long history and popularity of PE as a bearing surface has led to research in the development of tougher and more wear-resistant PE materials: the incorporation of short chopped carbon fibres in PE matrix (Poly II)[3,24], the extension of chain crystallite morphology with thicker lamellae and higher crystallinity (Hylamer)[25], and the creation of a three-dimensional molecular network by the crosslinking. Among them, only the crosslinking successfully improved the wear resistance and suppressed the periprosthetic osteolysis in the clinical setting[26,27]. It is therefore noteworthy that the MPC grafting onto the crosslinked PE surface further increased the wear resistance over the conventional crosslinked PE.

Considering that MPC is a biocompatible polymer, we next examined biological responses to particles using *in vivo* and *in vitro* models. The MPC polymer was grafted using a solvent-evaporation technique onto the surface of polystyrene (PS) particles whose size was approximately 500 nm in diameter, based on the result above (Fig. 2d) and previous findings[5,28] that the mean particle size from clinically failed prostheses is around 500 nm with >90% of particles less than 1 μm. The XPS spectra of C_{1s}, P_{2p} and N_{1s} on the surface of the PS particles grafted with MPC were quite similar to that of the MPC-PE liner surface as shown in Fig. 1b (data not shown). Although the surface electrical potential (ζ-potential) of the surface of non-grafted PS particles determined using electrophoretic light scattering was around –66.0 mV, the MPC grafting neutralized the potential to –2.5 mV, as we reported previously[29]. These results indicate that the MPC polymer was stably immobilized on the surface of the particles.

We first compared the *in vivo* bone resorption induced by PS particles with and without the MPC grafting using an established *in vivo* murine calvarial model[7,30]. When non-treated PS particles were injected beneath the calvarial periosteum, notable stimulations of tartrate-resistant acid phosphatase (TRAP)-positive osteoclast formation and bone resorption with inflammatory reaction were observed (Fig. 4a). However, subperiosteal injection of the MPC-grafted particles did not induce bone resorption. This effect was confirmed by histomorphometric analysis: the osteoclast number and the eroded surface of the calvarial bone that were increased