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Chondrocyte acting as phagocyte to internalize polyethylene wear particles and leads to the elevations of osteoarthritis associated NO and PGE_2

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ABSTRACT

It remains a mystery about the role of chondrocyte or cartilage on the coexistence of ultra-high molecular weight polyethylene (UHMWPE) wear particles from partial joint arthroplasty. An inverted co-culture system was performed to investigate the interactions between chondrocytes and UHMWPE wear particles. It was first time observed that chondrocytes can engulf UHMWPE particles and release osteoarthritis associated pro-inflammatory factors. TEM observation and flow cytometric analysis demonstrated the phagocytosis of particles by chondrocytes. It was found that polyethylene particles may reduce the viability of chondrocytes, and enhance the secretion of nitric oxide (NO) and PGE_2. In conclusion, all these phenomena may contribute to further cartilage degeneration after partial joint arthroplasty surgery. It is first identified in this study that the chondrocyte acts as phagocyte to internalize wear particles and leads to the elevations of precursor mediators of osteoarthritis.

Partial joint arthroplasty is a surgical treatment for partial damage of cartilage involving in only one compartment of the knee [1–4]. Ultra high molecular weight polyethylene (UHMWPE) is the most common bearing material to cushion impact forces on the partial joint prostheses. UHMWPE wear particles are generated, released, and accumulated in the joint surroundings and may interact with the adjacent cartilage. However, progressive degeneration of the cartilage remaining in the joint is commonly seen several years after the surgery [5–7]. It has been reported that the osteoarthritis in the opposite cartilage compartment is the major reason causing the failure of the partial joint arthroplasty [5–7].

It has been recognized that while UHMWPE are generated and released, macrophages are the first-line defending cells for these foreign materials [8]. As macrophage engulfing UHMWPE polyethylene particles, mediators which are capable of inducing osteoarthritis are released [8–13]. In addition, there remains the possibility of interactions between UHMWPE particles and adjacent chondrocytes or cartilage tissues. Surprisingly, Castillo et al. stated that chondrocytes have the ability to engulf latex particles and cell debris by cytoplasmic projections in vitro [14]. However, whether the above inflammatory mediators leading to osteoarthritis could be released by chondrocytes after engulfing foreign particles is still not known.

Osteoarthritis is related to pro-inflammatory substances as interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), and interleukin-17 (IL-17) secreted by macrophages and they further stimulate chondrocytes to releases matrix metalloproteinases, cathepsins, aggrecanases which decompose extra cellular matrix [15]. In addition, nitrogen oxide (NO) would interact with superoxide anions to form peroxynitrite and cause progression of cartilage degradation [16]. Prostaglandin E_2 (PGE_2), which intensifies inflammatory responses, is released by activated macrophages and damaged cells. It is a main catabolism of osteoarthritic joints generated during cartilage degradation and chondrocyte apoptosis [16,17].

In this study, a newly developed inverted in vitro cell culture system was designed to study the interactions between chondrocytes and UHMWPE particles. Lipopolysaccharide (LPS) was added to see if it can exaggerate phagocytosis ability and inflammatory responses of chondrocytes [18]. Flow cytometry and transmission electron microscopy methodologies were applied to observe the contact between UHMWPE particles and chondrocytes. Significant factors which are capable of inducing osteoarthritis such as PGE_2 and NO were also analyzed.

Materials and methods

Generation of UHMWPE particles. A micro-cutting process was facilitated to generate UHMWPE wear particles with uniform size and shape. Wedge-shaped features on the surface were designed for the micro-cutting process to generate UHMWPE particles [19–21]. The details of the surface-texture design principles

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have been described previously [21]. Raw GUR1050 UHMWPE materials obtained from United Orthopaedic Corporation, Taiwan, were used in this study. UHMWPE cylinder pins were machined to 6.35 mm in diameter and 25.4 mm in length with diamond turning on both end surfaces without polishing. A linear reciprocating wear test was carried out under a nominal contact pressure of 3 MPa, a stroke length of 19 mm, a frequency of 1.5 Hz, and an average sliding speed of 57.2 mm/s in purified water. After the wear tests, the UHMWPE particles were collected by repeated rinsing (purified water, adjusted to pH 5.5, with hydrochloric acid) of the sample, sample holder, and parts that came into contact with particles, into a sterilized beaker. Particles that were dispersed in the solution were collected on a 0.1 μm pore size membrane through a vacuum filtration process. The particles were further exposed to ultra-violet light overnight for sterilization purpose. The UHMWPE particles are averaged 5.65 ± 1.47 μm in length and 2.25 ± 0.78 in aspect ratio. The images of the UHMWPE particles are shown in Fig. 1.

Co-culture of chondrocytes with UHMWPE particles. A knee joint was harvested aseptically from an adult porcine within 6 h after slaughter. It was washed and sterilized with povidone iodine and 70% ethanol. The articular cartilage tissue on the distal femur was retrieved and cut into small pieces following the digestion process with 0.2% collagenase (Sigma, USA) in culture medium (DMEM with 10% fetal bovine serum and 50 μg/ml ascorbic acid) for 12 h. The suspended solution was

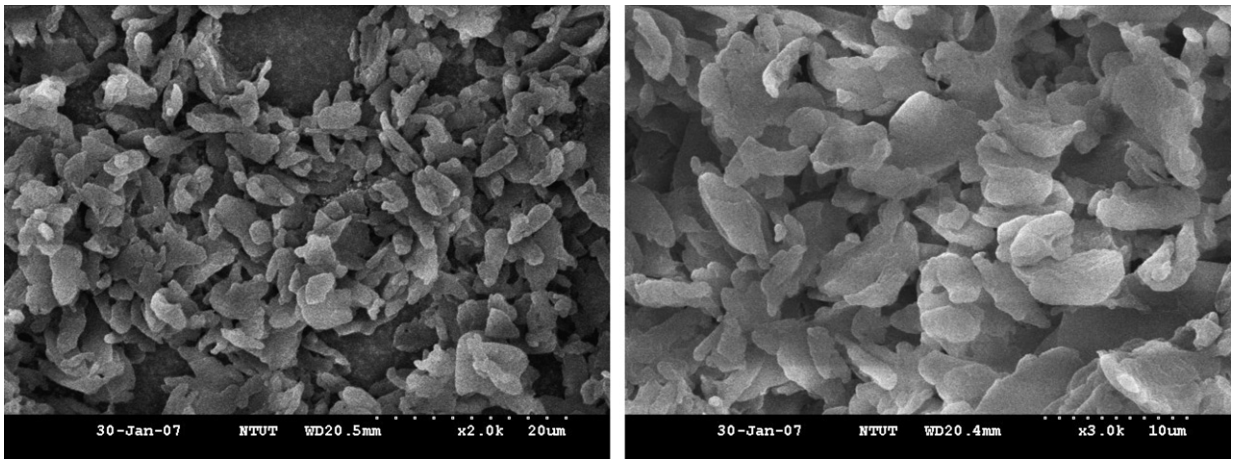

Fig. 1. Scanning electron microscopy (SEM) of UHMWPE wears particles generated by articulation of the microfabricated surface against UHMWPE material.

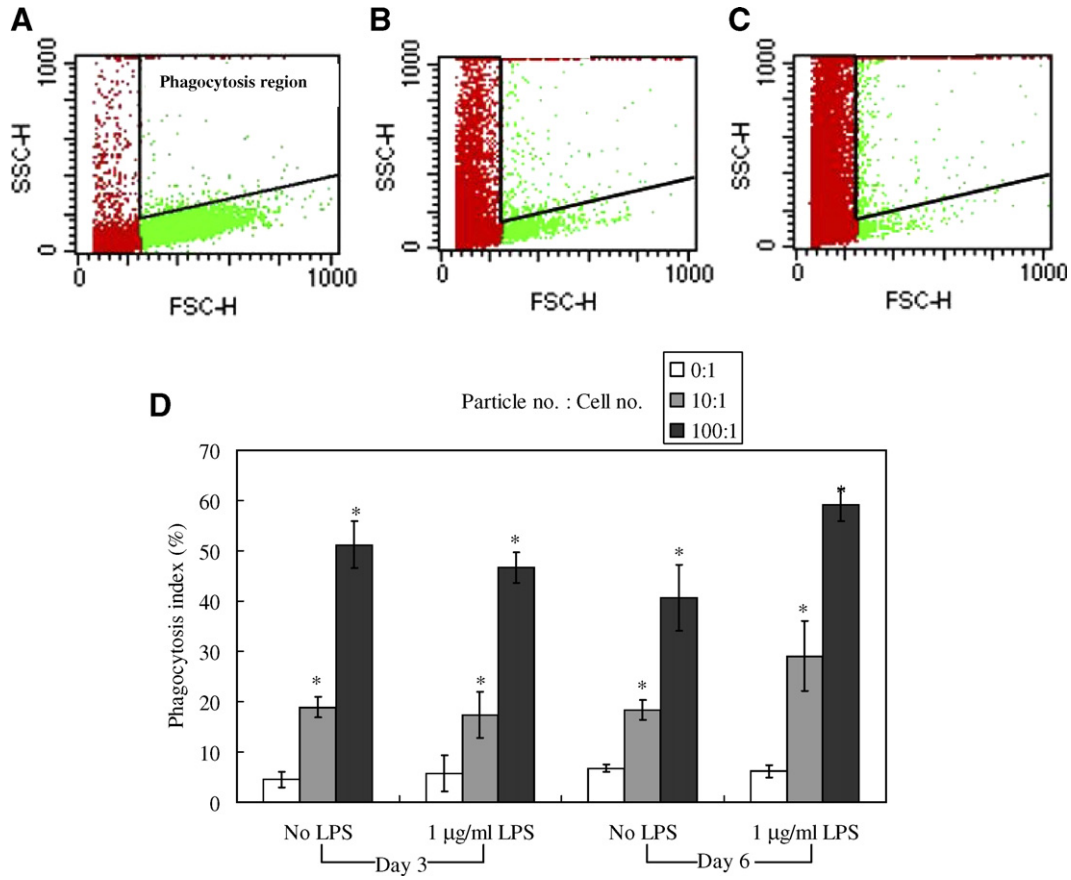

Fig. 2. Analysis of the cells by flow cytometry. Forward scatter (FSC) means the size of the cells and side scattering (SSC) means the granularity of cells. After phagocytosis, cell granularity and size increased. In the green region, the proportion of cells in the phagocytosis region was calculated as the phagocytosis index [8]. The UHMWPE particles were smaller and they concentrated in the red region, so the red region was excluded. (A) No UHMWPE particles. (B) Particle number: cell number = 10:1. (C) Particle number: cell number = 100:1. (D) Phagocytosis index of chondrocytes co-cultured with UHMWPE wear particles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)