



ORIGINAL ARTICLE

# Specific material effects of wear-particle-induced inflammation and osteolysis at the bone–implant interface: A rat model



Lisa K. Longhofer<sup>a</sup>, Alexander Chong<sup>a</sup>, Nora M. Strong<sup>b</sup>, Paul H. Wooley<sup>a,b</sup>, Shang-You Yang<sup>a,b,\*</sup>

<sup>a</sup> Department of Orthopaedics, University of Kansas School of Medicine–Wichita, Wichita, KS, USA

<sup>b</sup> Department of Biological Sciences, Wichita State University, Wichita, KS, USA

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## KEYWORDS

aseptic loosening;  
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wear debris

**Summary** *Introduction:* Wear particles produced from prosthetic joints may play critical roles in periprosthetic inflammatory reactions and osteolysis. The objective of this study was to quantify and compare the response to wear debris from different biomaterials at the bone–implant interface in a rat knee model.

*Methods:* Sixty rats were divided into titanium alloy (Ti–6Al–4V), cobalt chromium (Co–Cr), ceramic (Al<sub>2</sub>O<sub>3</sub>), ultrahigh molecular weight polyethylene (UHMWPE), and control (phosphate buffered saline) groups with 12 animals per group. A nonweight-bearing titanium rod was implanted into the right distal femur of each rat followed by intra-articular injections of the biomaterial particles to the surgical knees for up to 16 weeks. Micro-computed tomography scanning was performed monthly and at the time of sacrifice to determine bone densities around the bone–implant interface. Histological evaluations were executed to quantify local inflammatory reactions and osteoclastogenesis.

*Results:* Co–Cr particles resulted in the most severe reductions in bone density. UHMWPE and ceramic particles resulted in a rapid reduction in bone density followed by a recovery. Inflammatory pseudo-membranes were ubiquitously present close to the femoral condyle and pin insertion site. Ceramic particles significantly promoted periprosthetic tissue formation compared with the other groups ( $p < 0.05$ ). Cathepsin K positive cells were dominantly present at the peri-implant site following challenges of metallic alloy and ceramic particles.

*Conclusion:* Different biomaterials in particulate form exert different forms of adverse effects in terms of the amount of osteolysis and inflammatory reactions on bone tissue at

\* Corresponding author. Department of Orthopaedics, University of Kansas School of Medicine, 929 North Saint Francis Street, Wichita, KS 67214, USA.

E-mail address: [syang6@kumc.edu](mailto:syang6@kumc.edu) (S.-Y. Yang).

the bone–implant interface. It provides information for engineering more appropriate materials for arthroplasty components.

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## Introduction

Periprosthetic osteolysis following prosthetic joint arthroplasty has been a subject of increasing concern in the orthopaedic research community as well as a dominant limiting factor in the longevity of the prosthetic device. Depending on the distribution and severity, osteolysis can lead to aseptic loosening, periprosthetic fracture, and daunting reconstructive problems at revision surgery [1–3]. It is widely recognised that polyethylene wear debris is one of the main causes of long-term prosthesis loosening. The longevity of prosthetic joint replacements is often jeopardised by particulate wear debris associated aseptic loosening and osteolysis [4–6].

Small wear particulate debris generated at the periprosthetic site have been identified as a main causative factor leading to periprosthetic osteolysis because they often stimulate a range of inflammatory cellular responses (including foreign-body reactions), which may ultimately result in osteoclastogenesis and bone resorption [6]. The amount of particulate debris, the composition of debris, and the location of debris generation all must be considered when trying to resolve design issues and minimise particulate wear debris. Because osteolysis is predominantly a biologic response to particulate wear and corrosion products, alternative bearing surfaces and cross-linked ultrahigh molecular weight polyethylene (UHMWPE) have been developed in an attempt to reduce the incidence of wear-induced periprosthetic osteolysis. These alternative bearing surfaces currently include ceramic-on-UHMWPE, ceramic-on-ceramic, metal-on-metal, and metal-on-UHMWPE. However, it has been shown in many studies that even these alternative bearing surfaces as well as UHMWPE lead to periprosthetic osteolysis and inflammation [7,8]. In addition, the biologic response to debris generated from bearing surfaces has been highly debated in recent years [7].

Howie et al [9] examined the resorption of bone and the formation of a membrane at the interface between an acrylic cement implant and bone. A nonweight-bearing plug of methylmethacrylate was inserted through the knee joint into the distal part of the femur of the rat representing similarities to human joint prostheses. The resorption of bone that occurred around the plug after the injection of high-density polyethylene wear particles to the rat knee took place in the absence of mechanical causes for loosening. This rat model technique was more recently applied to the mouse and has successfully been used to study osteolysis, inflammatory responses, and cellular reactions to wear particles [10,11]. The model has repeatedly been shown to be a reliable representation to help further elucidate the problems with prosthetic joint prostheses [12–16]. To our knowledge, there is no published report using a rat model in which a

nonweight-bearing sterile titanium rod (eliminating the variable of mechanical loosening) placed in the distal part of a viable femur to investigate the effects of different particulate wear debris biomaterials on inflammatory reactions and the osteolytic process at the bone–implant interface, and no study to date has compared the effects of each of the particulate debris with the extent of osteolysis on the bone. This model theoretically allows evaluation of the biocompatibility of orthopaedic particulate biomaterials, and evaluation of the wear particles that cause resorption of bone and formation of connective tissue leading to loosening of prostheses simultaneously. We hypothesise that wear debris from different composition of the biomaterials act differently to promote local tissue response. The objective of this study was to test the hypothesis to quantify and compare the response with wear debris from different particulate biomaterials (UHMWPE, cobalt chromium, titanium alloy, and ceramic particles) at the bone–implant interface in a rat knee joint with a distal femur implant model.

## Materials and methods

### Experimental protocol and grouping

The animal protocol has been approved by the Institutional Animal Care and Use Committee. A total of 60 female Lewis rats with the body weight range of 200–225 g (Envigo, 800-793-7287) were used for this study. The animals were housed in cages of three for a total of 20 cages according to the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). They were kept in the Wichita State University animal facility 1 week prior to surgical implantation and randomly assigned to five groups ( $n = 12$  for each group): UHMWPE particles, cobalt–chromium alloy (Co–Cr) particles, titanium alloy (Ti–6Al–4V) particles, ceramic ( $Al_2O_3$ ) particles, and phosphate buffered saline (PBS). All groups had a titanium rod insert into the left distal femur with injections of the particles with carrier solution (PBS) as defined by each group respectively. Group 5 (control) had a titanium rod insert into the left distal femur with injection of PBS solution alone without any particles. This study was conducted over a 16-week period allowing the first 4 weeks for implant stability and healing to take place and the following 16 weeks for particle injections, assessment, and animal sacrifice.

### Biomaterial particles

Four orthopaedic biomaterials in particulate form were evaluated for reactions in the rat distal femur model alone

(Table 1), and the size and distribution of the particles were evaluated with scanning electron microscopy (SEM), as detailed previously [17]. Figure 1 exhibits the SEM images of the biomaterials used in the experiments. SEM imaging revealed all materials to be predominantly spherical in shape. The particles were washed in 70% ethanol solution to remove bound endotoxin, and the particle suspension was determined to be endotoxin-free using the Limulus assay (Endosafe; Charles Rivers, Charlestown, SC, USA). The particles were then suspended in sterile PBS at  $6.4 \times 10^5$  particles/mL for injection.

### Titanium-rod implantation surgery

The animals were anaesthetised with an intraperitoneal injection of a mixture of ketamine (90 mg/kg) and xylazine

(5 mg/kg), and buprenorphine (0.05 mg/kg, i.p.) for preventative analgesia. One orthopaedic surgeon performed all of the surgical procedures on the rats. After the animals were anaesthetised, the medial parapatellar incision of the left knee was incised and the patellae was dislocated laterally. Using a sterile technique, the intracondylar notch of the distal femur was exposed through a medial parapatellar arthrotomy. A 0.9-mm-diameter drill was used to drill through the intracondylar notch to access the distal femur medullar cavity. A 1.1-mm-diameter and 6.0-mm-long titanium rod with smooth surface was press fitted into the distal femur, and radiographs were taken immediately after surgery to evaluate the implant placement. After implant insertion, the medial parapatellar arthrotomy was repaired with 5.0 Vicryl sutures, and a 5.0 silk suture was used to close the skin with simple interrupted suture technique. Radiographs were taken with titanium rods *in situ* in both anteroposterior and lateral planes in order to verify the placement of the titanium rod (Figure 2).

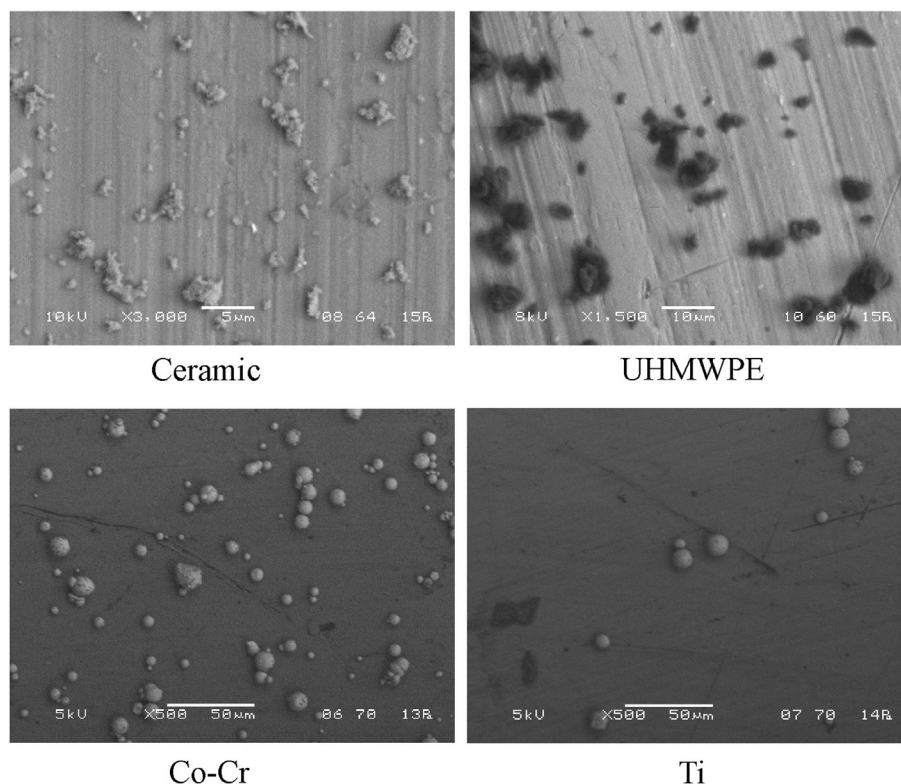
**Table 1** Experimental study groups.

| Group | Injection suspension | Mean diameter ( $\mu\text{m}$ ) | Size range ( $\mu\text{m}$ ) |
|-------|----------------------|---------------------------------|------------------------------|
| 1     | UHMWPE               | 1.6                             | 0.2–9.5                      |
| 2     | Co–Cr                | 1.5                             | 0.2–5.6                      |
| 3     | Ti–6Al–4V            | 1.4                             | 0.2–3.8                      |
| 4     | Ceramic              | 0.8                             | 0.2–6.3                      |
| 5     | PBS (control)        | NA                              | NA                           |

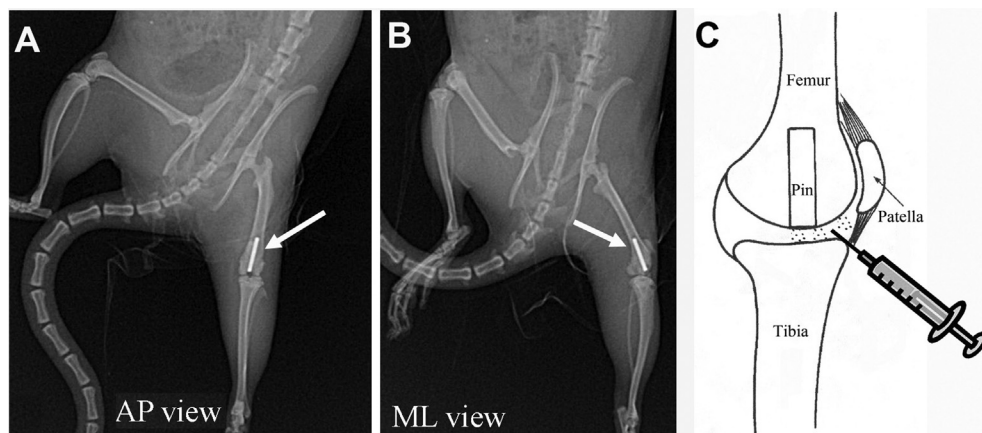
NA = not available; PBS = phosphate buffered saline; UHMWPE = ultrahigh molecular weight polyethylene.

### Particulate biomaterial introduction

Four weeks was allowed after the surgery to stabilise the implant and heal within the femoral canal, prior to the first wear particle injection (Figure 2C). Wear particles of different biomaterials in 50  $\mu\text{L}$  ( $6.4 \times 10^5$  particles/mL) were injected into the implanted knees every 2 weeks (Weeks 4, 6, 8, 10, 12, 14, and 16) for each of the five groups, respectively. Micro-computed tomography (CT) scanning was performed every 4 weeks on all the live rats starting from initial titanium rod insertion. Three rats from



**Figure 1** Scanning electron microscopy appearance of the particles used to interact at the periprosthetic site.



**Figure 2** (A, B) Typical radiograph images of the implanted titanium rod in the proximal femur through the rat intracondylar notch; and (C) schematic illustration of the experimental setup of the knee injections of particles with carrier solution.

each group were sacrificed at Weeks 4, 8, 12, and 16 to harvest the titanium pin-bearing femurs for histological analysis.

### Micro-CT scans and assessment

A detailed qualitative and quantitative 3-D evaluation was performed on each distal femur using a SCANCO microCT System (viva CT 40; SCANCO Medical, Zürich, Switzerland) with 10  $\mu\text{m}$  voxel size. Following acquisition and reconstruction, the image data were analysed with MicroCT Evaluation program V6.5-1 software to generate isosurfaces of the volume of interest, and to calculate the bone mineral density (BMD) of the femoral bones surrounding the titanium pins after establishment of a fixed lower threshold and upper exclusion to eliminate the artificial noise from soft tissue and the titanium pin [11]. The data recorded immediately after surgery were used as the baseline for comparison.

### Histological evaluation and image analysis

After each sacrifice, the left femur with pin implantation was harvested for histological processing. All femur specimens were decalcified in 10% ethylenediaminetetraacetic acid (EDTA), then paraffin-embedded and longitudinally sectioned after the implanted titanium pins were retracted. Haematoxylin and eosin (H&E)-stained histology sections were examined under a Zeiss light microscope. A computerised image analysis system with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA) was used to capture the digital photomicrographs and quantify the thickness of the pseudo-membranes [11,17]. Five random measurements along the bone–implant interface of each H&E-stained femur section were recorded and averaged according to the treatment groups.

To evaluate the influence of particles on peri-implant osteoclastogenesis, immunohistochemical staining against cathepsin K, an enzyme expressed by mature osteoclasts, was performed on the paraffin-embedded femur sections. Briefly, paraffin sections were deparaffinised in xylene, then rehydrated in graded alcohols and water. Next, 0.3% of

hydrogen peroxide was applied to diminish the endogenous peroxidase followed by microwave incubation to enhance the antigens. After blocking with 1.5% normal goat serum for 1 h, the sections were incubated overnight with anti-cathepsin K antibody (1:200 dilution, Cat# ab19027, Abcam.com) in a moisturised chamber at 4 °C. Biotin-conjugated secondary antibody and avidin–biotin enzyme reagents were sequentially applied for 30 min between extensive washes. The colour was developed by adding 3,3'-diaminobenzidine tetrahydrochloride. In negative control sections, an irrelevant antiserum was applied to replace the primary antibody. Digital images were captured and analysed using the Image-Pro software package to quantify the positive stained cells.

### Statistical analysis

The power analysis was performed prior to the experimentation using "PS Power and Sample Size Calculations (<http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize>). A one-way analysis of variance of SPSS software (Version 19.0; SPSS Inc., Chicago, IL, USA) with the least significant difference multiple comparisons *post hoc* analysis was used to determine if any observed differences between the different wear particles were significant. The level of significant difference was defined as  $p < 0.05$ .

### Results

The animals sustained the implantation surgery well, and all recovered to normal ambulation within 3 days after the implantation surgery. Weekly injection of particles did not result in obvious influence on mouse daily activities.

### Micro-CT evaluations

Micro-CT scans were performed on a monthly basis after biomaterial particle injections. All tested biomaterials provoked a reduction in periprosthetic BMD compared with the BMD values prior to particle stimulations, and the measurement in the saline control group. **Figure 3**

summarises the average percentage changes in bone density of the rat distal femur following exposure to different wear particles. This reduction was most severe in the group challenged with Co–Cr particles throughout the experimentation, followed by the group with titanium particle introductions. UHMWPE particles resulted in a rapid reduction in bone density at 4 weeks followed by a recovery in bone density levels, and a similar response pattern was seen using ceramic particles.

### Histological examination

Figure 4 shows examples of the typical histological appearance of peri-implant pseudomembranes generated at the interface between the implant and surrounding bone. Pit erosions at the rod implant contacted bone surface were rarely seen, and there was no noticeable difference among different particle-interaction groups. The inflammatory pseudo-membranes were ubiquitously present along the bone–implant interface, especially at the

distal femoral region (close to the femoral condyle and pin implant insertion site). Analysis of membrane thickness between the material groups indicated that both UHMWPE and ceramic particles dramatically resulted in the inflammatory periprosthetic tissue formation compared with the saline control and Ti alloy and Co–Cr groups. Figure 5 summarises the data distribution (pseudo-membrane thickness) among particle-challenged groups. Although the small sample size limited the data interpretation, it clearly showed that periodically interactions with ceramic or UHMWPE particles significantly promoted periprosthetic soft tissue formation ( $p < 0.05$ ) compared with the PBS controls. In addition, the ceramic particle-challenged group exhibited significantly thicker pseudo-membranes than many other particle-challenged groups (Ti–6Al–4V and Co–Cr groups) at the end of the experimentation (16 weeks). The UHMWPE particles also resulted in ubiquitous inflammatory tissue formation, although the data did not quite reach statistical significance compared with other particle groups at the end of the experiment ( $p = 0.056$ ).

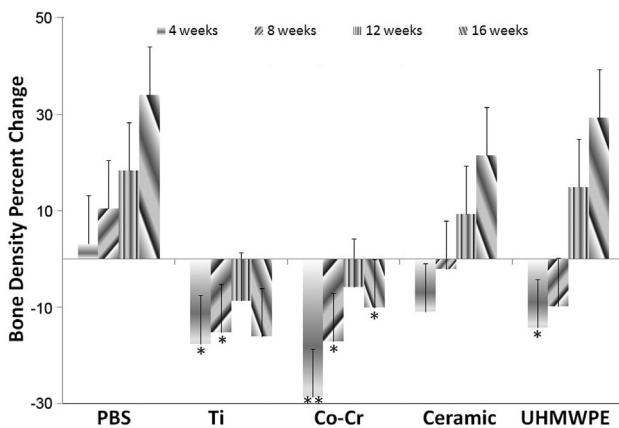


Figure 3 Quantification of bone mineral densities among groups at various time points using micro-computed tomography ( $*p < 0.05$ ,  $**p < 0.01$ ).

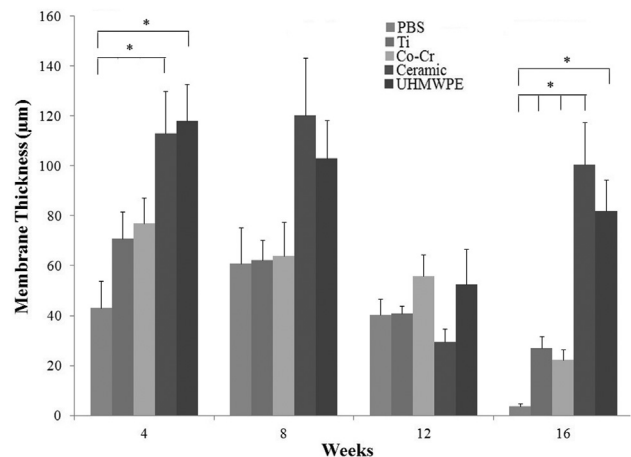


Figure 5 Quantification of the thickness of pseudo-membranes among particle-challenge groups during the experimentation period ( $*p < 0.05$ ).

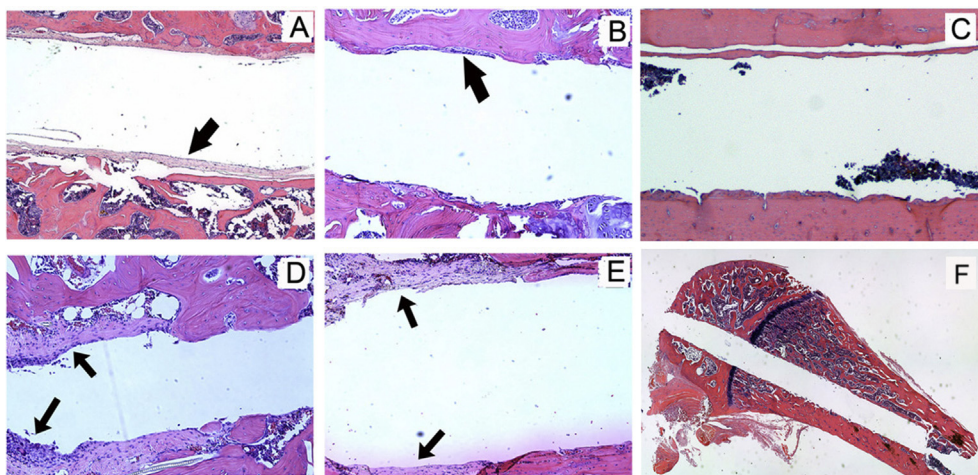


Figure 4 Typical histological appearances of peri-implant pseudo-membranes generated at the interface between the implant and surrounding bone. (A) Phosphate buffered saline control; (B) titanium alloy particles; (C) cobalt–chromium particles; (D) ceramic particles; (E) UHMWPE particles; and (F) a typical longitudinal section of the distal femur after the implanted titanium pins were retracted. All photomicrographs were taken at 100 $\times$  magnification, except panel F (20 $\times$ ).

## Osteoclastogenesis determination

Immunohistochemical staining against cathepsin K was performed to identify osteoclastic cells at the pin-implantation and particle stimulation site. The data suggested that there were significantly more cathepsin K positive cells in specimens from Ti–6Al–4V, Co–Cr, and ceramic particle-challenged groups, compared with the PBS control group (Figure 6), although no significant elevation was noticed in samples from the UHMWPE particles group. There was no significant difference between the ceramic group and the metal groups.

## Discussion

Wear particles produced from total joint replacements have been shown to stimulate host inflammatory reactions resulting in periprosthetic osteolysis. Most animal models that have been used to stimulate wear debris osteolysis and inflammatory responses have not effectively compared the outcomes of each of the particulate biomaterial debris on the implant–bone interface. This study quantified and compared the micro-CT results and the pathology of wear debris osteolysis provoked by different biomaterials (UHMWPE, Co–Cr, Ti–6Al–4V, and ceramic) at the bone–implant interface in a rat knee model.

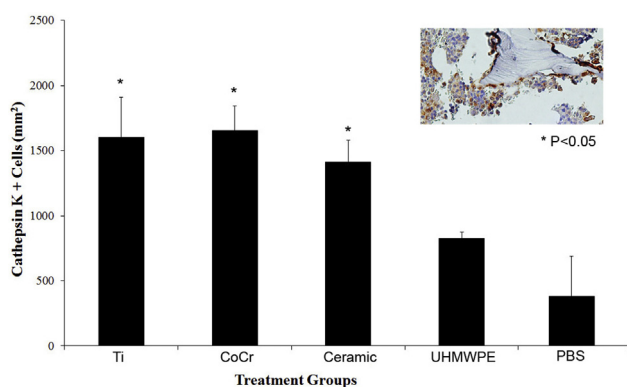
Shanbhag et al [18] conducted an *in vitro* study using commercially pure titanium, titanium–aluminium–vanadium, and UHMWPE wear debris, and reported that UHMWPE particles induced more severe osteolysis than other biomaterials. Howie et al [9] examined the resorption of bone and the formation of a membrane at the interface between an acrylic cement implant and bone using a nonweight-bearing plug of methylmethacrylate inserted through rat distal femur, and found that the resorption of bone that occurred around the plug after the injection of high-density polyethylene wear particles to the rat knee took place in the absence of mechanical causes for loosening. Previous studies from our laboratory used the murine

air pouch model and a mouse–human tissue chimera model to examine inflammatory reactions to UHMWPE, PMMA (polymethylmethacrylate), Co–Cr, and titanium particles *in vivo*, and found that all particulate biomaterials caused significant increases in membrane thickness compared with control (saline) air pouches, with the highest reaction seen in response to Ti–6Al–4V particles [8,17]. Pazzaglia et al [19] reported that metal wear debris is involved in loosening, and showed that there could be strong foreign-body reactions even when UHMWPE wear debris particles were absent. Several studies also reported aseptic loosening and massive bone resorption on cases of ceramic-on-ceramic total hip arthroplasty [20–22].

Our findings indicate that all biomaterials in particulate form exert an adverse effect on bone tissue at the implant interface, which is consistent with previous reports. However, notable differences were observed that may suggest mechanistic variations in response to different materials. Metallic alloy (Co–Cr and Ti) particles resulted in the most marked reduction in bone density, with effects sustained throughout the study period. However, this response did not correlate with the highest levels of periprosthetic inflammation. In contrast, ceramic particles provoked severe inflammation, but this response was not accompanied by a significant reduction in bone density. UHMWPE particles provoked notable levels of inflammation at the bone–implant interface that were correlated with significant bone loss during the early (4 weeks) postimplantation period. It remains to be determined whether biochemical differences (such as metallic ion dissolution) or physical variations between the particle populations contribute to the variations in the biological responses to wear debris observed in this study.

This study is limited in scope for several reasons. It is an animal model without a true prosthetic device and thus dissimilar to human joint prostheses; however, animal models represent an important tool in helping to understand the biological mechanisms associated with wear debris from joint replacements. The rat model is more cost-effective when compared with research involving larger animals. Another weakness of the present study is its small sample size. Although the power analysis prior to the experiment suggested a suitable sample size of six rats, expansion of the testing time points resulted in a half of sample size for the histological assessment. However, the data still reveal some intriguing findings on the significant difference of local inflammation and osteoclastogenesis among variant particle challenges. It remains to be determined whether biochemical differences (such as metallic ion dissolution) or physical variations between the particle populations contribute to the variations in biological responses to wear debris observed in this study. Further evaluation in living animals is required to support the conclusions of this study.

Our overall conclusions were that different biomaterials in particulate form exert different forms of adverse effect in terms of the amount of osteolysis and inflammatory reactions on bone tissue at the bone–implant interface. These conclusions have clinical implications and advance our overall understanding and knowledge of periprosthetic osteolysis and aseptic implant loosening for the different types of bearing surface biomaterials. This study has



**Figure 6** Immunohistochemical (IHC) staining against cathepsin K among particle-challenged groups to determine the influence of particles on peri-implant osteoclastogenesis. A computerised image analysis system was utilised to quantify the positive cells among specimens (\* $p < 0.05$ ). The inset figure shows an example of a typical IHC-stained section (200 $\times$ ).

potential influence on the choice of biomaterials, design of implants, and techniques of implantation of prostheses, and will help determine which types of bearing surfaces are inferior based on the biocompatibility of the type of wear particles generated, which should aim to minimise the wear of components.

## Conflicts of interest

All authors declare no conflicts of interest.

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