ANTIWEARABLE AND BIOCOMPATIBLE SURFACE OF ARTIFICIAL HIP JOINTS BY NANO-SCALED GRAFTING WITH PHSOPHOLIPID POLYMERS

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Introduction

The incidence of osteoarthritis and rheumatoid arthritis is on the rise due to the worldwide growth of elderly populations. Total joint replacement is the most significant advance in the treatment of these and other arthritic diseases affecting major joints of the upper and lower extremities. For example, the number of primary total hip replacements performed annually is estimated to be more than 1.3 million world-wide with 50-140 operations / 100,000 inhabitans in North American, European and Australian countries, and this is expected to continue to increase over at least the next three decades. However, despite improvements in implant design and surgical technique, periprosthetic osteolysis causing aseptic loosening of artificial joints remains as the most serious problem limiting their survivorship and clinical success. Up to 20% of patients with total hip replacement develop radiographic evidence of aseptic loosening within 10 years, about half of which require revision surgery due to pain and loss of function. However, there is no therapeutic intervention of the loosening other than the revision surgery, and the number of salvage operations whose outcomes are much poorer than the primary ones is also increasing.

Aseptic loosening relates to periprosthetic osteolysis caused by the foreign-body reaction of macrophages to wear particles (Fig. 1). Among the several potential sources of wear particles, the articulation between the acetabular liner and the femoral head is most significant. Moreover, recent studies revealed that periprosthetic osteolysis closely related to the rate of polyethylene (PE) liner wear and the characteristics of the particles. We hypothesized that creation of molecular assembly of phospholipids such as cell membrane provides excellent surface both biocompatible and low friction surfaces. That is, a grafting phospholipid polymer chains on the surface of PE liner will suppress wear and the wear particles, which will become biologically inert even when wear will be occurred. So, we prepare a novel PE liner grafted with 2-methacryloyloxyethyl phosphorylcholine (MPC[1], Fig. 2) and investigated its potential for the longevity of artificial joints.

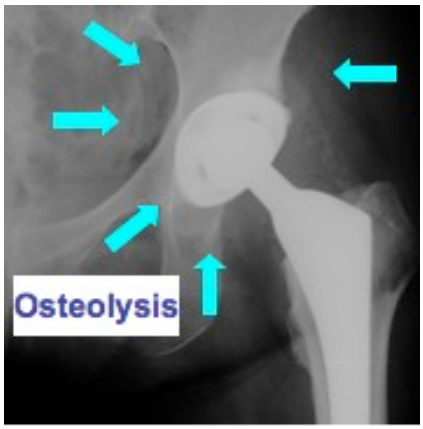

Fig. 1. Osteolysis induced by replacement with hip joints after 9-years implantation.

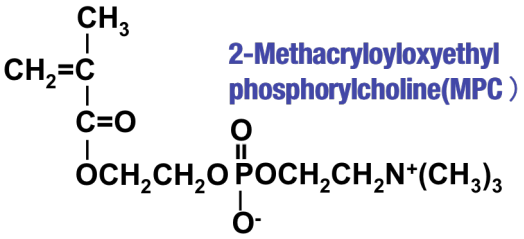

Fig. 2. Chemical structure of MPC inspired from natural phospholipids molecules at cell membrane [1].

Methods

Surface grafting of the MPC polymer on the PE liner was carried out by photoinduced polymerization using benzophenone as a photosensitizer [2]. The grafting was confirmed with X-ray photoelectron

spectroscopy (XPS), scanning electron microscopy (SEM) and wettability measurement.

For mechanical analyses, a 12-station hip joint wear simulator apparatus (MTS, MTS Systems Co. Ltd., Minneapolis, MN) with three kinds of PE liners in 42 mm acetabular cups: non-cross-linked PE liner (K-MAX®, Kobe Steel, Co., Ltd.), cross-linked PE liner (K-MAX Excellink®, *ibid*), and MPC-grafted K-MAX Excellink®, coupled to 22 mm cobalt-chromium-molybdenum alloy heads (K-MAX®HH-02, *ibid*), was mounted on the rotating blocks to produce biaxial or orbital motion. The simulator experiment was performed according to the international standard of "implants for surgery – wear of total hip-joint prosthesis –" established by ISO (#14242-1; 2002), which was proved to be closest to the physiological conditions. Briefly, a Paul-type loading profile, which is a physiological walking simulation with continuous cyclic motion and loading was applied (maximum force=280 kgf, frequency=1 Hz) in the lubricant of distilled water containing 25% bovine calf serum.

Friction torque between the liner and the femoral head was measured using a torque measuring instrument. The simulator was run up to 3 x 10^6 cycles, and the change of the lubricant and the gravimetric measurement of the liners were performed every 5 x 10^5 cycles. For the isolation of wear particles, the lubricant after the loading was incubated with 5 N NaOH solution in order to digest adhesive proteins that were degraded and precipitated, and the particles were collected and underwent sequential filtrations, as reported previously. The size of particles was defined as the maximum dimension under the SEM analysis.

Mouse macrophage-like cell line J774 cells (Riken Cell Bank, Saitama, Japan) were exposed to particles and cultured for 24 h. The supernatants were subject to cytokine and PGE_2 measurements using ELISA, and were used as the conditioned media for the following assays. For RANKL expression assay, mouse osteoblasts isolated from neonatal calvariae were cultured in the conditioned media for 24 h. RANKL expression in osteoblasts was measured using the semi-quantitative and real-time RT-PCR analyses. The information of the primers will be available upon request. For osteoclast formation assay, mouse primary osteoblasts above and bone marrow cells isolated from adult mouse long bones were cocultured in the conditioned media in the presence or absence of anti-TNF-α, anti-IL1, anti-IL-6 antibody, control non-immune serum, celecoxib, or osteoprotegerin. Cells were stained with TRAP, and those positively stained and containing more than three nuclei were counted as osteoclasts. For the statistical analysis, means of groups were compared by ANOVA and significance of differences was determined by post-hoc testing using Bonferroni's method.

Results and Discussions

The surface grafting on CLPE with the MPC polymer could proceed very well in this polymerization condition. The interface of grafted layer was observed with SEM and the picture is shown in Fig. 3. The thickness of the MPC polymer layer was 100-150 μm. The XPS analysis revealed that the surface was completely covered with the MPC polymer when the concentration of the MPC in feed solution was 0.5 M.

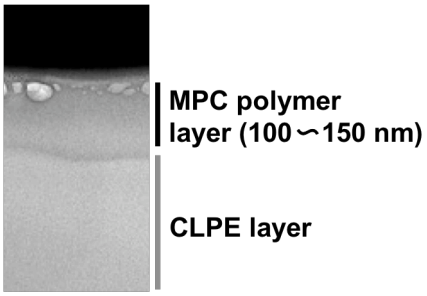

Fig. 3. Cross-section of interface between CLPE layer and MPC polymer layer grafted on the surface

The surface treated with the MPC polymer reduce contact angle of water dramatically. Throughout 1 x 10^7 cycles, the friction torque was about 90% lower in CLPE liner modified with poly(MPC) (CLPE/MPC) than CLPE liners. The friction torque of PE was as the same level as that of CEPL liner. That is, cross-linking is not affect to the surface lubrications. However, the wear of PE liner after 1 x 10^7 cycles was about 180mg, however, that of

CLPE liners showed a total weight loss of 38 ± 5 mg (Fig. 4). In contrast, MPC liners continued to gain weights, and showed a total weight gain of 2 ± 1mg. MPC liners also showed no or very little wear after correcting for weight gain due to water absorption, suggesting marked reduction in wears. The field emission-transmission electron microscope analysis showed that most of the liner surface was still covered by the MPC polymer layer even after the loading.

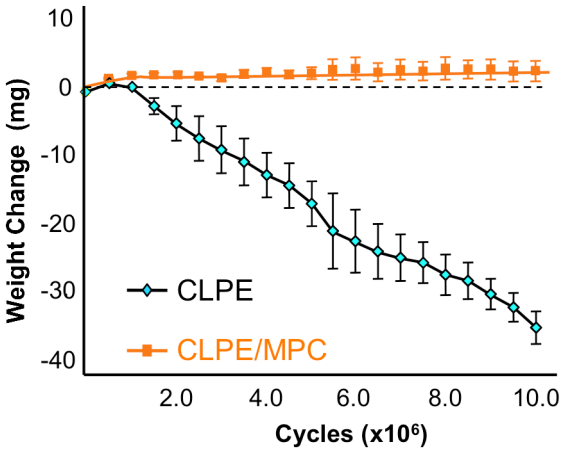

Fig. 4. Wear of CLPE/MPC polymer liners and non-treated CLPE liner during simulation experiments

In vitro murine osteolysis model, although large amounts of non-treated polymer particles were phagocytosed by macrophages, the MPC polymer particles were not taken into the cells, probably because biocompatible MPC polymer prevented macrophages from recognizing the particles as foreign bodies. Concentrations of TNF-α, IL-1, IL-6 and PGE_2 in the culture medium of mouse macrophage-like cell line J774 cells were stimulated by the exposure to non-treated particles up to 4-20 times those without the exposure; however, the exposure to the MPC polymer particles affected none of them (Fig. 5). In the mouse osteoblast culture, the receptor of NF-kB ligend(RANKL) expression was strongly induced by the conditioned medium exposed to non-treated particles, but not by that exposed to MPC polymer particles. Osteoclastogenesis from cultured bone marrow cells were about 7-fold increased by the conditioned medium of J774 cells exposed to non-treated polymer particles, and this stimulation was significantly inhibited by addition of anti-TNF-α, anti-IL-1 or anti-IL-6 antibody, a cyclooxygenase-2 (COX-2) inhibitor, and a RANKL inhibitor. Contrarily, the conditioned medium of exposed to MPC polymer particles did not increase osteoclastogenesis.

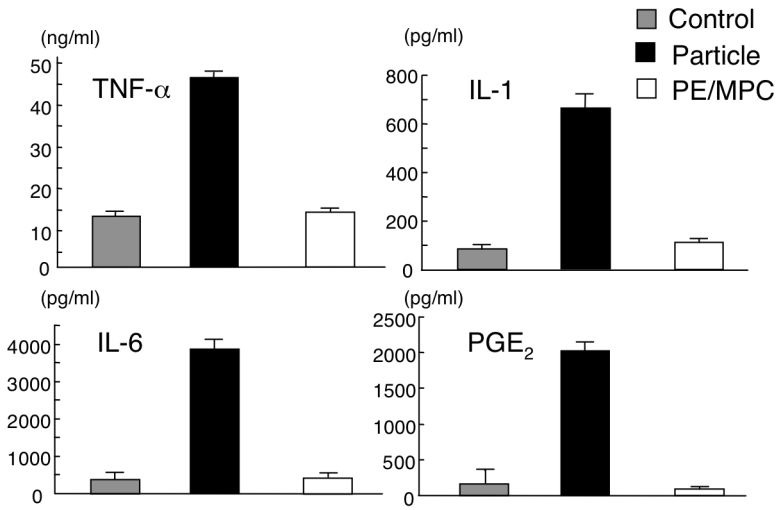

Fig. 5. Cell response by addition of PE/MPC polymer particles on cultured macrophage cells.

The treatment with the MPC polymer onto the surface of medical devices has already been approved to suppress biological reactions even when they are in contact with living organisms [3-6]. Hence, aiming at the prevention of periprosthetic osteolysis, the present study investigated the mechanical and biological effects of the MPC polymer grafting onto the surface of the PE liner of artificial hip joints.

The lines of results obtained in this study clearly demonstrate that the MPC grafting on the surface of PE liner markedly decrease the friction and the wear. In addition, even if wear particles are produced, they are biologically inert in respect to phagocytosis by macrophahes and subsequent bone resorptive actions. Furthermore, the MPC grafting can successfully inhibit the bone resorptive response

to wear particles to levels similar to those of recently developed pharmacological therapies such as cytokine antagonists, COX-2 inhibitors and osteoprotegerin, suggesting that this grafting will surpass the pharmacologic therapies that possibly cause serious side effects during a long period of administration after surgery.

Conclusions

Grafting of highly hydrophilic and biocompatible phospholipids polymer chains on PE liner of artificial hip joints provide long stability and safety based on suppression of wear between liner and stem. Photoinduced grafting technique is useful for this process. It will be applied for preparation of new-generation not only artificial hip joints but also every part, which is necessary to have lubrication in medical devices.

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