

From the Department of Orthopedics,
Osaka City University Medical School
1-5-7 Asahimachi Abeno-ku Osaka 545, Japan
Tel: + 81-6-645-2161 Fax: + 81-6-646-6260

Wear and osteolysis in total joint replacements

Yoshinori KADOYA, Akio KOBAYASHI and Hirotsugu OHASHI

ACTA ORTHOPAEDICA SCANDINAVICA SUPPLEMENTUM NO. 278, VOL. 69, 1998

SCANDINAVIAN UNIVERSITY PRESS



Oslo - Copenhagen - Stockholm - Boston

Printed in Sweden
Wallin & Dalholm
1998

Abstract

This presentation summarizes the results of our recent studies on the pathogenesis of osteolysis around total joint arthroplasties. First, interface tissues with adjacent bone were retrieved and histopathologically investigated with reference to the cells on the bone surface. Secondly, polyethylene particles were extracted with the tissue digestion method and characterized with scanning electron microscopy. Finally, an animal model for osteolysis was created and various interface conditions were compared concerning their resistance to particle migration.

Histopathological examinations demonstrated that active bone formation, regarded as a repair process, was the commonest feature, even in revised cases. They also highlighted the role played by macrophages, not as cells producing inflammatory mediators which could activate osteoclasts, but as cells primarily responsible for the bone loss in osteolytic lesions. Among the particle species present, only polyethylene particles were shown to play a significant role in macrophage recruitment and subsequent osteolysis.

A quantitative extraction of polyethylene particles showed a significant difference in the "number" of particles between osteolysis positive and negative cases whereas the "sizes" of particles were similar in these two groups. The critical number of particles for osteolysis was around 1×10^{10} particles / g tissue and the cellular reaction against phagocytosable particles accumulated over this concentration may be the prerequisite for progression of osteolysis.

The animal model for osteolysis indicated that the progression of osteolysis depends on the integrity of the bone-implant interface. We suggest that the solid fixation of the prosthesis performed by current techniques (e.g., improved cementing technique, hydroxyapatite coating) is beneficial for preventing particle migration and subsequent osteolysis.

Clinical relevance

Osteolysis induced by particulate wear debris from implant materials has been recognized as the major cause of long-term failure in total joint replacements. However, the development of preventive measures for this phenomenon has not been successful because the mechanism in which wear particles cause osteolysis is not quite clear.

On the basis of results obtained in this study, we believe that the basic strategy for addressing the problem of osteolysis is to reduce the "number" of accumulated wear particles in the interface tissues. This could be achieved either by improving the materials or the geometry of the articulating counterface. Another possibility is to increase the integrity of the bone-implant interface to prevent particle migration. It is important to note that pre-clinical testing of materials and prosthetic designs should include an analysis of the characteristics of the particle generated (e.g., size and number).

The widespread bone formation, even in revised cases, is encouraging in view of "conservative treatment" of aseptic loosening. Assuming that bone loss in aseptic loosening is not a remorseless process, some form of intervention, whether mechanical or pharmacological, might be possible to tip the balance more in favour of bone formation than resorption.

A comprehensive understanding of the bone reactions in osteolysis, including the basic mechanisms of bone loss, shown in this study, are decisive for the development of preventive measures that may minimize the clinical impact of this phenomenon.

Introduction

Osteolysis induced by particulate wear debris from implant materials has been recognized as the major cause of long-term failure in total joint replacements (TJR).^{11, 13, 25, 29, 32, 33} Wear particles of polyethylene (PE), metal, and bone cement (polymethylmethacrylate: PMMA) become deposited in the tissues between the implant and bone, forming a granulomatous tissue layer or membrane (interface tissues).^{23, 29, 37, 38} At present, it is widely believed that these particles are phagocytosed by macrophages in the tissues, and these cells in turn release inflammatory mediators which stimulate osteoclastic bone resorption.^{2, 11, 13} However, the mechanism in which wear particles cause osteolysis is still unclear because of several unanswered questions as listed below:

1. What are the essential effects of numerous particles?
2. Which are the most critical factors for osteolysis?
 - Particle species (PE, metal, PMMA).
 - Particle characteristics (size, volume or number of particles).
3. Which cells are primarily responsible for the bone loss?
 - Macrophages or osteoclasts.
4. Can we reproduce the osteolytic lesions in the animal model?

In this review, we summarise the results of our recent studies which had been addressed to these questions.^{14-17, 19, 20, 26, 27} These concern 3 topics.

Study 1. Histopathological studies on the retrieved interface tissues.

Study 2. Extraction and characterization of the wear particles.

Study 3. Animal model for osteolysis.

First, the background of each study is summarized. Then materials, methods and results are separately described. Finally, various aspects of problems including the possible methods of prevention are discussed.

Study 1. Histopathological studies of the retrieved interface tissues

This part of the study concerned the cellular mechanism of bone resorption in osteolysis with some novel approaches to the study of this field. The first original

point was that not only the whole interface membrane but also the adjacent bone was retrieved. The second point was that on frozen sections of these tissues macrophages and osteoclasts were immunohistochemically labelled. Finally, the length of the bone surfaces having these cell types adjacent to it was measured and the influence of certain parameters (the presence of osteolysis and of different particulate species) were analysed. We also investigated the microstructures of macrophages facing the bone surface to evaluate their direct bone resorption activity.

Study 2. Extraction and characterization of the wear particles

Little is known about the nature of wear particles, because most of them are too small for the resolution of conventional light microscopy. Consequently, we do not yet know which are the most critical factors for osteolysis—for example, the size, volume or number of particles. To investigate this, we extracted PE particles from the interface tissues around aseptically loosened TJR by a high-performance digestion method. The retrieved PE particles were studied with scanning electron microscopy (SEM) and the size and shape were quantified with a computerized image analysis system. Then the results were analysed with reference to the presence of osteolysis.

Study 3. Animal model for osteolysis

The lack of a reproducible animal model for osteolysis has been a major problem in studying this field. We have tried to establish a model with repeated administrations of particulate biomaterials into rabbit knee joints, where implants had been previously inserted. Using this model, we evaluated the influence of PE and alumina particles on the progression of osteolysis. This model also enabled us to compare various bone-implant interfaces (e.g., loosened cement, well-fixed cement and hydroxyapatite-coated implants) regarding the efficiency of the sealing effect, which reflects the resistance to particle migration and subsequent osteolysis.

Study 1

Histopathological study of the retrieved interface tissues

We examined 24 specimens obtained in 17 revision operations on aseptically loosened total hip replacements (THR, $n = 11$) and total knee replacements (TKR, $n = 6$). The specimens included the intact interface between the membrane and the adjacent bone (Figure 1, 2). The original joint diseases were mainly osteoarthritis ($n = 11$) and rheumatoid arthritis ($n = 4$).

Assessment of wear particles in the interface tissues

The specimens were immediately frozen in liquid nitrogen and serial sections (6–8 μm , at least 70 sections from each specimen) were cut using a heavy-duty cryostat (1720 digital cryostat, Leitz, Germany). Every tenth section was stained with hematoxylin and eosin (H&E) and the presence of wear particles was assessed using a grading system.²⁹

Since fine PE particles were invariably found somewhere in the sections when viewed at a higher magnification, we divided the presence of PE particles into two grades as follows: Grade \pm was used to indicate that PE particles were present but limited in amount and distribution. Grade + indicated the presence of abundant PE particles. Since metal and PMMA particles were rarely abundant and widespread, sections were categorized Grade + when these particles were present, and Grade – when they were absent.

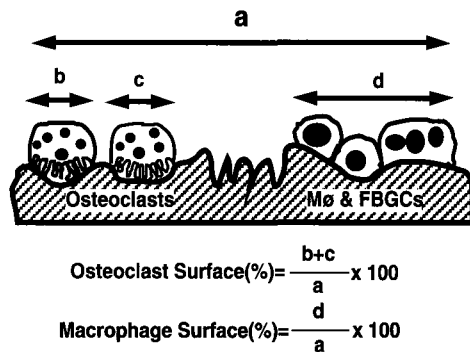
Assessment of radiographic osteolysis

The presence of osteolysis was noted in the radiographs taken immediately before the revision surgery. Osteolysis was defined as an appearance of cystic bone erosion that was absent on the immediate post-operative radiograph. Osteolysis was recorded as “positive” only when the specimen was retrieved from such a cystic bone erosion (i.e. non-linear radiolucencies around the component).

Immunohistochemical labelling and histomorphometry

On the remaining serial sections, macrophages were labelled with monoclonal antibody CD11b (Dako). Osteoclasts were labelled with monoclonal antibody to vitronectin receptor (23C6, a gift from Dr. M A Horton). Osteoblasts were identified as cells on the bone surface, with alkaline phosphatase activity. The bone surface against which each cell type was present

was measured using an image analysis and digitizing system. At least two different levels in serial sections were measured. The mean length of the bone surface measured in all the specimens was 17 (SE 1.0) mm. The results were calculated as the percentage of stained surface compared to the total bone surface measured as illustrated below. Measurement results were collated in groups according to the presence of osteolysis and the different particulate species present in the fibrous interface. Then the difference observed between groups was statistically analysed with the nonparametric Mann-Whitney U-test.



Electron microscopic (EM) study

Two specimens which had shown a large proportion of the bone surface to be in contact with macrophages were chosen for EM study and they were processed in the standard manner.

Results

Histology of the interface tissue and adjacent bone

The morphology of interface tissues, including the bone-granuloma junction, was satisfactorily preserved in undecalcified frozen sections (Figures 1 and 2). The most frequently observed feature was the bone-forming surface, identified by alkaline phosphatase activity. This appearance is referred to below as an “osteoblast surface.” Interface membrane consisted of fibrovascular tissue, with varying numbers of infiltrating macrophages (Figure 2). These macrophages contained abundant PE particles and were frequently in direct contact with the bone surface with or

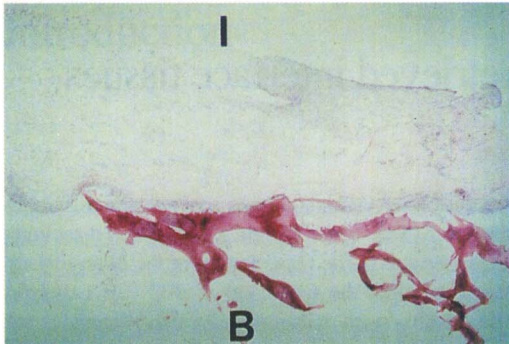


Figure 1. An example of the interface tissue. The morphology, including the bone-membrane junction, is satisfactorily preserved. I) implant side, B) bone side, H&E stain x50.

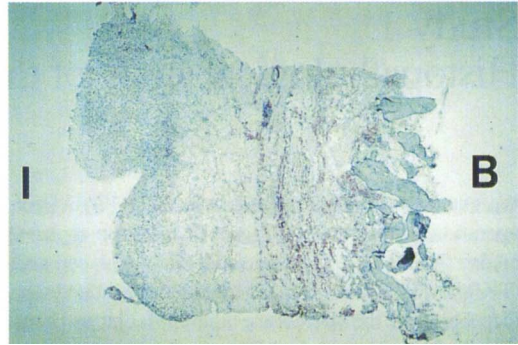


Figure 2. Another example of the retrieved interface tissue with adjacent bone. This section was stained with a macrophage marker (CD 11b). Note that labelled cells (stained red) are distributed throughout the interface membrane. B) bone side, I) implant side, immunostaining with CD 11b x30.

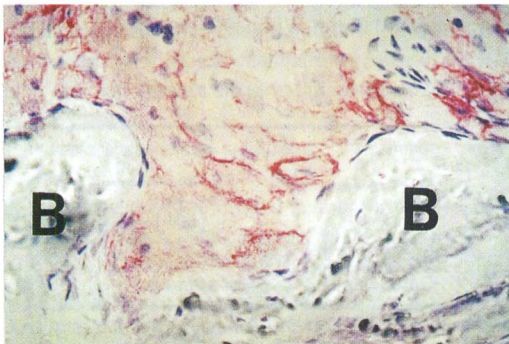


Figure 3. Macrophages and foreign body giant cells labelled with CD11b antibody (stained red). They are in direct contact with the bone surface, with or without the interposition of lining cells (resting osteoblasts). B) bone, immunostaining with CD11b, x200.

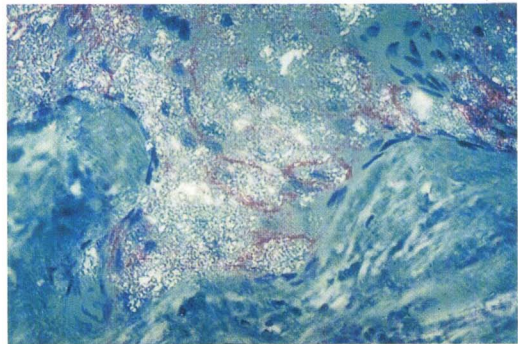


Figure 4. Polarized picture of the same field shown on the left. These cells contain abundant fine birefringent PE particles indicating that these cells are macrophages, x200.

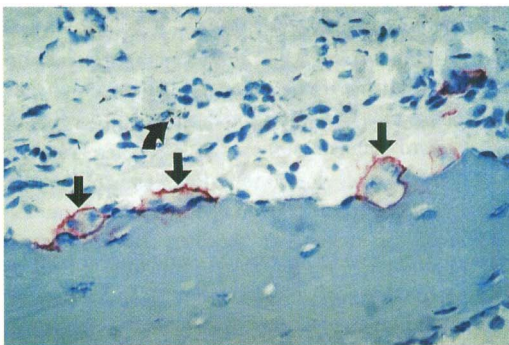


Figure 5. Osteoclasts on the bone surface (arrows) clearly identified by their membranous expression of vitronectin receptor. Note the presence of fine metal particles adjacent to the bone surface (curved arrows). Immunostaining with 23C6, x600.

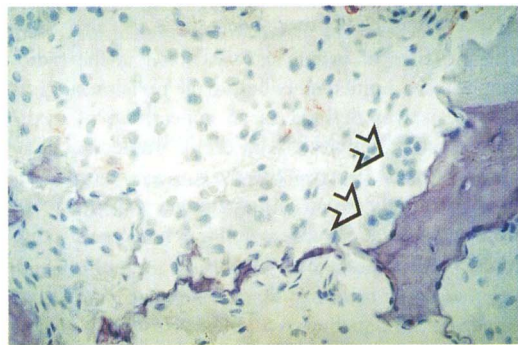


Figure 6. Osteoclasts (white arrows) are completely negative for CD 11b antibody. Immunostaining with CD11b, x600.

without the interposition of lining cells (resting osteoblasts) (Figure 3 referred to below as a “macrophage surface”). Osteoclasts were identified by their mem-

branous expression of vitronectin receptor and distinguishable from macrophages because they are negative for CD11b antibody¹⁴ (Figures 4 and 5, referred

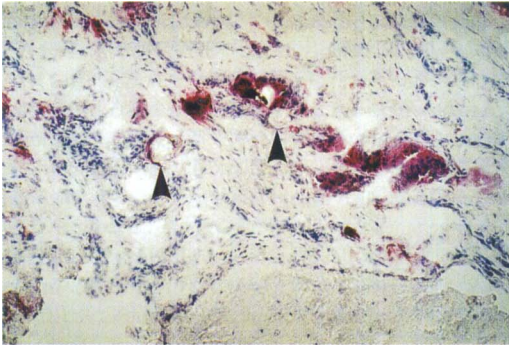


Figure 6. PMMA particles in the interface tissue (arrowheads). They are generally large (from 30 to several hundred μm) and round or oval in shape. These particles mainly provoke a foreign body giant cell reaction with some surrounding fibrosis and are hardly ever seen in the cytoplasm of macrophages, H&E, x200.

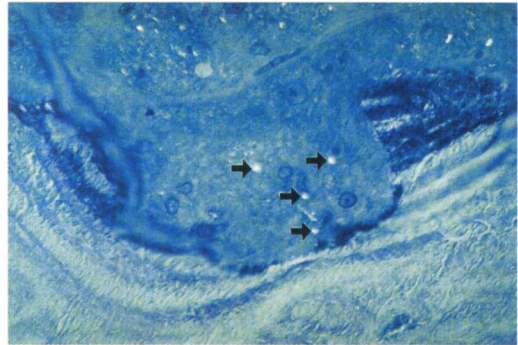


Figure 7. Polarized picture of macrophage-bone interface at semi-thin section level. Note that they contain intracytoplasmic, fine birefringent PE particles (arrows) and are morphologically eroding the bone surface, toluidine blue, x600.

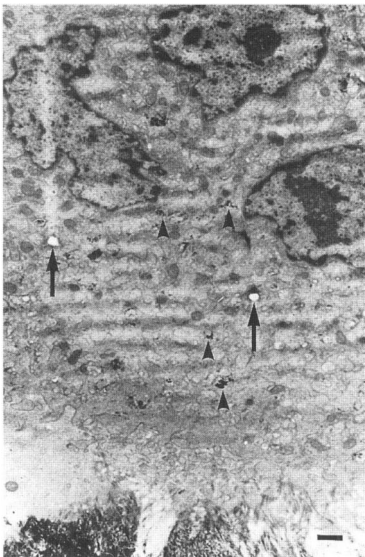


Figure 8. TEM picture of foreign body giant cells in contact with the bone surface (bottom). Note the presence of submicron particles of PE inferred from the voids in cells (arrows) and numerous metal particles which can be seen directly (arrowheads), x7800, bar = $1\mu\text{m}$.

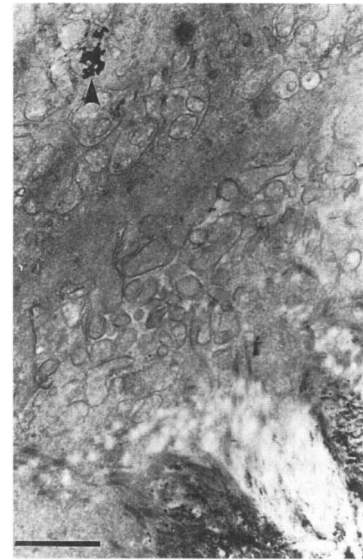


Figure 9. Many of the foreign body giant cells showed a folded surface membranous structure facing the bone (lower right), which appears to be undergoing dissolution. Metal particles are present (arrowhead), x22400, bgr = $1\mu\text{m}$.

to below as an "osteoclast surface"). PMMA particles mainly provoked a giant cell reaction with some surrounding fibrosis and were hardly ever seen in the cytoplasm of macrophages (Figure 6).

In semi-thin sections for EM study, the cells with intracytoplasmic, fine birefringent PE particles were morphologically eroding the bone surface (Figure 7). On EM examination, the presence of submicron particles of PE was inferred from the presence of voids in cells with abundant mitochondria and intracytoplasmic vacuoles. These particle-containing cells were in close contact with the bone surface, which appeared to be undergoing dissolution. Submicron metal particles could be seen directly and they were frequently

membrane-bound (Figure 8). At higher magnification, many of these cells showed a folded surface membranous structure facing the bone (Figure 9).

The results of histomorphometry (Table 1, Figures 10 and 11)

An osteoblast surface was by far the commonest feature, measuring mean 33 (SE 5.2)% of the total bone surface. An osteoclast surface was measured to be mean 7.7 (SE 1.8)%. The macrophage surface was the second commonest feature with mean 19 (SE 5.2)% of the total bone surface measured (Figure 10).

Tissues retrieved from implantation sites with radiographic osteolysis had a significantly larger macro-

Table 1. Summary of histomorphometry, mean \pm SE (median)

	n	Macrophage surface (%)	Osteoclast surface (%)
Total	24	19 \pm 5.2 (9.3)	7.7 \pm 1.8 (6.4)
Osteolysis -	12	5.3 \pm 1.3 (3.5) ^a	9.7 \pm 4.1 (7.1)
Osteolysis +	12	33 \pm 8.6 (21) ^a	6.2 \pm 1.0 (5.4)
PE \pm	7	3.2 \pm 1.2 ^b (2.7)	7.0 \pm 1.9 (8.7)
PE +	17	26 \pm 6.7 ^b (12)	7.9 \pm 2.3 (6.1)
Metal -	14	23 \pm 7.3 (11)	5.1 \pm 1.0 ^c (3.2)
Metal +	10	15 \pm 7.2 (3.5)	13 \pm 4.7 ^c (8.7)
PMMA -	20	18 \pm 5.7 (9.2)	8.3 \pm 2.1 (6.8)
PMMA +	4	24 \pm 14 (15)	3.9 \pm 2.5 (2.7)

^a p = 0.007, ^b p = 0.01, ^c p = 0.03; Mann-Whitney U-test

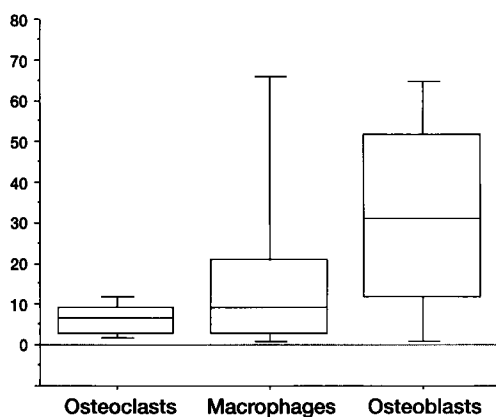
% of the total bone surface

Figure 10. Overall proportions of the bone surface in contact with each cell type. The upper T-bar of the box plot indicates the 90th percentile, the box is the 75th, median and 25th percentile, while the lower T-bar indicates the 10th percentile.

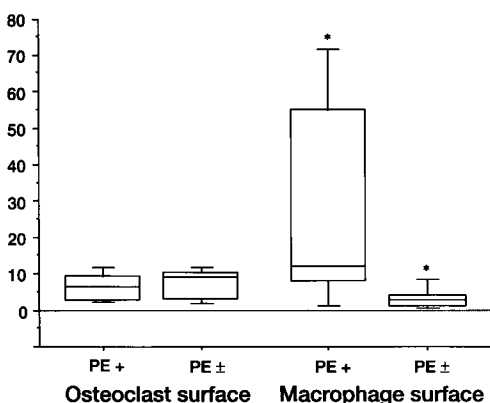
% of the total bone surface

Figure 11b. The influence of polyethylene (PE) particles. *P = 0.01; Mann-Whitney U-test.

phage surface (mean 33 [SE 8.6]%; n = 12) than those without osteolysis (5.3 [SE 1.3]%; n = 12) (p = 0.007; Figure 11a). Tissues from osteolytic sites tended to have less osteoclast surface (6.2 [SE 1.0]%) than those without osteolysis (9.7 [SE 4.1]%), although this difference was not statistically significant.

In the tissues with abundant PE particles, the macrophage surface was significantly more extensive (26 [SE 6.7]%; n = 17) than those with fewer PE particles (7.9 [SE 2.3]%; n = 7) (p = 0.01; Figure 11b). In contrast, no significant difference was observed in the amount of osteoclast surface between these two groups.

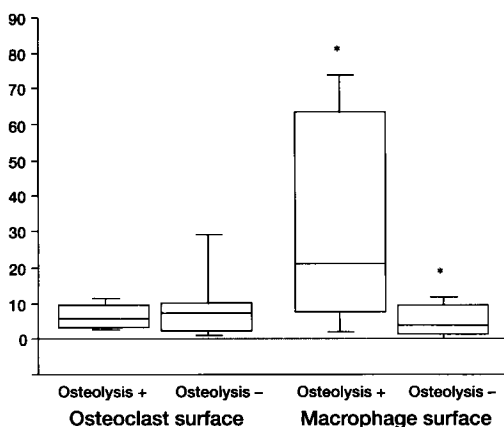
% of the total bone surface

Figure 11a. Comparison between osteolysis positive and negative groups. *P = 0.007; Mann-Whitney U-test.

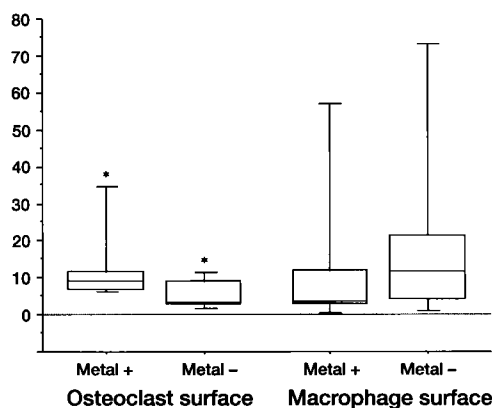
% of the total bone surface

Figure 11c. The influence of metal particles. *P = 0.03; Mann-Whitney U-test.

In the tissues where metal particles were present, the osteoclast surface was significantly larger (13 [SE 4.7]%; $n = 10$) than in those without metal particles (5.1 [SE 1.0]%; $n = 14$) ($p = 0.03$; Figure 11c). The tissue with metal particles showed a tendency to have a smaller macrophage surface, although this difference did not reach statistical significance ($p = 0.4$).

PMMA particles were found in only 4 sections in very limited amounts. The presence of PMMA particles in the tissue ($n = 4$) had no significant influence on either the osteoclast or macrophage surface.

Since the association of macrophage surface with PE particles could be caused by other factors, a stepwise

regression analysis was carried out. This showed that PE particle was the only significant variable ($p = 0.04$) and the presence of metal, PMMA particles as well as the duration of the implantation had no significant relation to the macrophage surface. The same analysis was carried out for the osteoclast surface and metal particles. This showed that the relationship of metal particles and osteoclast surface was of borderline statistical significance ($p = 0.07$) and the presence of PE, PMMA particles and duration of implantation showed no significant relationship to the osteoclast surface.

Study 2

Extraction and characterization of the wear particles

PE particles were extracted from 18 interface tissues from revisions of 10 TKRs and 8 THRs according to a refinement of the basic method of Campbell et al.^{5,6} The weight of the tissues and volume of the solutions applied were accurately measured for the calculation of the number of PE particles. The extraction methods are summarized in Figure 12. Briefly, the tissues were digested by 5N sodium hydroxide (NaOH). The resulting sample was applied to a sucrose density gradient (5, 10, 20 and 50%) and ultracentrifuged at 40,000 rpm (106,000 g). The top layer was collected and hot distilled water added to dilute the sucrose. After ultrasonication for 10 min., the solution was applied to 2 layers of isopropanol-water mixture (density: 0.90 and 0.96 Mgm⁻³), and ultracentrifuged again. Particles at the interface of the two layers were collected, and distilled water was added to dilute appropriately and disperse the PE particles homogeneously on the filter during filtration through a 0.1µm polycarbonate filter. The filter was dried and attached to a carbon stub, then coated with gold for scanning electron microscopy (SEM). At least 100 particles were counted in the SEM photographs for each sample, and the particle size was defined by the equivalent circle diameter (ECD: the diameter of a circle having the same area as the measured feature) with a computerized image analyser.

The purity of extracted PE particles was validated by Fourier transform infrared spectrometer (FTIR), energy-dispersive X-ray analysis (EDAX) and a control study using capsule tissue retrieved at primary TJR. FTIR showed a typical spectrum of PE and EDAX revealed few contaminations of the particle species (i.e., metal, PMMA and bone). Furthermore, it was confirmed that very few organic particulate debris were contaminated in the control study. Thus we concluded that the vast majority of the particles we extracted were, indeed, PE.

Another control study, using commercially available PE powder and capsule tissue, enabled us to determine the "PE particle retrieval ratio" of this extraction method. The number of the particles per 1 g peri-implant tissue was calculated by the number of PE particles in the SEM photographs, the area of SEM photographs, the dilution ratio and retrieval ratio with this extraction method. The presence of osteolysis was evaluated using the same methods as used in Study 1.

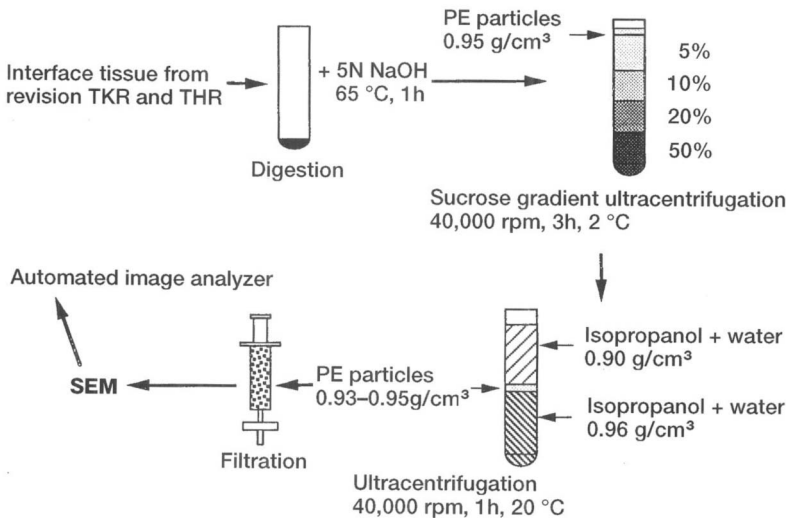


Figure 12. The methods for polyethylene particle extraction.

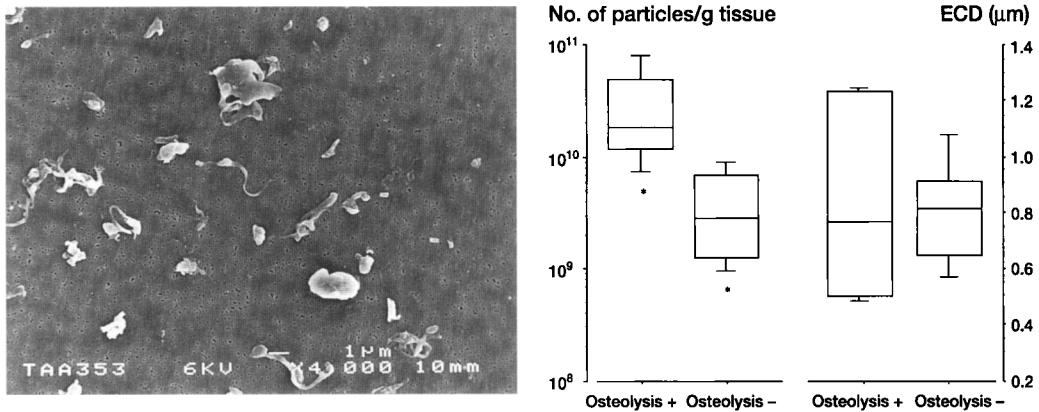


Figure 13. SEM photograph of retrieved PE particles. The pore size of the filter is $0.1\mu\text{m}$. Note that the majority of the particles are submicron in size. Some particles have an elongated shape. * $P = 0.002$; Mann-Whitney U-test.

Results

The ECDs of all 18 cases ranged from 0.48 to $1.32\mu\text{m}$ (mean = 0.82 [SE 0.06] μm ; median = $0.82\mu\text{m}$; $n = 18$). The average number of particles was $1.42 \times 10^{10}/\text{g}$ tissue (median = 7.01×10^9 ; $n = 18$). The number of particles in the osteolysis positive group (n

= 6) was significantly greater than that in the osteolysis negative group ($n = 12$; $p = 0.002$; Mann-Whitney U-test), whereas no significant difference between these two groups was observed in ECD (Figure 13).

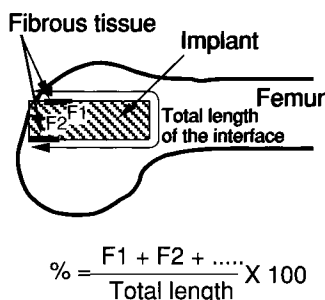
Study 3

Animal model for osteolysis

Part 1. Establishment of a rabbit model for osteolysis

A hole (3.5mm in diameter) was drilled in the intercondylar notch of the femur in 9 Japanese white rabbits (male, BW = 3 kg). The cavity was washed with saline and then doughy PMMA (Surgical Simplex-P®, Howmedica) was pressurized into it using a small syringe. 5 mg of PE particles (less than 150 µm in diameter), dispersed in sodium hyaluronate, were injected into both knee joints (6 animals, 12 knees). This procedure was repeated at 2, 4, 6, 8, 10 and 12 weeks after the implantation. 3 other animals (6 knees) served as controls, since sodium hyaluronate alone was injected at the same interval. All animals were killed 2 weeks after the last injection (14 weeks after the implantation).

The distal part of the femur was retrieved and fixed in 10% buffered formalin. After decalcification, they were embedded in paraffin and sagittal sections were cut at the centre of the implant. H&E stained sections were examined with a microscope, special attention being paid to the amount of interposed fibrous tissue. The length of interposed fibrous tissue against the total length of bone-cement interface was measured with the digitizing system connected to a personal computer. The results were calculated as the percentage of interposed fibrous tissue between PMMA and bone compared to the total length of the interface (see below) and statistically analysed by the Mann-Whitney test.



Results

Most PE particles were observed in the thickened synovial membrane forming small nodules. Some thin fibrous tissue was invariably found between PMMA and bone (Figure 14). PE particles elicited intense macrophage reactions and were associated with resorption on the adjacent bone surface (Figure 15). The extent of fibrous tissue interposition in a PE particle-injected knee increased to mean 16 (SE 10)% of the total bone-implant interface compared to that of the control group (mean 8.3 [SE 7.7]%) and this difference approached statistical significance ($p = 0.08$).

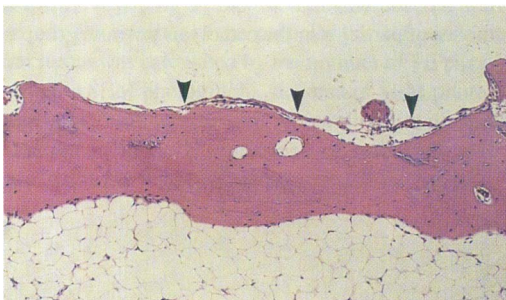


Figure 14. Thin fibrous layer (arrowheads) interposed between PMMA plug (upper half, dissolved in embedding procedure) and bone (lower half), H&E, x100.

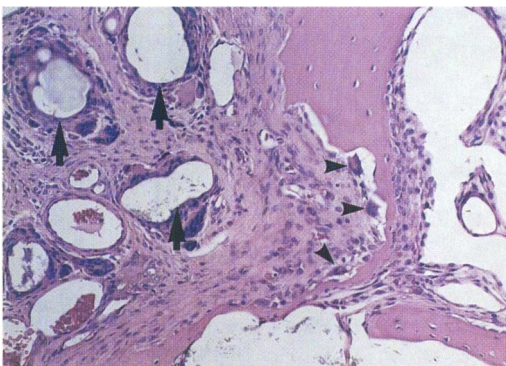


Figure 15. Picture of the bone-PMMA interface near the articular surface. PE particles (arrows) are surrounded by foreign body giant cells and intense macrophage reactions are observed. Osteoclastic bone resorption is present on the adjacent bone surface (arrowheads), H&E, x200.

Part 2. Resistance of bone-implant interface to osteolysis

19 rabbits were operated on using the same method as in Part 1 (drill size was increased to 3.8 mm in diameter) and they were divided into three groups as follows;

1. PMMA plug group (PLUG group, 6 rabbits, 12 knees): PMMA was pre-polymerized into cylindrical form (3.5 mm Ø x 10 mm) and then implanted.
2. PMMA injection group (INJECTION group, 9 rabbits, 18 knees): Doughy PMMA was pressurized into the holes using a small syringe.
3. Hydroxyapatite plug group (HA group, 4 rabbits, 8 knees): A porous hydroxyapatite plug (3.5 mm Ø x 10 mm, Sumitomo Pharmaceutical Co., Japan) was implanted. The pore size was 50–300 µm and the porosity was 42%.
4. Control group (4 knees in PLUG group, 10 knees in INJECTION group and 4 knees in HA group): Each material was implanted and no particles were challenged.

50 mg of PE and alumina particles (diameter: 170 ± 18 µm and 2 ± 1 µm, respectively) were implanted in the knee joints with capsulotomy (PE in 12 knees, alumina in 8 knees). The number in each group is shown in Figure 16). This procedure was started at 1 month after the implantation and repeated 6 times at 1-month intervals. All animals were killed at 7 months after implantation and analysed in the same manner as in Part 1.

Results

Thin fibrous tissue was frequently observed adjacent to PMMA in the PLUG group. When PMMA was injected with pressure (INJECTION group), interdigitation with cancellous bone was obvious and direct bone-PMMA contact was observed to some extent. In the HA group, widespread new bone formation into the porous structure of the hydroxyapatite plug was observed and fibrous tissue interposition was hardly seen.

The results of histomorphometry are shown in Figure 16. Even in the absence of the particles, the PLUG group had the most widespread fibrous interposition and the HA group had the least ($p < 0.05$). When the PE particles were administered, the fibrous interposition was significantly increased only in the PLUG group ($p < 0.05$), while it remained unchanged in the INJECTION and the HA groups. In contrast, alumina particles did not change the extent of interposed fibrous tissue in all groups. PE particles induced significantly more widespread interposed fibrous tissue than did the alumina particles in the PLUG group ($p < 0.05$). In summary, significant differences in the extent of fibrous tissue interposition were observed according to the condition of the interface as well as to the species of challenged particles.

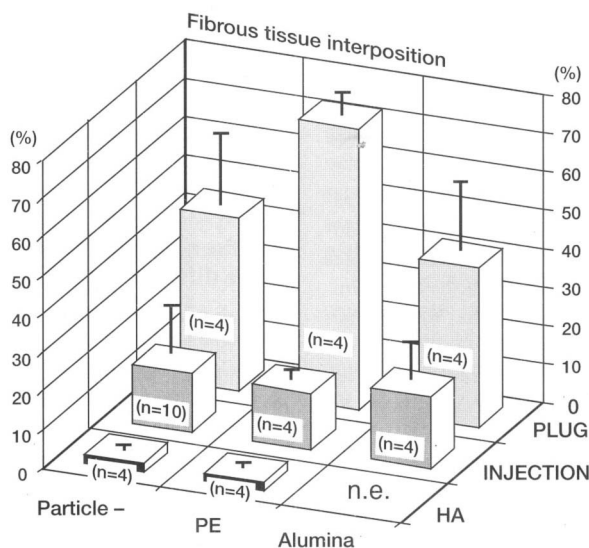


Figure 16. The results of histomorphometry. n.e. = not examined in this study.

Discussion

The results of our studies can be discussed under different headings as follows;

- 1) What are the effects of numerous particles?
- 2) Are the responses to particles characteristic of the different species of material?
- 3) Is osteolysis related to the number or size of particles?
- 4) In view of the above, what can be done to modify the osteolytic process?

What are the effects of numerous particles?

Although there is a large volume of literature on the morphology of interface tissues in aseptic loosening, most attention has been paid to the cells producing various cytokines or other biochemical molecules. Only a few papers have described the morphology of the bone to some extent^{9, 23, 37} and none of them have dealt with the cellular activity on the bone surface. One reason is the difficulty of obtaining the tissue samples including bone at revision surgery. Another difficulty is the processing and sectioning of hard tissue. The method employed in this study was to use undecalcified frozen sections of bone and this is, so far as we know, the first study that has investigated the cellular events taking place on the bone surface and quantitatively analysed them.

With these methods, we addressed an essential question: Is bone resorption mediated by osteoclasts or macrophages? Concerning this point, various problems should be addressed separately.

The first one is whether macrophages are capable of resorbing bone. Several recent *in vitro* studies have shown that inflammatory macrophages can resorb bone albeit at a low rate.^{3, 28} The important findings in this study are that direct macrophage-bone contact is frequently present *in vivo* and that these cells have microstructures which are compatible with an ability to resorb bone directly.

The next question is: How much bone surface is in contact with these cells. No literature which deals with this question. Here we have demonstrated first that the bone surfaces covered by these non-osteoclastic cells, on average, comprise about one fifth of the total bone surface which is by far larger than ex-

pected. Considering this observation, we suggest that the amount of bone removed by these cells could be substantial, even if the bone resorption rate of each cell might be low.

The final important question is: Which cells are predominant in the area where osteolysis is in progress? We have demonstrated that the existence of radiographic osteolysis is closely related to the proportion of the bone surface covered by macrophages. In contrast, no significant difference was observed in the proportion of osteoclastic bone resorption between the groups with and without osteolysis. Combining these findings, we believe that macrophages play a more significant role than osteoclasts especially in the progression of osteolytic lesions.

These observations, in turn, give rise to a question about the role played by some of the inflammatory mediators produced by activated macrophages, which are now commonly believed to result solely in osteoclast activation and subsequent osteolysis. These inflammatory mediators (especially cytokines) have been shown to be produced by many kinds of cells and have diverse actions on various cells. Considering this apparent redundancy and diversity of cytokine action, it is, at least, not a wise approach to relate their presence solely to osteoclast activation and bone loss.

We again emphasize that a correct understanding of these cellular events taking place at the bone surface, as distinct from elsewhere in the interface membranes, must form the basis for an understanding of aseptic loosening.

Are the responses to particles characteristic of the different species of material?

It is well known that tissue reactions to particulate types of foreign materials vary depending on the species, number, shape and size of them.^{8, 35, 36} Although increasing evidence has suggested that submicron PE particles are central to the pathogenesis of osteolysis,^{32, 33} the relative contribution of each of the particulate species to the overall bone loss is still controversial. We have demonstrated in Study 1 that among the three particulate species studied, only PE particles have a significant influence on the area of the macrophage attachment to the bone surface and that, in con-

trast, PE particles have no significant effect on the extent of osteoclastic bone resorption. These observations, combined with the close relationship between osteolysis and macrophage-bone contact also shown in this study,^{15, 16} permit two important conclusions. First, PE particles are primarily responsible for macrophage recruitment and attachment onto the bone surface which leads to osteolysis. Secondly, the significance of macrophages in the context of bone loss is not as cells producing inflammatory mediators which could activate osteoclasts, but as cells which could resorb bone directly.

Several studies have demonstrated the significant role of metal particles in the failure of implants.^{1, 12, 18, 24, 30} The more complex prostheses become, by modularity and supplementary screw fixation of the components, the more likely the generation of metal particles from their junctions. The observed influence of metal particles on osteoclastic bone resorption can be interpreted in two ways. One possibility is that the fixation stability of those implants around which metal particles are present might be different from those without metal particles. Metal particles might be produced predominantly in more unstable implants (e.g., with gross movement between components and bone), in contrast to PE particles which could be generated from the articulation surface even in well-fixed implants. Thus enhanced osteoclastic resorption could be explained as being related to the enhanced remodelling around more unstable implants. The significantly shorter duration of the implantation of prostheses surrounded by metal particles indicates the premature failure of the implants and may support this hypothesis (data not shown). Another possibility is that metal particles have different effects on osteoclast recruitment and activation. It is reported from our group that an immunological response²¹ and the production of inflammatory mediators, such as IL-1 β ² is more apparent in the presence of metal particles, especially titanium. If this is the case, it is conceivable that these mediators change the coupling process into a resorption-dominant state, which might explain extensive bone loss in metallosis.

PMMA particles were considered to be a main cause of the foreign body reactions and subsequent bone loss observed around cemented implants.³² Many investigators have suggested the importance of PMMA fragmentation in implant loosening and associated osteolysis.^{11, 28, 38} However, there is a paucity of data on the actual size and shape of these particles because they are readily soluble in the organic solvent used in routine histological processing procedures. So far as we know, only a few studies of intracytoplasmic PMMA particles have been done using reliable meth-

ods.^{11, 22} Since the sections used in this study were not treated in organic solvent, PMMA particles remained undissolved, and we could observe their actual size and shape. Three important points about the nature of PMMA particles have been demonstrated. First, the presence of PMMA particles is not a common finding even in the interface tissues around loosened cemented implants. Even if they are present, their amount and distribution are quite limited. Secondly, most of these particles are relatively large, oval or round in shape, and are usually surrounded by foreign body giant cells rather than phagocytosed by macrophages. Thirdly, the presence of PMMA particles has no significant influence on either macrophage or osteoclast coverage of the bone surface. We cannot completely exclude the possibility that small PMMA particles might be missed with the methods used in this study. However, we believe it is unlikely, because the tissues retrieved from cemented prostheses, as a whole, showed no significant difference in the proportions of osteoclast or macrophage surfaces, compared to those from uncemented ones (data not shown). Combining these observations, we conclude, at least in the tissue specimens examined in this study, that PMMA particles cannot be regarded as a potent stimulatory factor for bone resorption. We suggest that the importance of fragmented PMMA in the context of osteolysis may have been over-emphasized without paying enough attention to their actual size and distribution.

Is osteolysis related to the number or size of particles?

Light microscopic examinations under polarized light or with oil red O staining demonstrated the presence of fine PE particles.^{10, 29, 31, 34} However, most PE particles are too small to be characterized by light microscopy. Hence, it is necessary to employ higher-resolution methods, such as extraction and characterization with SEM, which we used in this study.

The accurate characterization of retrieved PE particles has some important consequences. First, detailed examination of particles provides some information on the wear mechanism in TJR as a basis for improving the articulating materials and geometry. Secondly, although there is a large literature concerning the biological reaction against biomaterial particles *in vivo* and *in vitro*, the particles used in such studies have not always been similar to those seen in the failed TJR. An elucidation of the nature of wear particles would provide useful information for such studies, and the particles themselves, retrieved by such a high-

performance method as we used in this study, could be available and more appropriate in such experiments.

We have demonstrated that PE particles accumulated in the interface tissues are extremely small (mean = 0.82 μm) and that they are present in large amounts (range: 5.2×10^8 to 9.2×10^{10} / g tissue, mean = 1.4×10^{10}). A more important point shown in this quantitative extraction is that a significant difference was observed in the "number" of PE particles between osteolysis positive and negative cases, but the "size" of particles was similar in these two groups. The critical number of PE particles for osteolysis is around 1×10^{10} particles/g tissue because tissues containing PE particles above this number ($n = 5$) invariably originated from osteolytic lesions. Thus we suggest that the cellular reaction against phagocytosable PE particles which are accumulated over 1×10^{10} particles/g tissue could be the prerequisite for the progression of osteolysis.

In view of the above, what can be done to modify the osteolytic process?

From the results in this study, we believe that the basic strategy for addressing the problem of osteolysis is to reduce the "number" of accumulated wear particles in the interface tissues. Here we suggest three possibilities to reduce the number of wear particles, as follows: First, improvement of the materials and geometry of the articulating counterface might reduce the number of wear particles generated. Secondly, although there are some reports concerning the dissemination channel of particles through lymphatics to the adjacent and remote lymph nodes^{4,7} the clearance system of wear particles and its efficacy remain unknown. Thus, it might be possible that activation of this clearance system reduces the number of wear particles accumulated in the interface tissue. Finally, as demonstrated in Study 3, the access pathway of the wear particles into the interface between the bone and implant may be important. If a bioactive

surface with osseointegration (e.g., hydroxyapatite coating) has a more efficient sealing effect at the entrance of the particles than the "gold standard" cement fixation, such an interface might reduce the number of accumulated particles at the bone-implant interface.

So far as the role of inflammatory mediators is concerned, it is perhaps not wise to relate them solely to osteoclast activation and bone loss, neglecting their other possible roles. We believe their essential significance in interface tissues, as a response to wear particles, should be in the regulation of cell growth in the context of inflammation rather than their effects on bone resorption. Furthermore, we believe that the response to particulate debris is so vital a function in maintaining life that attempts to intervene in this process by pharmacological methods might be dangerous.

A comprehensive understanding of the basic mechanisms of bone loss and its relationship to particulate species demonstrated in this study is critical for the development of preventive measures that may minimize the clinical impact of these phenomena.

Acknowledgements

The authors thank Mr. M A R Freeman (Consultant Orthopaedic Surgeon, London Hospital Medical College, London, UK.), P A Revell (Professor, Department of Histopathology, Royal Free Hospital School of Medicine, London, UK.), Yoshiki Yamano (Professor, Department of Orthopaedic Surgery, Osaka City University Medical School, Osaka, Japan) and W. Bonefield (Director, Interdisciplinary Research Centre in Biomedical Materials, Queen Mary and Westfield College, London, UK) for their continuing inspiration and help in conducting this study.

Figures 1-4 are reproduced from *Clin Orthop* 1997; 340: 118-129 by Kadoya et al. with permission.

Figures 7-9 are reproduced from *J Orthopaedic Res* 1996; 14: 473-82 by Kadoya et al. with permission.

Figure 12 is reproduced from the *Proceedings of the Institution of Mechanical Engineers* 1997; Part H, volume 211, H1: 11-5, by Akio Kobayashi et al. by permission of the Council of the Institution of Mechanical Engineers.

References

1. Agins HJ, Alcock NW, Bansal M et al. Metallic wear in failed titanium-alloy total hip replacements. A histological and quantitative analysis. *J Bone Joint Surg* 1988; 70-A: 347-56.
2. Al-Saffar N, Revell PA. IL-1 production by activated macrophages surrounding loosened orthopaedic implants: A potential role in osteolysis. *British J Rheumatol* 1994; 33:309-16.
3. Athanasou NA, Quinn J, Bulstrode CJK. Resorption of bone by inflammatory cells derived from the joint capsule of hip arthroplasties. *J Bone Joint Surg (Br)* 1992; 74B: 57-62.
4. Bauer TW, Saltrarelli M, McMahon JT, Wilde AH. Regional dissemination of wear debris from a total knee prosthesis. A case report. *J Bone Joint Surg (Am)* 1993; 75A: 106-11.
5. Campbell P, Ma S, Schmalzried T, Amstutz HC. Tissue digestion for wear debris particle isolation. *J Biomed Mater Res* 1994; 28: 523-6.
6. Campbell P, Ma S, Yoon B, McKellop H, Schmalzried T, Amstutz HC. Isolation of predominantly submicron-sized UHMWP particles from periprosthetic tissues. *J Biomed Mater Res* 1995; 29: 127-31.
7. Case CP, Langkamer VG, James C, Palmer MR, Kemp AJ, Heap PF and Solomon L. Widespread dissemination of metal debris from implants. *J Bone Joint Surg (Am)* 1993; 76A: 701-12.
8. Cohen J. Assay of foreign-body reaction. *J Bone Joint Surg (Am)* 1959; 41A: 152-66.
9. Freeman MAR, Bradley GW, Revell, PA. Observations upon the interface between bone and polymethylmethacrylate cement. *J Bone Joint Surg (Br)* 1982; 64B: 489-93.
10. Guttmann D, Schmalzried TP, Jasty M, Harris WH. Light microscopic identification of submicron polyethylene wear debris. *J Appl Biomater* 1993; 4: 303-7.
11. Holowitz SM, Doty SB, Lane JM, Burstain AH. Studies of the mechanism by which the mechanical failure of polymethylmethacrylate leads to bone resorption. *J Bone Joint Surg (Am)* 1993; 75A: 802-13.
12. Huo MH, Salvati EA, Lieberman JR, Betts F, Bansal M. Metallic debris in femoral endosteolysis in failed cemented total hip arthroplasties. *Clin Orthop* 1992; 276: 157-68.
13. Jasty M, Jiranek W, Harris WH. Acrylic fragmentation in total hip replacements and its biological consequences. *Clin Orthop* 1992; 285: 116-28.
14. Kadoya Y, Al-Saffar N, Kobayashi A, Revell PA. The expression of osteoclast markers on foreign body giant cells. *Bone and Mineral* 1994; 27: 85-96.
15. Kadoya Y, Al-Saffar N, Revell PA et al. Bone loss in aseptic loosening. Bone resorption by osteoclasts or macrophages. *Trans ORS* 1995; 20: 215.
16. Kadoya Y, Revell PA, Kobayashi A et al. The bone formation and bone resorption in failed total joint arthroplasties. Histomorphometric analysis with histochemical and immunohistochemical technique. *J Orthop Res* 1996; 14: 473-82.
17. Kadoya Y, Revell PA, Al-Saffar N, Kobayashi A, Scott G, Freeman MAR. Wear particulate species and bone loss in failed total joint arthroplasties. *Clin Orthop* 1997; 340: 118-29.
18. Kelley SS, Johnston RC. Debris from cobalt-chrome cable may cause acetabular loosening. *Clin Orthop* 1992; 285: 140-6.
19. Kobayashi A, Bonfield W, Kadoya Y, Yamac T, Freeman MAR, Scott G, Revell PA. The size and shape of particulate polyethylene wear debris in total joint replacements. *Proc Instn Mech Engrs* 1997; 211 (Part H): 11-5.
20. Kobayashi A, Freeman MAR, Bonfield W, Kadoya Y, Yamac T, Al-Saffar N, Scott G, Revell PA. Number of polyethylene particles and osteolysis in total joint replacements—a quantitative study with tissue digestion method. *J Bone Joint Surg (Br)* 1997; 79B: 844-8.
21. Lalor PA, Revell PA, Gray AB et al. Sensitivity to titanium. A cause of implant failure? *J Bone Joint Surg (Br)* 1991; 73B: 25-8.
22. Lennox DW, Schofield BH, McDonald DF, Riley LH. A histologic comparison of aseptic loosening of cemented, press-fit and biologic ingrowth prostheses. *Clin Orthop* 1987; 225: 171-91.
23. Linder L, Lindberg L, Carlsson Å. Aseptic loosening of hip prostheses: A histologic and enzyme histochemical study. *Clin Orthop* 1983; 175: 93-104.
24. Lombardi AV, Mallory TH, Vaughn BK, Drouillard P. Aseptic loosening in total hip arthroplasty secondary to osteolysis induced by wear debris from titanium-alloy modular femoral heads. *J Bone Joint Surg (Am)* 1989; 71A: 1337-42.
25. Maloney WJ, Jasty M, Rosenberg A, Harris WH. Bone lysis in well-fixed cemented femoral components. *J Bone Joint Surg (Br)* 1990; 72B: 966-70.
26. Ohashi H, Kobayashi A, Yamano Y, Iwaki H. Histological evaluation of bone-cement interface affected by polyethylene particles in rabbit knee. *J Material Science: Materials in Medicine* 1994; 5: 610-2.
27. Ohashi H, Kobayashi A, Kadoya Y, Yamano Y. Effect of particles and condition of the interface on fibrous tissue interposition: A particle challenge model in rabbit. *J Material Science: Materials in Medicine* (submitted).
28. Quinn J, Joyner C, Triffitt JT, Athanasou NA. Polymethyl methacrylate-induced inflammatory macrophages resorb bone. *J Bone Joint Surg (Br)* 1992; 74B: 652-8.
29. Revell PA, Weightman B, Freeman MAR, Vernon Roberts B. The production and biology of polyethylene wear debris. *Arch Orth Trauma Surg* 1978; 91: 167-81.

30. Salvati EA, Betts F, Doty SB. Particulate metallic debris in cemented total hip arthroplasty. *Clin Orthop* 1993; 293: 160-73.
31. Savio JA, Overcamp LM, Black J. Size and shape of biomaterial wear debris. *Clin Mater* 1994; 15: 101-47.
32. Schmalzried TP, Kwong LM, Jasty M et al. The mechanism of loosening of cemented acetabular components in total hip arthroplasty: analysis of specimens retrieved at autopsy. *Clin Orthop* 1992; 274: 60-78.
33. Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total joint hip arthroplasty: the role of polyethylene wear debris and the concept of effective joint space. *J Bone Joint Surg (Am)* 1992; 74A: 849-63.
34. Schmalzried TP, Jasty M, Rosenberg A, Harris WH. Histologic identification of polyethylene wear debris using Oil Red O stain. *J Appl Biomater* 1993; 4: 119-25.
35. Stinson NE. The tissue reaction induced in rats and guinea-pigs by polymethyl-methacrylate (Acrylic) and stainless steel (18/8/Mo). *Br J Exp Pathol* 1963; 45: 21-9.
36. Stinson NE. Tissue reaction induced in guinea-pigs by particulate polymethyl-methacrylate, polythene and nylon of the same size range. *Br J Exp Pathol* 1964; 46: 135-46.
37. Willert HG, Ludwig J, Semlitsch M. Reaction of bone to methacrylate after hip arthroplasty. A long-term gross, light microscopic, and scanning electron microscopic study. *J Bone Joint Surg (Am)* 1974; 56A: 1368-82.
38. Willert HG, Bertram H, Buchhorn GH. Osteolysis in alloarthroplasty of the hip. The role of bone cement fragmentation. *Clin Orthop* 1990; 258: 108-21.