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Polyethylene wear particles play a role in development of osteoarthritis via detrimental effects on cartilage, meniscus, and synovium

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S U M M A R Y

Objective: While ultra-high molecular weight polyethylene (UHMWPE) wear particles are known to cause periprosthetic osteolysis, its interaction with other intra-articular tissues in the case of partial joint arthroplasties is not well understood. We hypothesized that UHMWPE particles *per se* would interact with intra-articular tissue, which by acting as inflammatory reservoirs, would subsequently induce osteoarthritic (OA) changes. Our goal was to assess the inflammatory response, phagocytic activity, as well as apoptosis of intra-articular cells in the presence of UHMWPE particles *in vitro*, and the *in vivo* response of those tissues after intra-articular injection of particles in a murine model.
Design: Three cell types were used for the *in vitro* study; chondrocytes, meniscal fibrochondrocytes, and synoviocytes. Each cell type was cultured with two different concentrations of UHMWPE particles. Pro-inflammatory cytokine production, phagocytosis, and apoptosis were analyzed. *In vivo* experiments were done by injecting two concentrations of UHMWPE particles into normal and murine OA model knee joints.
Results: In vitro experiments showed that UHMWPE particles increase pro-inflammatory cytokine and mediator (IL-1β, IL-6, TNF-α, Nitric Oxide, and Prostaglandin E2) production, phagocytosis of particles, and apoptosis in all cell types. *In vivo* experiment showed degeneration of cartilage and meniscus, as well as synovitis after particle injection.
Conclusions: UHMWPE wear particles *per se* exert detrimental effects in cartilage, synovium, and meniscus of the knee joint resulting in pro-inflammatory cytokine release, phagocytosis of particles and apoptosis. Particles induced and exacerbated OA changes in a murine model.
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Introduction

Ultra-high molecular weight polyethylene (UHMWPE) is the bearing material of choice for total knee arthroplasties (TKA) and partial knee replacements. Continuous joint motion creates UHMWPE particles by abrasive, surface fatigue related, and/or adhesive mechanisms[1]. Particles are primarily phagocytosed by macrophages, which lead to release of inflammatory cytokines. Cytokines stimulate osteoclast precursors leading to periprosthetic osteolysis and joint replacement failure[2]. This scenario has been extensively studied in TKA. Studies on the interaction of such particles with other joint structures such as cartilage, meniscus, and synovium, however, are lacking. These interactions occur in partial knee replacements such as unicompartmental knee arthroplasty (UKA), in which only one compartment of the knee is replaced.

While partial joint replacements such as UKA are on the rise, survival rates of these implants have fallen short of TKA[3,4]. One of the most important failure modes in UKA is progression of osteoarthritis (OA) in the non-operated compartment[3,5]. Some authors have hypothesized this to be a result of transfer of increased forces to the uninvolved compartment[6].

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Recent evidence, however, support the possibility of a wear particle-related biological process involved in OA of the non-operated compartment, adding to the previous mechanical hypothesis. Possibilities of inflammatory reaction by intra-articular cell sources other than macrophages in the knee have been studied. Castillo *et al.* found that chondrocytes act as non-professional phagocytes, capable of engulfing cell debris *in vitro*[7]. Chang *et al.* have shown that chondrocytes are capable of phagocytosing UHMWPE particles which lead to elevations of inflammatory mediators such as NO and PGE2 *in vitro*[8]. Yet the link between UHMWPE wear particles and OA in the knee joint has not been established.

In an effort to clarify the interaction between UHMWPE particles and the non-replaced compartments in partial joint replacement surgeries, we hypothesized that UHMWPE particles *per se* would interact with intra-articular tissue, which by acting as inflammatory reservoirs, would subsequently induce OA changes. Our goal was to assess the inflammatory response, phagocytic activity, as well as apoptosis in chondrocytes, synoviocytes, and meniscal fibrochondrocytes in the presence of UHMWPE particles *in vitro*. We have also sought to assess the biological response of cartilage, synovium and meniscus after intra-articular injection of UHMWPE particles in a murine model.

Materials and methods

UHMWPE wear particle production

A slightly modified version of the micro-cutting procedure for generation of UHMWPE particles was constructed[9]. Briefly, photolithography patterning and etching was done to create a silicon wafer surface with micro-cutting edges [Fig. 1(A)]. Two 8 mm height, right side UHMWPE articular surfaces from uni-compartmental knee system (MIS M/G, Zimmer, Warsaw, IN) were used to manufacture the polyethylene particles. These inserts consisted of gamma sterilized (37 kGy), GUR 1050 UHMWPE. Inserts were cut to make cylindrical pins in the size of 6 mm in diameter and 25 mm in length. Linear reciprocating wear test cycles were carried out on the silicon wafer surface using a custom made wear tester (Pacific Engineering, Yeosu, Korea). This was done under a contact pressure of 3 MPa, a stroke length of 19 mm, a frequency of 1.5 Hz, and a sliding speed of 57 mm/s in purified water mixed with 12.6 mg/ml bovine serum albumin (Millipore, Billerica, MA). After the wear test, UHMWPE particles were collected by repeated rinsing with purified water. Digestion of albumin and other endotoxins was done by addition of 5 N NaOH and kept at 65°C for 24 h. Particles were collected on a 0.1 μm pore size membrane by a vacuum filtration process. Analysis of particle size and shape was done with scanning electron microscopy (SEM). Diameters and aspect ratio (AR) were calculated on 500 particles in five different occasions on an identical sample by digital image processing software (Image J, National Institute of Health)[9,10]. Particles averaged 5.21 μm ± 1.85 μm (mean ± SD) in length and 1.58 ± 0.42 (mean ± SD) in AR [Fig. 1(B)]. Size distribution was as follows; 6.77% for <0.1 μm, 43.62% for <1.0 μm, and 97.65% for <10 μm (Supplementary Fig. 1). Particles were tested for endotoxins by Limulus Ameboyte Lysate kit (Sigma–Aldrich, St. Louis, MO). Results were negative for endotoxins (<0.01 μE)[9,11–13]. Particles were sterilized under ultra-violet light for 48 h before use.

Cell harvesting and culture

Knee joints were harvested from four different, 8-week-old male Wistar rats (Orient Bio, Seongnam, Korea). Articular cartilage from the femoral condyles, infra-patellar synovial membrane, and both menisci from each knee were dissected and minced, followed by digestion with 0.2% collagenase type II (Gibco, Rockville, MD) for cartilage, and 0.2% collagenase type I (Gibco, Rockville, MD) for synovium and menisci in different culture media for 4 h. Cartilage, synovium, and menisci were suspended in DMEM (Hyclone, USA), α-MEM (Hyclone, Waltham, MA), and DMEM F12 (Gibco, Rockville, MD) media, respectively. In each medium, 10% fetal bovine serum and antibiotics were added. The suspended solutions were centrifuged at 1700 rpm for 10 min. The supernatant was drained and cells were seeded and expanded. Passage 2 cells were chosen and seeded in 24-well culture plates in densities of 1×10^5 cells/well. The ratios of particle number to cell number were chosen as 0:1 (negative control), 1:1 (low dose), and 10:1 (high dose), respectively. Lipopolysaccharide (LPS) is known to incite cytokine and mediator production, while decreasing proteoglycan synthesis in intra-articular cells[14,15]. It was used as a positive control, in a concentration of 1 μg/ml. An inverted cell culture system was used to maximize contact between cells and particles[16]. Cultures were carried out for 3 days.

Analysis of inflammation

Inflammatory cytokines, IL-1β, IL-6, TNF-α, and mediators, nitric oxide (NO) and prostaglandin E2 (PGE2), all related with OA progression, were chosen for analyses[17]. Concentrations were measured with rat IL-1β (Rat IL-1β Platinum ELISA), IL-6 (Rat IL-6 Platinum ELISA), TNF-α (Rat TNF-α Platinum ELISA) ELISA kits (eBioscience, San Diego, CA), NO Assay Kit (Abcam, Cambridge, UK), and PGE2 EIA kit (Cayman Chemical, Ann Arbor, MI). Protocols provided by the manufacturer were followed.
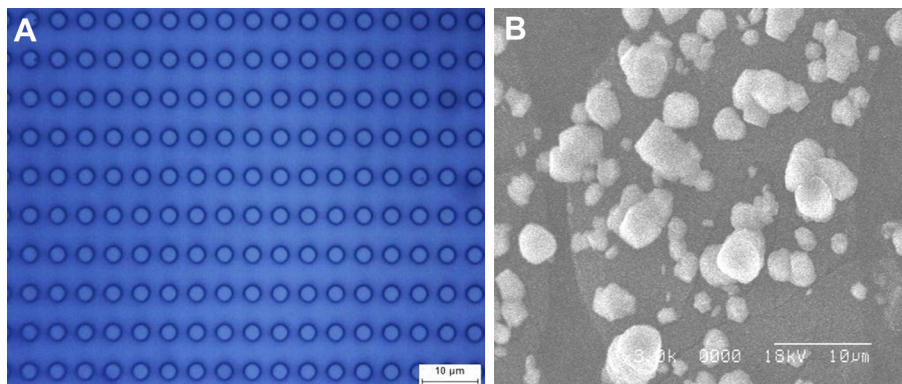

Fig. 1. (A) Microscopic view of the silicon wafer surface. Each micro-cutting edge was 5 μm apart, with 1.3 μm in height. (B) SEM of UHMWPE wear particles generated by micro-cutting process.

Phagocytosis: phagocytosis index measurement by flow cytometry

Phagocytosis index is a calculation based on changes in cell granularity (side scattering), which increases if phagocytosis occurs[18]. Cells were suspended in 350 μl of phosphate buffered saline (PBS) and 150 μl of propidium iodide (PI) solution (Invitrogen, Grand Island, NY)[18]. Cells were incubated at 37°C for 5 min, avoiding light exposure, and were analyzed with flow cytometry.

Phagocytosis: confocal microscopy

In order to visualize phagocytosis of each cell type, confocal microscopy was taken from the high dose groups. UHMWPEs are auto-fluorescent under FITC wavelengths, thus visible under confocal microscopy[19]. Cells were fixed with 4% paraformaldehyde-PBS for 30 min at room temperature. They were counterstained with PI for 2 min and centrifuged for 5 min at 1000 rpm. After suspension in PBS, cells were placed onto a slide and observed with confocal microscopy. Digitalized confocal images were processed with UltraVIEW system (PerkinElmer, Waltham, MA).

Apoptosis: TUNEL assay

Analysis of apoptosis was done for each group as previously described using APO-BrdU TUNEL assay kit (Invitrogen, Grand Island, NY) by flow cytometry[20]. Extra care was taken to preserve the cell-UHMWPE pellets between washing steps, which have the propensity to float.

Animals

For our *in vivo* study, 10-week-old male Wistar rats (Orient Bio, Seongnam, South Korea), weighing approximately 300 g, were used. The experimental protocol was approved by the Animal Care and Use Committee of our institution. The rats were divided randomly into the following groups (Table I). In groups 1 and 2, UHMWPE were injected in normal knees at two different doses (Low; 10^7 particles/100 μl, High; 10^8 particles/100 μl). In groups 3, 4, and 5, surgical OA models were made. This was based on the observation that patients receiving UKA have established OA in the knee, albeit single compartmental cartilage degeneration[3]. For groups 4 and 5, UHMWPE injections at two different doses were made after establishment of OA (6 weeks after surgical OA induction). For the groups requiring injection (groups 1, 2, 4, 5), UHMWPE were dispersed in PBS according to their respective dosages. Intra-articular injections were carried with BD Ultra-Fine needle Insulin Syringe (BD Biosciences, San Jose, CA). Surgical OA models (groups 3, 4, 5) were made as follows. Rats were anesthetized with a mixture of Zoletil (50 mg/ml; Virbac laboratories, Carros, France) and xylazine hydrochloride (Rompun; Bayer, Ansan, Korea) (Zoletil: Rompun = 1:2). Both legs were shaved and aseptically prepped. On the left knee, a medial parapatellar approach

arthrotomy was done to expose the joint. The medial collateral ligament and the mid portion of the medial meniscus were transected as described previously[21]. The wound was closed layer by layer. A sham operation was performed in the right knee. For groups 1 and 2, the end points were 6 weeks after injection. For group 3, the end point was 12 weeks after OA surgery. For groups 4 and 5, injections of UHMWPE were given 6 weeks post-operatively, and the end point was 6 weeks after injections. At the same time, the same amount of PBS was injected in the sham-operated right knees. After the respective end points, animals were sacrificed and knee joints were harvested. Specimen were fixed in 10% formalin for 1 week, and decalcified with 0.5% nitric acid for 24 h. Each right knee served as controls.

Histological assessment

Processing and paraffin embedding were done for each specimen. Serial 10 μm sagittal sections were made from the lateral compartment of the knee. Eight sections obtained at 20 μm intervals were placed on slides. Slides were stained with Safranin-O/Fast Green (SAFO) for cartilage and meniscus analysis, and hematoxylin/eosin (HE) for synovium analysis. The slides were mounted and observed under microscopy. For cartilage degeneration analysis, Osteoarthritis Research Society International (OARSI) OA grading system was used (grade 0–6; normal, grade 0; surface intact, grade 1; surface discontinuity, grade 2; vertical fissures, grade 3; erosion, grade 4; denudation, grade 5; and deformation, grade 6)[22]. For histopathologic analysis of knee meniscus, the grading system proposed by Pauli was used (0–18 points; normal tissue, 0–4 points; mild degeneration, 5–9 points; moderate degeneration, 10–14 points; and severe degeneration, 15–18 points)[23]. For analysis of synovitis, we used the histological score proposed by Krenn (0–9 points; no synovitis, 0–1 point; low-grade synovitis, 2–4 points; and high grade synovitis, 5–9 points)[24]. Each scoring was measured by three independent researchers, two of whom did not participate in this study. Each observer scored each image twice, with intervals of at least 2 weeks. While scoring, the participants were blinded to information of the study (i.e., group). For each scoring system, intraclass correlation coefficients (ICC) of intra- and inter-observer agreement were calculated.

Statistical analysis

Each *in vitro* experiment was carried out at least four times, each time using cells from different donor rats. As for the *in vivo* experiment, six animals were assigned for each group. Data are presented as mean ± 95% confidence intervals. Data were compared by Kruskal–Wallis test with Dunn's multiple comparison test.

Table I
Outline of *in vivo* analysis

Group no.	Group name	Number of animals.	Treatment
Group 1	Normal + low dose UHMWPE	6	Low dose UHMWPE particles were injected in the left knee (10^7 particles/100 μl). One hundred μl of PBS was injected in the right knee as controls.
Group 2	Normal + high dose UHMWPE	6	High dose UHMWPE particles were injected in the left knee (10^8 particles/100 μl). One hundred μl of PBS was injected in the right knee as controls.
Group 3	Surgical OA	6	OA surgery was done in the left knee. The right knee received sham operation as controls.
Group 4	Surgical OA + low dose UHMWPE	6	OA surgery was done in the left knee. The right knee received sham operation as controls. After 6 weeks, low dose UHMWPE particles were injected in the left knee (10^7 particles/100 μl). One hundred μl of PBS was injected in the right knee as controls.
Group 5	Surgical OA + high dose UHMWPE	6	OA surgery was done in the left knee. The right knee received sham operation as controls. After 6 weeks, High dose UHMWPE particles were injected in the left knee (10^8 particles/100 μl). One hundred μl of PBS was injected in the right knee as controls.

UHMWPE: Ultra high molecular weight polyethylene.

Results

Inflammatory cytokine and mediator production in all cell types increase after co-culture with UHMWPE

Production of IL-1β was increased in high dose groups of chondrocytes, meniscal fibrochondrocytes, and synoviocytes compared to controls ($P = 0.0051$, $P = 0.0252$, $P = 0.0081$, respectively). Production of IL-6 was also increased in high dose groups of the above three cells ($P = 0.0186$, $P = 0.0083$, $P = 0.0132$, respectively). TNF-α, another cytokine measured, also showed increase in production for high dose groups in all cell types ($P = 0.0155$, $P = 0.0167$, $P = 0.0459$) (Fig. 2). As for mediator production, PGE2 increased for high dose groups in all cell types when compared to controls ($P = 0.0228$, $P = 0.0077$, $P = 0.0027$). Synoviocytes, in particular, showed a five-fold increase of PGE2 production in high dose group when compared to the control group. For NO, increased levels were observed in all high dose groups for all cell types when compared to controls ($P = 0.0186$, $P = 0.0287$, $P = 0.0198$). Again, the high dose group of synoviocytes demonstrated a five-fold increase compared to the control group (Fig. 2).

Intra-articular cells exhibit phagocytosis toward UHMWPE particles

Phagocytosis index increased in a particle dose dependant manner in all cell types. High dose treated groups showed the largest percentage of increase for chondrocytes, meniscal fibrochondrocytes, and synoviocytes ($P = 0.0124$, $P = 0.0149$, $P = 0.0101$, respectively) (Fig. 3). Confocal images confirmed the above result. In each cell type, UHMWPE wear particles were observed inside the cell. Features of apoptosis, such as chromatin condensation and membrane blebbing were also noted [Fig. 4(A–C)].

Apoptosis after co-culture of intra-articular cells and UHMWPE

TUNEL assay results showed a higher percentage of TUNEL positive cells in high dose groups of the three cells when compared to controls ($P = 0.0081$, $P = 0.0049$, $P = 0.0132$). These results suggest apoptosis occurs in all cell types when co-cultured with UHMWPE particles (Fig. 5).

In vivo study

Controls of all groups (right knees of each specimen treated with PBS injection and/or sham surgery) displayed no morphological differences among each other. In cartilages, group 1 showed early OA changes such as edema and clustering of chondrocyte in the superficial zone. Group 2 showed decreased matrix staining, in addition to disorientation of chondron columns. Both groups 1 and 2 showed no change in the smoothness of cartilage surface but lessened matrix formation was observed; this was especially noticeable in group 2. Group 3, the surgically-induced OA group, showed matrix fissures. Group 4 showed matrix loss. Group 5 exhibited extensive surface denudation. For menisci, groups 1 and 2 showed early degenerative changes such as increase in cellularity and matrix staining when compared to controls. Groups 3 and 4 showed surface fibrillation and hypocellular areas. Group 5 exhibited hypocellularity with disorganization of collagen fibers. For synovium, groups 2 and 5 (both high dose UHMWPE injected groups) showed typical high grade inflammatory OA synoviopathy, with giant cells
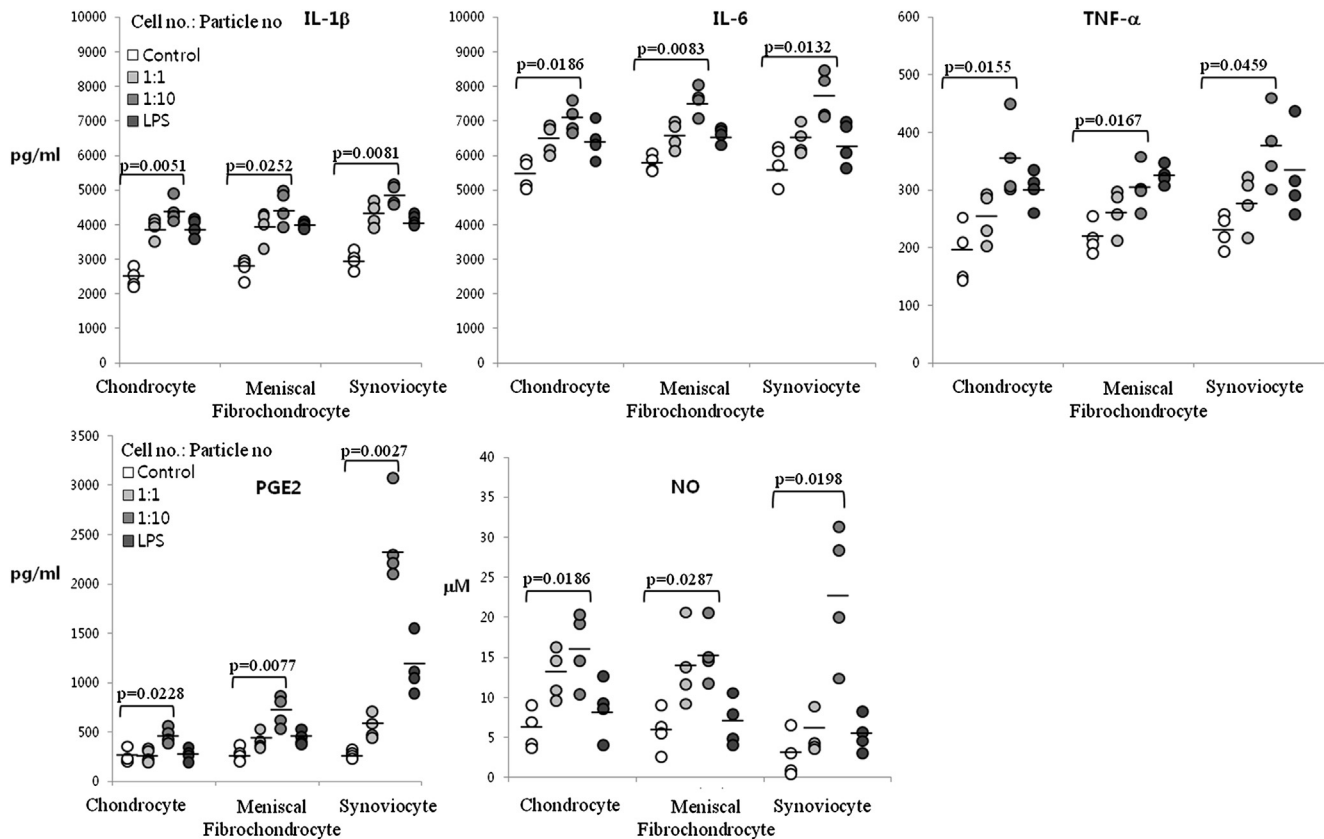

Fig. 2. Dot plot representing concentration of inflammatory cytokines IL-1β, IL-6, TNF-α, and inflammatory mediators PGE2 and NO after co-culture of chondrocytes, meniscal fibrochondrocytes, and synoviocytes with UHMWPE. Differences among groups with $P \leq 0.05$ are enumerated.
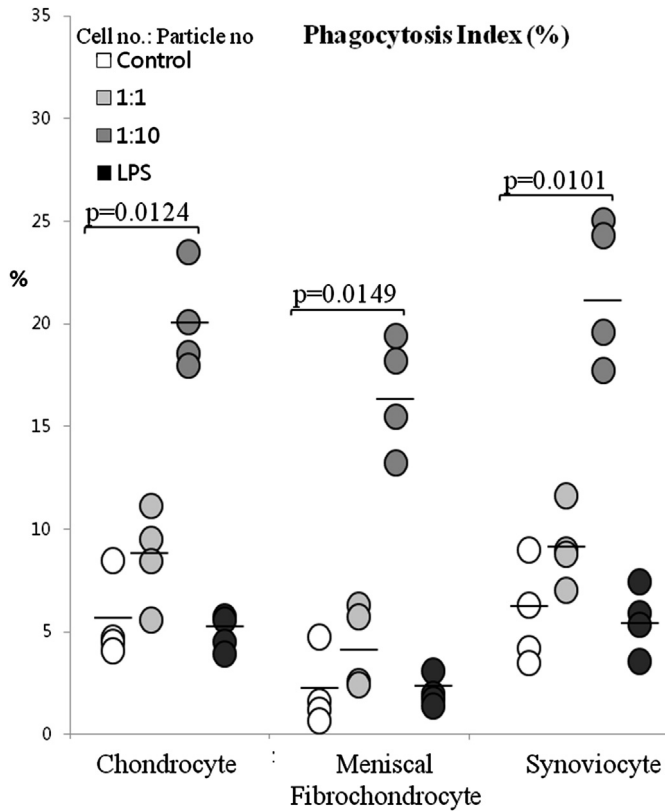
Fig. 3. Phagocytosis index analysis by flow cytometry. Dot plot represents the percentage of cells showing phagocytic activity, determined by increase of side scatter by FACS analysis. Differences among groups with $P \leq 0.05$ are enumerated.
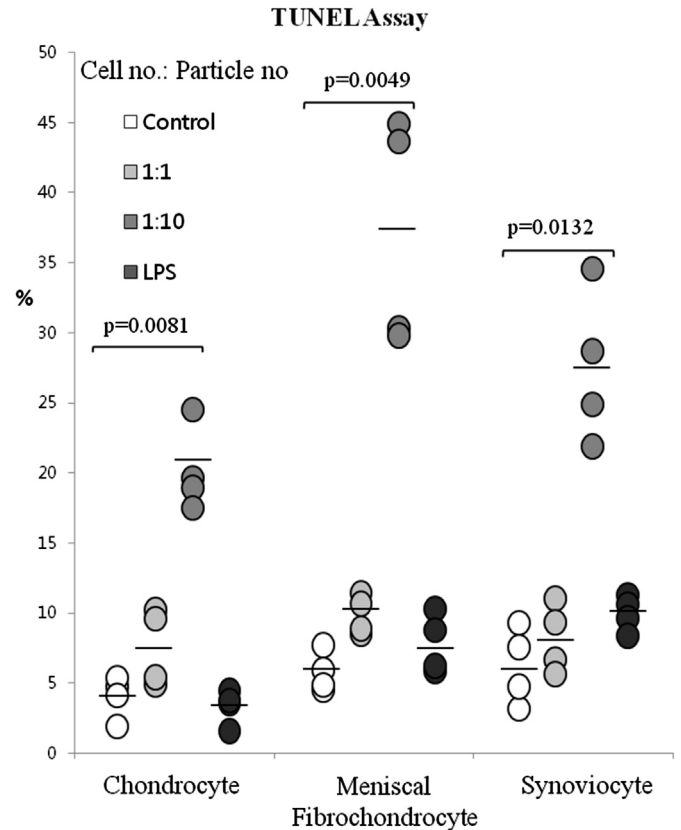

Fig. 5. Apoptosis after co-culture of intra-articular cells and UHMWPE by TUNEL assay. The percentage of TUNEL positive cells are shown in the dot plot. Differences among groups with $P \leq 0.05$ are enumerated.

encircling UHMWPE (Fig. 6)[25]. Quantifications of histological changes are represented in Fig. 7. The respective intra- and inter-observer ICC of each scoring system were as follows; OARSI OA scoring (0.78/0.91), meniscus scoring (0.79/0.89), and synovitis scoring (0.83/0.95). For cartilage, groups 2 and 5 each showed more degeneration related changes compared to control and group 3, respectively ($P = 0.024$, $P = 0.0049$). For meniscus, group 5 resulted in higher degeneration scores compared to groups 3 and 4 ($P < 0.001$, $P < 0.001$). As for synovium, group 2 showed increased synovitis over control and group 1 ($P < 0.001$, $P < 0.001$). Group 5 also showed increased synovitis scores over groups 3 and 4 ($P < 0.001$, $P < 0.001$).

Discussion

Arthritic change in the remaining articular compartment after partial joint replacement surgery such as UKA is an important,

emerging concern. While previous theory suggests increased postoperative mechanical loading in the remaining compartment as the cause of this phenomenon, our results provide a biological explanation caused by UHMWPE particles as another reason for OA in the remaining compartment. Since all intra-articular tissues such as cartilage, meniscus, and synovium are known to be involved in the pathogenesis of OA, this study aimed at the analysis of the responses of such tissues to UHMWPE particles. To the best of our knowledge, this is the first report to investigate the relationship between UHMWPE particles and individual intra-articular tissue responses eventually leading to OA. We first demonstrated cellular response to UHMWPE particles; phagocytosis of particles, apoptosis, and inflammatory cytokine production, *in vitro*. Finally, we were able to induce, as well as progress OA changes in a murine model by injecting UHMWPE particles.
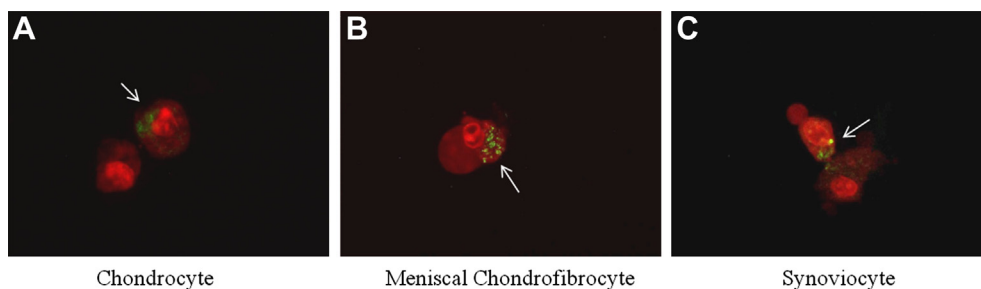

Fig. 4. Confocal microscopy of chondrocyte, meniscal fibrochondrocyte, and synoviocyte co-cultured with UHMWPE. Images of PI stained cells were merged with FITC fluorescence images. UHMWPE particles, which show FITC fluorescence (white arrows), are seen inside each cell type, suggestive of phagocytosis. Chromatin condensation, are noted in all cell types, suggestive of apoptotic activity (A) Chondrocytes showing engulfment of UHMWPE. (B) Meniscal fibrochondrocytes showing engulfment of UHMWPE. (C) Synoviocytes showing engulfment of UHMWPE. Note the membrane blebbing, suggestive of apoptotic activity. x600.
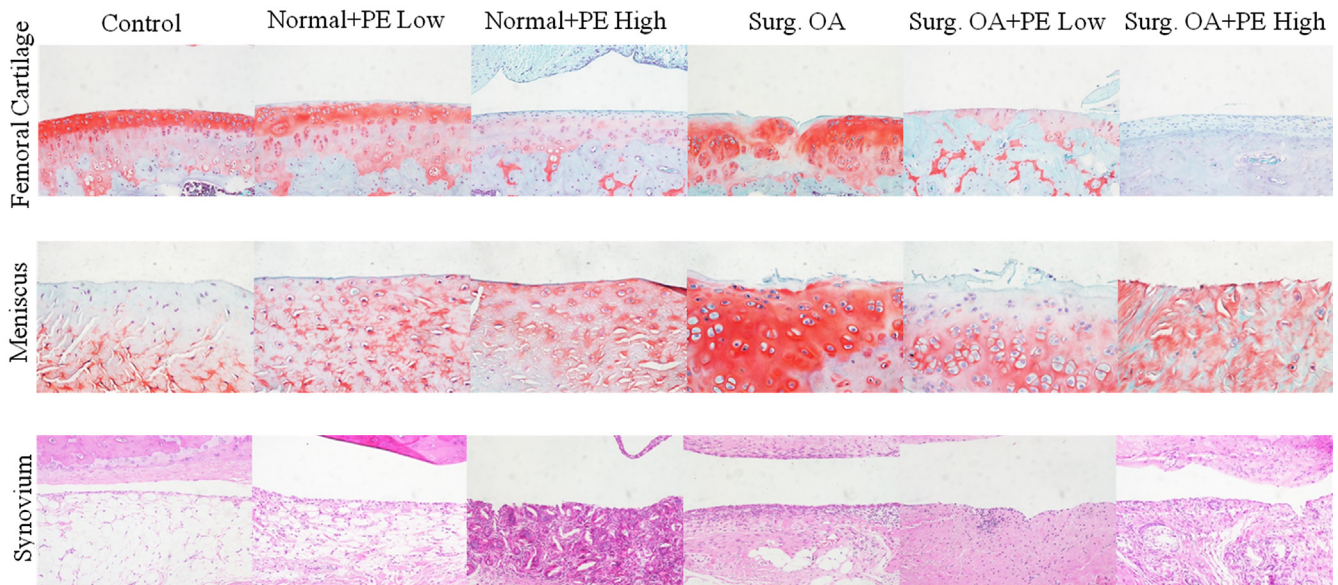
Fig. 6. Representative histopathological results of all groups. Upper row shows cartilage slides, stained in SAFO. Meniscus slides, also stained in SAFO are shown in the middle row. Synovium slides, stained in HE, are shown in the lower row. X200.

Our histopathological results demonstrate that UHMWPE particles may be an inciting factor for OA in the remaining cartilage after UKA. UHMWPE particles *per se* induced OA changes in the normal rat knee, while exacerbating the disease progression in OA models. Significant differences were observed in high dosage UHMWPE groups, in not only *in vivo*, but also *in vitro*. This suggests an existence of a critical particle dose to cause degradation of intra-articular tissues and initiate OA related changes[18]. Inflammatory synoviopathy with giant cells, in addition, were observed in the high dose groups only (groups 2 and 5), indicating a stronger inflammatory process involved. Another interesting histological finding was found on normal knees with UHMWPE injection (groups 1 and 2). While the morphological integrity of articular cartilage and meniscus were fairly maintained, considerable changes in the matrices of these tissues were found. Such particle–tissue interactions inflicting matrix changes may have accelerated tissue damage in the OA models groups (groups 4 and 5). Overall, deformation of inner matrices of cartilage and meniscus were notable in all UHMWPE injected groups (groups 1, 2, 4, and 5).

Considerable amount of studies indicate that proinflammatory cytokines (IL-1β, IL-6, TNF-α) and mediators (NO and PGE2) play a key role during inflammation and tissue destruction in OA[26]. We have shown an increase of cytokines (IL-1β, IL-6, TNF-α) and mediator (NO and PGE2) production in chondrocytes, meniscal fibrochondrocytes, and synoviocytes. Our results suggest that these cells can each act as inflammatory reservoirs, capable of producing cytokines and mediators when coming into contact with UHMWPE particles. All cells exhibited such responses in a particle dose dependent manner. Synoviocytes, in particular, secreted more inflammatory mediators than the other two cells. Zysk *et al.* have also observed strong synovial inflammatory responses after polyethylene particle injection in mice[27]. We therefore expect the synovium to play a lead role in such inflammatory response.

Synoviocytes and chondrocytes have already been reported to have phagocytosis capability. This study shows that meniscal fibrochondrocytes also have this ability. Castillo *et al.* previously reported that chondrocytes act as "non professional phagocytes" capable of engulfing latex particles and cartilage detritus[7]. Chang
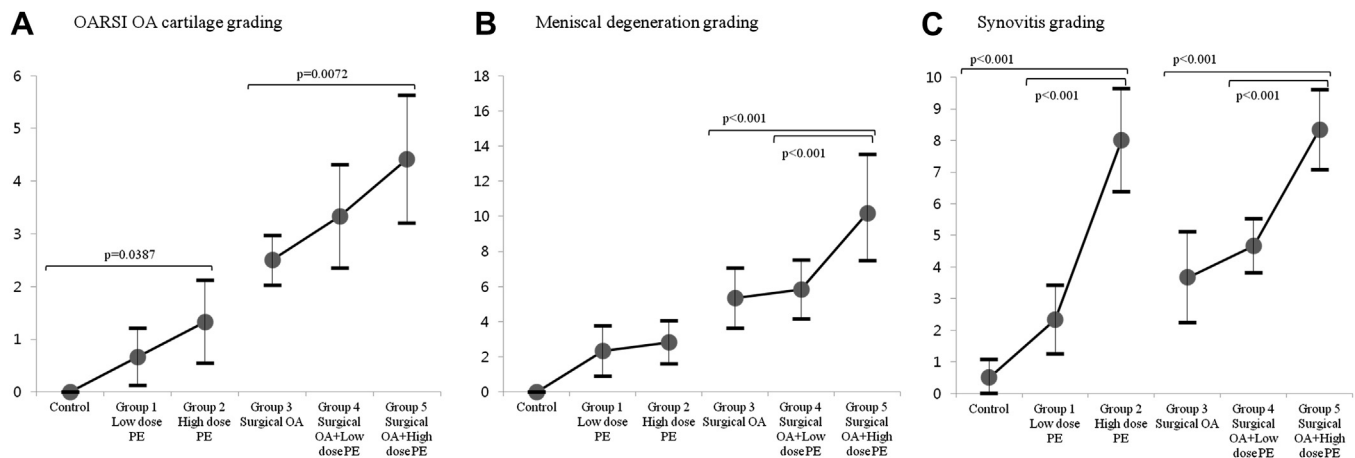

Fig. 7. (A) Quantitative representation of cartilage status by OARSI scoring. (B) Quantitative representation of degenerative changes of menisci. (C) Quantitative representation of synovitis. Error bars represent 95% confidence intervals. Differences among groups with $P \leq 0.05$ are enumerated.

et al. reported that chondrocytes engulfed UHMWPE particles, and subsequently secreted inflammatory factors such as NO and PGE2[8]. This study shows that meniscal fibrochondrocytes and synoviocytes also show similar trends with regard to UHMWPE particles, ultimately resulting in apoptosis.

Apoptosis is regarded as one of the main, inherent mechanisms of OA. Trauma, inflammation, and aging all cause chondrocyte apoptosis, reducing matrix synthesis. This process eventually leads to cartilage damage. Moreover, it disrupts cell–matrix interaction and consequently ignites the apoptotic cascade again, initiating a destructive vicious cycle[28]. Our TUNEL assay results show a UHMWPE dose dependent increase of apoptosis in all three cells. This process was also visualized by chromatin condensation and apoptotic blebbing in confocal images. This vicious cycle of apoptosis may be escalated as the concentration of UHMWPE particles increases, due to particle accumulation in the intra-articular space. Long-term wear of UHMWPE is thus expected to have serious adverse effects on the articular cartilage.

Some limitations exist in our study. The single injection-murine model used in here may not entirely reflect the chronic production of wear particles in patients with joint replacements[6]. Many prosthetic wear animal models exist[1]. Continuous intramedullary infusion of polyethylene particles by osmotic pumps in a murine model has been achieved by several authors[29,30]. We did not place this device in the knee joint, due to concerns that the pumps themselves would cause irritation. If possible, a sophisticated animal model incorporating a more continuous infusion of UHMWPE in the knee joint should be utilized in future studies.

Particle size and shape used in this study may not entirely reflect particles generated after partial arthroplasties. First of all, UHMWPE particle size differs according to the manufacturing/isolation process (i.e., simulator or *in vivo* retrieval), anatomical region of joint prosthesis (i.e., total hip arthroplasty or TKA) and end point of study (i.e., osteolysis, macrophage response, *in vivo* tissue response etc.). UHMWPE particles isolated from *in vitro* hip simulators appear to be predominantly submicron and round, with occasional fibrils[31]. Studies utilizing micro-cutting technique as the manufacturing process, the method used in our study, produce a mean diameter size comparable to our results[8,9]. Polyethylene retrieval studies during revision knee arthroplasty offer the most accurate representation of the actual particle size produced in the patient. Shanbhag *et al.* reported wear particle analysis data from total knee replacements and the mean size was 1.7 ± 0.7 μm, with ranges from 0.1 μm to 200 μm[10]. The distribution of particles were as follows; 36% <1 μm, 90% <3 μm[10]. Compared to our data, there is about <4 μm difference in mean size, while the distribution difference is <10% for <1 μm sized particles. Secondly, as for particle shape, our overall AR was 1.58 ± 0.42 (mean $\pm$ SD). Globular particles constituted 79.3% of the measurements (AR 1–2.39) while elongated fibular shape particles constituted 19.23% of the measurements (AR > 2.4–5). A retrieval study from hip revision arthroplasties reported an AR of 1.17 ± 0.06–3.59 ± 0.50[32]. Our AR is comparable to a retrieval study from TKA patients (AR 1.7)[10]. Morphologically more elongated particles with a higher AR are more commonly found in hip arthroplasty retrieval/simulator studies rather than knee arthroplasty related studies.

Controversy exists on the 'critical' size and shape of UHMWPE wear particles capable of inciting inflammatory reactions. Some authors suggest that particle size of 0.2–0.8 μm induce the strongest biologic responses[33,34]. Hallab *et al.* discusses that the internalization of small particles in the nanometer sizes (<150 nm) occurs by endocytosis for particles, where as larger particles (>150 nm–10 μm) are phagocytosed in the traditional manner, inside phagosomes of macrophages. They found that the most inflammatory particles were not the smallest particles tested (i.e.,

0.3–0.7 μm), but rather they were the larger 10–13 μm particles barely able to be phagocytosed by the cells[35]. Other authors have observed that polyethylene debris too large to be ingested provoke foreign body giant cell reactions[36,37]. This process itself is capable of inciting production of mediators that stimulate osteolysis[36,37]. These larger sized particles have been found in *in vivo* human tissue from revision arthroplasties[38]. As for shape, Sieving *et al.* have reported that sharp, elongated shape particles activated a more pronounced inflammatory response in a murine air pouch model[32]. These results, however, have yet to be confirmed in a synovial joint environment. In summary, we believe that both small (<1 μm) and large (>10 μm) particles induce inflammatory reactions in the knee joint. Our data show a near even distribution between <1 μm and >1 μm sized particles. Both phagocytosis of small particles as well as foreign body giant cell reaction in the synovium were shown in the confocal microscopic images and histological images, respectively. The size of UHMWPE particles used in this study covered both the small, phagocytosable particles and large, indigestible particles, both of which are related to *in vivo* inflammatory reactions. Further studies are required to elucidate the effect of particle size and shape in OA induction described in our study.

Clinical relevance of our study is that it provides an alternate, biochemical explanation for the development of OA and early failure in partial arthroplasties. Both clinicians and patients should note that UHMWPE particles not only cause osteolysis and implant loosening, but may also cause OA in the non-replaced compartments, leading to revision surgery[5]. Advancements of bearing materials may, in turn, reduce revision rates of partial joint arthroplasties, which currently fall behind total joint replacements[3,5].

UHMWPE wear particles *per se* exert detrimental effects on chondrocytes, synoviocytes, and meniscal fibrochondrocytes of the knee joint by phagocytosis of particles and apoptosis, resulting in pro-inflammatory cytokine release. Relationship between UHMWPE wear particles and OA was observed in a murine model, suggesting that UHMWPE wear particles play a role during OA development in partially replaced joints.

Author contributions

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Competing interest statement

None of the authors have any other conflict of interest related to this study.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.09.013.

References

1. Catelas I, Wimmer MA, Utzschneider S. Polyethylene and metal wear particles: characteristics and biological effects. Semin Immunopathol 2011;33:257–71.
2. Maloney WJ, Smith RL. Periprosthetic osteolysis in total hip arthroplasty: the role of particulate wear debris. J Bone Joint Surg Am 1995;77A:1448–61.
3. Berger RA, Meneghini M, Jacobs JJ, Sheinkop MB, Della Valle CJ, Rosenberg AG, et al. Results of unicompartmental knee arthroplasty at a minimum of ten years of follow-up. J Bone Joint Surg Am 2005;5:999–1006.
4. Lyons MC, MacDonald SJ, Somerville LE, Naudie DD, McCalden RW. Unicompartmental versus total knee arthroplasty database analysis: is there a winner? Clin Orthop Relat Res 2012;470:84–90.
5. Manson TT, Kelly NH, Lipman JD, Wright TM, Westrich GH. Unicondylar knee retrieval analysis. J Arthroplasty 2010;25: 108–11.
6. Scott RD, Cobb AG, McQueary FG, Thornhill TS. Unicompartmental knee arthroplasty. Eight-to 12-year follow-up evaluation with survivorship analysis. Clin Orthop 1991;271: 96–100.
7. Castillo EC, Kourí JB. A new role for chondrocytes as non-professional phagocytes. An in vitro study. Microsc Res Tech 2004;64:269–78.
8. Chang CH, Fang HW, Ho YC, Huang HT. Chondrocyte acting as phagocyte to internalize polyethylene wear particles and leads to the elevations of osteoarthritis associated NO and PGE2. Biochem Biophys Res Commun 2008;369:884–8.
9. Fang HW, Hsu SM, Sengers JV. Generation of narrowly distributed ultra-high-molecular-weight polyethylene particles by surface texturing techniques. J Biomed Mater Res B Appl Biomater 2003;67:741–9.
10. Shanbhag AS, Bailey HO, Hwang DS, Cha CW, Eror NG, Rubash HE. Quantitative analysis of ultrahigh molecular weight polyethylene (UHMWPE) wear debris associated with total knee replacements. J Biomed Mater Res 2000;53:100–10.
11. ISO 17853. Wear of Implant Materials-Polymer and Metal Wear Particles – Isolation and Characterization 2010. TC 150/SC4.
12. Campbell P, Ma S, Yeom B, McKellop H, Schmalzried TP, Amstutz HC. Isolation of predominantly submicron-sized UHMWPE wear particles from failed total joint arthroplasties. J Biomed Mater Res 1995;29:127–31.
13. Tipper JL, Ingham E, Hailey JL, Besong AA, Fisher J, Wroblewski BM, et al. Quantitative analysis of polyethylene wear debris, wear rate and head damage in retrieved Charnley hip prostheses. J Mater Sci Mater Med 2000;11:117–24.
14. Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Relat Res 2004;427:27–36.
15. Sun JS, Wu CX, Tsuang YH, Chen LT, Sheu SY. The in vitro effects of dehydroepiandrosterone on chondrocyte metabolism. Osteoarthritis Cartilage 2006;14:238–49.
16. Fang HW, Ho YC, Yang CB, Liu HL, Ho FY, Lu YC, et al. Preparation of UHMWPE particles and establishment of inverted macrophage cell model to investigate wear particles induced bioactivites. J Biochem Biophys Methods 2006;68:175–87.
17. Umlauf D, Frank S, Pap T, Bertrand J. Cartilage biology, pathology, and repair. Cell Mol Life Sci 2010;67:4197–211.
18. Catelas I, Huk OL, Petit A, Zukor DJ, Marchand R, Yahia L. Flow cytometric analysis of macrophage response to ceramic and polyethylene particles: effects of size, concentration, and composition. J Biomed Mater Res 1998;40:600–7.
19. Niki Y, Matsumoto H, Otani T, Suda Y, Yatabe T, Yoshimine F, et al. Flow cytometric technique for the detection of phagocytosed wear particles in patients with total joint arthroplasty. Biomaterials 2003;24:3715–24.
20. Darzynkiewicz Z, Galkowski D, Zhao H. Analysis of apoptosis by cytometry using TUNEL assay. Methods 2008;44:250–4.
21. Prasadam I, Mao X, Wang Y, Shi W, Crawford R, Xiao Y. Inhibition of p38 pathway leads to OA-like changes in a rat animal model. Rheumatology 2012;51:813–23.
22. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, et al. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
23. Pauli C, Grogan SP, Patil S, Otsuki S, Hasegawa A, Koziol J, et al. Macroscopic and histopathologic analysis of human knee menisci in aging and osteoarthritis. Osteoarthritis Cartilage 2011;19:1132–41.
24. Krenn V, Morawietz L, Burmester GR, Kinne RW, Mueller-Ladner U, Muller B, et al. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. Histopathology 2006;49:358–64.
25. Oehler S, Neureiter D, Meyer-Scholten C, Aigner T. Subtyping of osteoarthritis synoviopathy. Clin Exp Rheumatol 2002;20: 633–40.
26. Buckwalter JA, Lotz M, Stoltz JF. Osteoarthritis, Inflammation and Degradation: a Continuum. Amsterdam: IOS Press; 2007163–75.
27. Zysk SP, Gebhard H, Plitz W, Buchhorn GH, Sprecher CM, Jansson V, et al. Influence of orthopedic particulate biomaterials on inflammation and synovial microcirculation in the murine knee joint. J Biomed Mater Res B Appl Biomater 2004;71B:108–15.
28. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum 1998;41:1632–8.
29. Kim KJ, Kobayashi Y, Itoh T. Osteolysis model with continuous infusion of polyethylene particles. Clin Orthop Relat Res 1998;352:46–52.
30. Ma T, Huang Z, Ren PG, McCally R, Lindsey D, Smith RL, et al. An in vivo murine model of continuous intramedullary infusion of polyethylene particles. Biomaterials 2008;29:3738–42.
31. Scott M, Morrison M, Mishra SR, Jani S. Particle analysis for the determination of UHMWPE wear. J Biomed Mater Res B 1995;73:325–37.
32. Sieving A, Wu B, Mayton L, Nasser S, Wooley PH. Morphological characteristics of total joint arthroplasty-derived ultra-high molecular weight polyethylene (UHMWPE) wear debris that provoke inflammation in a murine model of inflammation. J Biomed Mater Res A 2003;64:457–64.
33. Ingham E, Fisher J. Biological reactions to wear debris in total joint replacement. Proc Inst Mech Eng 2000;214:21–37.
34. Green TR, Fisher J, Matthews JB, Stone MH, Ingham E. Effect of size and dose on bone resorption activity of macrophages by in vitro clinically relevant ultra high molecular weight polyethylene particles. J Biomed Mater Res 2000;53:490–7.
35. Hallab NJ, McAllister K, Brady M, Jarman-Smith M. Macrophage reactivity to different polymers demonstrates particle size- and material-specific reactivity: PEEK-OPTIMA(®) particles versus UHMWPE particles in the submicron, micron, and 10 micron size ranges. J Biomed Mater Res B Appl Biomater 2012;100:480–92.

36. Voronov I, Santerre JP, Hinek A, Callahan JW, Sandhu J, Boynton EL. Macrophage phagocytosis of polyethylene particulate in vitro. J Biomed Mater Res 1998;39:40–51.

37. Howie DW, Mantney B, Hay S, Vernon-Roberts B. The synovial response to intra-articular injection in rats of polyethylene wear particles. Clin Orthop 1993:352–7.

38. Benz EB, Federman M, Godleski JJ, Bierbaum BE, Thornhill TS, Spector M. Transmission electron microscopy of intracellular particles of polyethylene from joint replacement prostheses: size distribution and cellular response. Biomaterials 2001;22: 2835–42.