Initial response of human bone marrow–derived stem cells after contact with ultrahigh-molecular-weight polyethylene (UHMWPE) material: An in vitro study on cell viability and interleukin-6 expression

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Introduction

Ultrahigh-molecular-weight polyethylene (UHMWPE) is a thermoplastic polymer useful in biomaterial applications; it has high biocompatibility, chemical stability, impact strength, and wear resistance.1 UHMWPE is known to be prone to generate wear particles after long-term use in joint arthroplasty surgery.2–4 Polyethylene debris induces inflammatory responses from macrophages, osteoclasts, osteoblasts, and fibroblasts. These cells produce cytokines that stimulate osteoclast activity and promote osteolysis.5–10 It was reported earlier that variation in the shape of UHMWPE particle debris could induce different cellular responses. UHMWPE debris with sharp fibular-shaped particles induced a substantial increase in cellular infiltration and increased expression of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the murine air pouch model when compared to globular-shaped particles of a similar volume.11 Furthermore, Sieving et al.12 reported variation in cellular response to various shapes and surface roughness of UHMWPE particle. Their investigation suggested that both shape and surface roughness influence the severity of specific inflammatory responses, and the rough surface debris induce a marked effect on adverse tissue responses when combined with particles that have a sharp, elongated shape.13 Although the effects of polyethylene debris to several human cells have been well studied, little is known about its cellular effect at the initial contact as a block material (non-particle debris). This study aimed to investigate the response of human bone marrow–derived stem cells to a block material of UHMWPE that resembles the initial situation when total arthroplasty prosthesis was implanted in a human joint.

Materials and Methods

Human bone marrow–derived stem cell isolation, culture, and immunohistochemistry

This study was approved by the Ethics Commission of Prof. Dr. R. Soeharso Orthopaedic Hospital, Solo, Indonesia. Approximately 10mL bone marrow suspension was harvested from the femoral intramedullary canal of patients who received total hip arthroplasty surgery after obtaining written consent. It was collected in a 20-mL tube (Falcon; BD Bioscience, Bedford, MA, USA) containing an equal volume of heparinized (10 U/mL) phosphate-buffered saline (PBS) to avoid clotting. The mixture of heparinized PBS and bone marrow was kept at 4°C before further processing. The culture processes were conducted in the Laboratory of Cell Culture at the Department of Physiology, School of Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

The obtained bone marrow was loaded and centrifuged at 2500rpm for 10min at room temperature. The upper layer of mononuclear cells was collected and washed with PBS twice and with Dulbecco’s modified Eagle’s medium (DMEM) once. The isolated cells were suspended in DMEM, supplemented with 15% PBS and 1% antibiotic–antimycotic solution (Sigma-Aldrich, St. Louis, MO, USA), and seeded into 25cm² flasks. The cells were then incubated at 37°C, 5% CO₂. The nonviable cells were removed by washing twice with PBS on the third day, and new medium was applied. We used inverted microscope to evaluate the cell density and morphology. When 80% of cells confluence was reached, they were harvested using 0.25% trypsin (Sigma-Aldrich) and then subcultured. We used the third passage of cells for subsequent studies [Figure 1]
Immunohistochemistry staining was used for confirmation of the obtained stem cells. A 4% paraformaldehyde solution was used for fixation of the cells. It was blocked to avoid a nonspecific antibody binding. Then an overnight incubation with primary antibodies at 4°C was carried out. For specific cell evaluation, anti-CD-44 and anti-CD-19 were used for human bone marrow stem cell (hBMSC) (Bioss, Woburn, MA, USA). Mesenchymal lineage stem cells were confirmed with the positive result of CD-44 and hematopoietic lineage stem cells with that of CD-19. A fluorescence microscope (Eclipse E400; Nikon, Tokyo, Japan) was used in this process.

**Cytotoxicity study of UHMWPE on hBMSC and IL-6 expression**

A 96-well microplate was used for re-culture of the obtained stem cells. One 96-well microplate was prepared for each incubation period: 24, 48, and 72h. The UHMWPE implants were placed in the wells before seeding the stem cells into the wells. The implants consist of two different sizes: UHMWPE with size $2 \times 2$mm (size A) and with size $4 \times 2$mm (size B). Both of them had 1mm thickness [Figure 2]. After seeding of $1 \times 10^4$ cells, the plates were incubated at 37°C, 5% CO2 for 24, 48, and 72h. An inverted microscope was used for the evaluation of cells. Before the cytotoxicity study, we collected the culture medium for the measurement of IL-6 expression.

A 50-$\mu$L solution containing 5mg/mL of 3-(4,5-dimethylthiazol 2-yl)-2,5-diphenyltetrazolium (MTT) reagent in PBS was added to each well of 96-well microplate, which will be evaluated for cytotoxicity study, and then reincubated for 4h in 5% CO2 and 37°C. Viability of the cells was evaluated with colorimetric assay after incubation for 24, 48, and 72h with UHMWPE implant treatment. To each well, 50 $\mu$L dimethyl sulfoxide was added and reincubated for 5min at 37°C to accomplish the cytotoxicity test process. We measured the optical density of each well using enzyme-linked immunosorbent assay (ELISA) reader in 620-nm wavelength. With a significance of 95%, the data were tabulated and analyzed using t-test (version 17; SPSS, Chicago, Illinois, USA). Viable cell percentage was calculated using the IC50 formula. The amount of IL-6 on culture medium was quantified by an immunoassay kit (ELISA; Koma Biotech, Yeongdeungpo-gu, Seoul, Korea).

**Results**

**Human bone marrow–derived stem cell cultures and cytotoxicity study**

Visual microscopic evaluation was carried out in all the study groups before performing MTT assay using an inverted microscope (Eclipse E400; Nikon). We found that bone marrow–derived stem cells attached to the implant in all the experiment groups [Figure 3]. According to the results of MTT assay, there was no statistical difference in the optical cell density of all treatment groups compared to control group at 24, 48, and 72h of incubation period [Table 1], [Table 2], [Table 3], [Table 4].
Figure 3: Microscopic analysis of hBMSC around UHMWPE implants. Stem cells attached to the implant in all groups (×10). Size A, 24h (A); size B, 24h (B); size A, 48h (C); size B, 48h (D); size A, 72h (E); size B, 72h (F)

Table 1: Mean and standard deviation of cell optical density of all study groups at 24, 48, and 72h of incubation period

| Cell viability | 24h | 48h | 72h |
|---------------|-----|-----|-----|
| Size A        | 2.2 ± 0.91 | 1.2 ± 0.27 | 1.8 ± 0.13 |
| Size B        | 1.4 ± 0.47 | 1.6 ± 0.23 | 1.4 ± 0.13 |

Table 2: Difference of mean optical density between incubation periods of cell control group

| Incubation period | Control group | Size A | Size B |
|-------------------|---------------|--------|--------|
| 24h               | -             | -0.39  | -0.47  |
| 48h               | -             | -0.38  | -0.45  |
| 72h               | -             | -0.38  | -0.45  |

Statistical analysis showed that at the control groups significant difference (P < 0.05) was observed between 24 and 72h group, also between 48 and 72h group. No significant difference was observed between 24- and 48-h groups. In the treatment group, significant difference was found in size A and size B implants between 48 and 72h and between 24 and 72h of incubation period, respectively. At 24, 48, and 72h, all treatment groups showed >50% cells that were viable [Table 5].

Table 5: Percentage of stem cell viability after contact with UHMWPE after each incubation period

| Incubation period | Size A | Size B |
|-------------------|--------|--------|
| 24h               | 120%   | 141%   |
| 48h               | 107%   | 110%   |
| 72h               | 115%   | 116%   |

Table 2: Difference of mean optical density between incubation periods of size A implant group

| Incubation period | Size A |
|-------------------|--------|
| 24h               | -0.38  |
| 48h               | -0.37  |
| 72h               | -0.37  |

Table 3: Difference of mean optical density between incubation periods of size B implant group

| Incubation period | Size B |
|-------------------|--------|
| 24h               | -0.32  |
| 48h               | -0.30  |
| 72h               | -0.30  |

Expression of IL-6

According to the results of ELISA, we found lower expression of IL-6 in hBMSC compared to control group at 48h of incubation period both in size A and size B implants. We found higher IL-6 expression compared to control group at 72h of incubation period in size A implant [Figure 4].

Figure 4: Expression of IL-6 after all incubation periods (pg/mL)

Discussion
A study by Chiu et al. [14] evaluated the effect of UHMWPE particle on murine bone marrow osteoprogenitor cells; the study showed that UHMWPE particles inhibit the osteogenic activity of osteoprogenitor cells, which may result in reduced periprosthetic bone regeneration and repair. Exposure of both cell population to UHMWPE particles resulted in a dose-dependent decrease in mineralization, proliferation, alkaline phosphatase activity, and osteocalcin production when compared with control cells cultured on collagen matrix without particles. [14] Another study by Preedy et al. [15] reported the changes in cell elasticity and spring constant of rat mesenchymal stem cells (MSCs) exposed to wear particles occurred in the first 24h of contact. The particle concentration ranging from 0.5 to 50mg/L did not play a significant role. [15] Cell viability study by Lin et al. [16] using cultured mouse MSCs resulted in a decrease of cell viability after contact with UHMWPE particle wear. Huang et al.[17] examined the effect of UHMWPE particle wear on chemotaxis of human MSCs. They found that chemokines released by macrophages stimulated by wear particles can have an effect on the migration of macrophages and MSCs.

Different to previously reported studies, our study involved an hMSC response examination to a block of UHMWPE material (non-particle) resembling the real intraoperative condition when a block of UHMWPE material was implanted in a human joint during joint replacement surgery. We believe this method could explain the early cell response after contact with UHMWPE material. In our study, we found there was a decrease in cell viability at the 48-h incubation period of smaller implant; however, there was still high cells viability at 72-h incubation period. This finding was consistent with the expression of IL-6 in smaller implant group (48h), which was the lowest IL-6 expression compared to other incubation period. No modulation on IL-6 expression was seen after 24h of contact with UHMWPE material as compared to control group. IL-6 expression was downregulated at 48h of contact with UHMWPE. However, it was re-increased at 72h of incubation period. The pattern of IL-6 expression in the experiment group was different from the result in the control group.

In this study, the cell viability and IL-6 expression after contact with a larger (size B) UHMWPE material showed an inconsistent pattern. Despite stable, excellent cell viability during all incubation periods, a marked decrease of IL-6 expression was observed at the 72-h incubation period. This finding indicates that the cellular response to UHMWPE may vary with size and volume of the UHMWPE block material, and further study is needed in this regard. For comparison, Rader et al. [18] reported that the volume of UHMWPE wear particles is an important factor for the release of cytokines. Certain volume of wear particle is needed for maximum release of inflammatory mediators (TNFα, IL-1, IL-8). [18]

**Conclusions**

hBMSC showed a high cell viability after initial contact with UHMWPE material. Modulation of IL-6 expression was present at the initial stage as a response to foreign material.

**Financial support and sponsorship**

This study was funded by PUSNAS research grant of the Ministry of Research, Technology and Higher Education of Indonesia, based on the agreement letter: 022/SP2H/LT/DRPM/II/2016 on 17 February, 2016.

**Conflicts of interest**

There are no conflicts of interest.

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