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The biological response of macrophages to PMMA particles with different morphology and size

R. Yoshioka[a], Y. Nakashima[b], Y. Fujiwara[c], Y. Komohara[c], M. Takeya[c], Y. Nakanishi[b,*]

[a]*Graduate School of Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan*
[b]*Faculty of Advanced Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan*
[c]*Faculty of Life Science, Kumamoto University, Kumamoto 860-8555, Japan*

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Abstract

We used polymethylmethacrylate (PMMA) particles to investigate the relationship between particle properties and biological responses of macrophages. In a previous study, we reported that biological response of these immune cells was activated by a specific PMMA particle size. In this study, biological responses of macrophages to particle parameters other than and in conjunction with size were evaluated. However, particle size as a biologically active factor of the biological response has not been characterized in detail. Here, macrophage viability and proinflammatory cytokine production were investigated to elucidate the relationship between particle size, added volume, and added surface area, and the biological response of macrophages. Decrease of cell viability was observed when relatively large particles were tested (5.6–19.3 μm). Production of proinflammatory cytokines was elicited by 5.6- and 9.6-μm particles. Cell death occurred when the added volume exceeded 1×10^5 μm^3 per cell. Proinflammatory cytokines were produced upon stimulation with added volume between 1×10^5 and 4.5×10^5 μm^3 per cell. Cell death was elicited when the added surface area per cell exceeded 1×10^5 μm^2 and proinflammatory cytokines were produced with the added surface area per cell between 1×10^5 and 3×10^5 μm^2. These results suggested that biologically active factors exert their effect through added volume and added surface area rather than through particle size. This work will contribute to biological responses after total joint replacement since particles generated in the joint as a result of load bearing lead to tissue reaction and joint loosening.

Keywords: Biological response; Macrophages; PMMA particles; Viability; Proinflammatory cytokine

1. Introduction

In our previous study, we investigated the relationship between "particle size" and biological response of macrophages [1]. The results of that study indicated that a specific particle size elicits the biological response of macrophages. However, additional particle parameters should be investigated and further research is required for their biological evaluation. Particle morphology, i.e., added volume and added surface area, should be explored and the relationship between these parameters and biological response of macrophages should be examined. Furthering the research into the relationship between particle morphology and size, and the biological response of macrophages is highly relevant, e.g., for artificial joint research.

Artificial joint replacement is an effective surgical intervention for end-stage arthropathy, such as osteoarthritis and rheumatoid arthritis [2,3]. However, several studies have reported that the failure rate of artificial joint replacement increases after 10–20 years post implantation [2–8]. Artificial joint loosening, caused by osteolysis, has been proposed as one of the causes. Osteolysis is associated with wear debris from ultra-high molecular weight polyethylene (UHMWPE) [9–12]. The mechanism of osteolysis involves macrophages, a type of

*Correspondence to: Faculty of Advanced Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan.
E-mail address: y-naka@mech.kumamoto-u.ac.jp (Y. Nakanishi).
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phagocytic white blood cells. Macrophages release proinflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) [2,4,9,10,13], after phagocytosing wear debris, resulting in the stimulation of osteoclasts. Stimulated osteoclasts, in turn, cause osteolysis [3,5,11,14]. Phagocytosis of debris by macrophages thus plays an important role in osteolysis. Ingram et al. [11] reported that particles with sizes 0.1–10 μm activate the biological response of macrophages. Amer et al. [2] reported stimulatory effect of particles sized 0.2–10 μm. In contrast, Koseki et al. [15] and Sabokbar et al. [14] reported that particles smaller than 1.0 μm also elicit biological response of these immune cells. In this regard, it is not clear whether particle size plays a *bona fide* role in the biological response of macrophages. Inhibition of the biological response of macrophages is expected to be attained by maintaining a balance between the enlargement and minimization of UHMWPE wear debris [16]. In the current study, we investigated the effect of particle size, added volume, and surface area on the biological response of macrophages by evaluating cell viability and proinflammatory cytokine production.

2. Materials and methods

2.1. Preparation of narrow-dispersion polymethylmethacrylate (PMMA) particles

To elucidate the relationship between particle morphology and proinflammatory cytokine production, narrow-dispersion PMMA particles (Soken Chemical & Engineering Co., Ltd., Japan) were used [17]. Fig. 1 shows scanning electron microscope (SEM) images of PMMA particles. The following mean particle sizes were used in the current study: 0.16, 0.43, 0.8, 1.6, 5.6, 9.6, and 19.3 μm; 0.16- and 0.43-μm particles were composed of cross-linked PMMA and the remaining particles were composed of non-cross-linked PMMA. PMMA particles were washed with 70% ethanol before concentration adjustment, as described [18]. The concentration of PMMA particles was adjusted with phosphate-buffered saline [PBS (-)] prepared by dissolving sodium di-hydrogen phosphate dihydrate (199-02825, Wako Pure Chemical Industries, Ltd., Japan), di-sodium hydrogen phosphate dodecahydrate (196-02835, Wako Pure Chemical Industries, Ltd., Japan), and sodium chloride (192-13925, Wako Pure Chemical Industries, Ltd., Japan) in distilled water.

2.2. Preparation of human monocyte-derived macrophages (HMDMs)

Cell assays were performed using HMDMs isolated as described previously [1]. Briefly, HMDMs were obtained from healthy volunteer donors. Informed written consent was obtained from all donors. Fig. 2 shows a schematic of monocyte isolation from the blood. PBS (-) containing 1 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid disodium salt dehydrate (EDTA; 34501861, Dojindo Molecular Technologies, Inc., Japan) was added to the collected blood and the samples were centrifuged at 200*g* for 35 min using a general purpose refrigerated centrifuge (5920, KUBOTA Co., Japan) to separate blood cells and serum. The supernatant (blood serum) was removed and PBS (-) with EDTA was added to the pellet. Lymphoprep™ (1114547, Axis-Shield Diagnostics Ltd., UK) was measured out in another tube, according to manufacturer's instructions, and the suspension of blood cells in PBS (-) with EDTA was slowly added, to prevent mixing. The tube was centrifuged at 200*g* for 35 min to separate red blood cells, mononuclear cells, and blood platelets. The supernatant (blood platelets) was removed and mononuclear cells were collected. PBS (-) with EDTA was added to the collected mononuclear cells. The samples were centrifuged at 200*g* for 10 min to separate monocytes, lymphocytes, and other cell types. The supernatant (lymphocytes, etc.) was removed and Dulbecco's modified Eagle's medium (DMEM; 041-29775, Wako Pure Chemical Industries, Ltd., Japan) supplemented with 2% fetal bovine serum (FBS; 172012-500 ML, 12E183-A, Sigma), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (168-23191, Wako Pure Chemical Industries, Ltd., Japan) were added. The culture medium was divided between two 100-mm PRIMARIA™ tissue culture dishes (353803, BD Falcon, USA). Granulocyte-macrophage colony-stimulating factor (GM-CSF; 075-04114, Wako Pure Chemical Industries, Ltd., Japan) was added to one dish, and macrophage colony-stimulating factor (M-CSF; 139-13613, Wako Pure Chemical Industries, Ltd., Japan) was added to the other dish. The dishes were incubated for 7 d at 37 °C and 5% CO_2. After incubation,

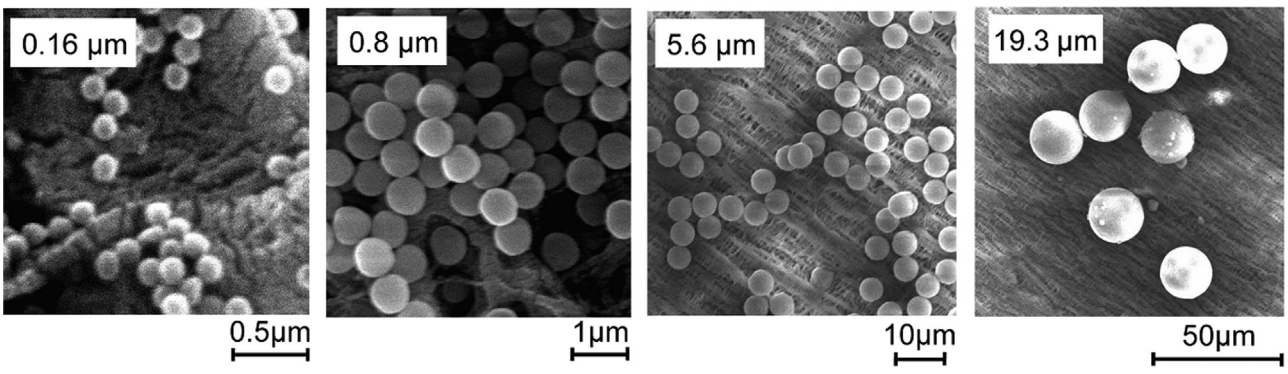

Fig. 1. SEM images of PMMA particles. Particle sizes are as labeled and scale bars are shown and reproduced by permission from Chikaura [1].