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Effect of particle size on biological response by human monocyte-derived macrophages

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Abstract

Ultra-high molecular weight polyethylene (UHMWPE) wear particles from artificial joints induce osteolysis and the subsequent loosening of implants. Studies have reported that particles in the size range of 0.1–10 μm are the most biologically active in macrophage immune response. To develop prosthetic joints with greater longevity and durability, it is crucial to understand the deleterious effects of wear particles. In this study, to evaluate the effects of particle size on the activities of human monocyte-derived macrophages (HMDMs), seven differently sized particles of polymethylmethacrylate (PMMA), in the range of 0.1–20 μm, were prepared. Viability and the secretion of cytokines were evaluated after phagocytosis of each size particles by HMDMs. Differences in the viability of HMDMs after phagocytosis of particles sized 0.16–9.6 μm were statistically significant. Proinflammatory cytokine production of both tumor necrosis factor-α and interleukin-6 by HMDMs was strongly induced by 0.8 μm PMMA particles. Consistent with the fact that macrophages are known to respond to pathogens measuring approximately 1.0 μm in size, in this study, PMMA particles measuring 0.8 μm in size induced an immune response. This work provides fundamental data for the designing of surface profiles of prosthetic joints, Which may expect the lower incidence of immune response.

Keywords: Particle size; Biological response; Artificial joint; Macrophage; Cytokines

1. Introduction

Several studies have reported the phagocytosis of ultra-high molecular weight polyethylene (UHMWPE) wear particles from artificial joints by macrophages [1–5]. Macrophages release cytokines that stimulate osteoclasts, thereby resulting in bone resorption [2,6,7]. UHMWPE wear particles induce osteolysis and the subsequent loosening of implants, which are the main factors of aseptic revision. Phagocytosis of particles by macrophages represents an important component of the cellular response to implants. UHMWPE particles within a size range of 0.1–10 μm are known to be the most biologically active [8–10]. In particular, particles measuring less than 1.0 μm in size are thought to induce biological responses and stimulate macrophages to produce inflammatory mediators such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [2,4,11,12]; these study findings indicate that the size of the wear particles is an important parameter. In this regard, previous studies have proposed that the nano-level surface texture of the Co–Cr–Mo alloy would ensure both, a larger size of UHMWPE wear particles and minimization of the wear of UHMWPE, which is expected to inhibit the biological activity of macrophages [13]. However, it is not clear that particle size affects on biological response. With an aim to design a surface profile to ensure lower incidence of osteolysis and aseptic loosening of implants, this study investigated the effect of the particle morphology, especially the particle size, on immune responses of human monocyte-derived macrophages. Furthermore, this study attempted to determine the particle size

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that induces the most hazardous biological response by analyzing viability and the secretion of proinflammatory cytokines in macrophages after they phagocytosed particles of each size.

2. Materials and Methods

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

To unveil the relationship between particle size and biological response, narrow-dispersion (PMMA) particles (Soken Chemical & Engineering Co., Ltd., Japan) were used instead of UHMWPE wear particles. Spherical particles with a mean size of 0.16, 0.43, 0.8, 1.6, 5.6, 9.6 and 19.3 μm were prepared. The 0.16 and 0.43 μm particles were composed of cross-linked PMMA and the others were composed of non-cross-linked PMMA. Fig. 1 showed the scanning electron micro scope (SEM; JSM-6390LV, JEOL Ltd., Japan) images of PMMA particles. Particles of each size were washed with 70% ethanol. The particles were suspended in phosphate-buffered saline (PBS (-)), which made by dissolving sodium dihydrogenphosphate dihydrate (199-02825, Wako Pure Chemical Industries, Ltd., Japan), disodium hydrogenphosphate 12-Water (196-02835, Wako Pure Chemical Industries, Ltd., Japan) and sodium chloride (192-13925, Wako Pure Chemical Industries, Ltd., Japan) in distilled water, in order to obtain an initial concentration of 50 μg/μL. To disperse the aggregated fine particles, the suspensions were treated with ultrasonic waves using Bioruptor (UCD-250, Cosmo Bio Co., Ltd., Japan).

2.2. Preparation of macrophages

Human monocyte-derived macrophages (HMDMs) obtained from healthy volunteer donors were used in the present study. Informed written consent was obtained from all donors. The preparation procedure for the cells is shown in Fig. 2. Peripheral blood (35 mL) was collected from healthy donors. Fifteen milliliter of PBS (−) with 1 mM ethylenediamine-N, N,N′,N′-tetraacetic acid, disodium salt, dihydrate (EDTA 2NA; 34501861, Dojindo Molecular Technologies, Inc., Japan) was

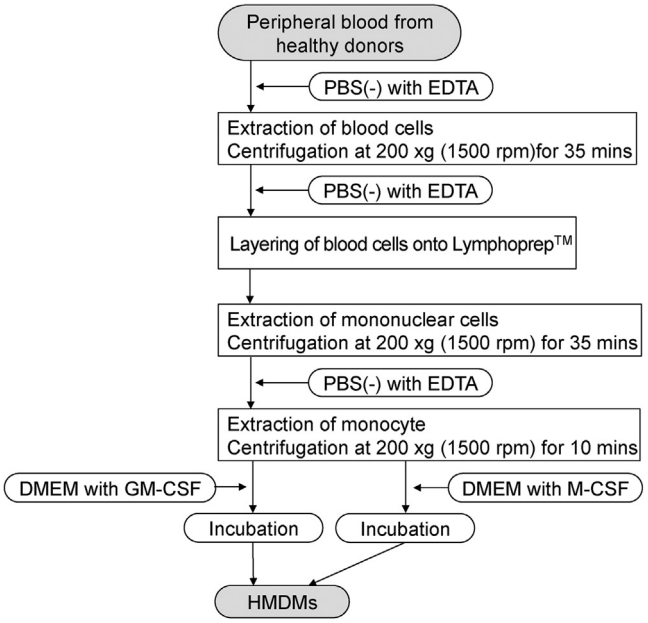

Fig. 2. Procedure for the preparation of HMDMs.

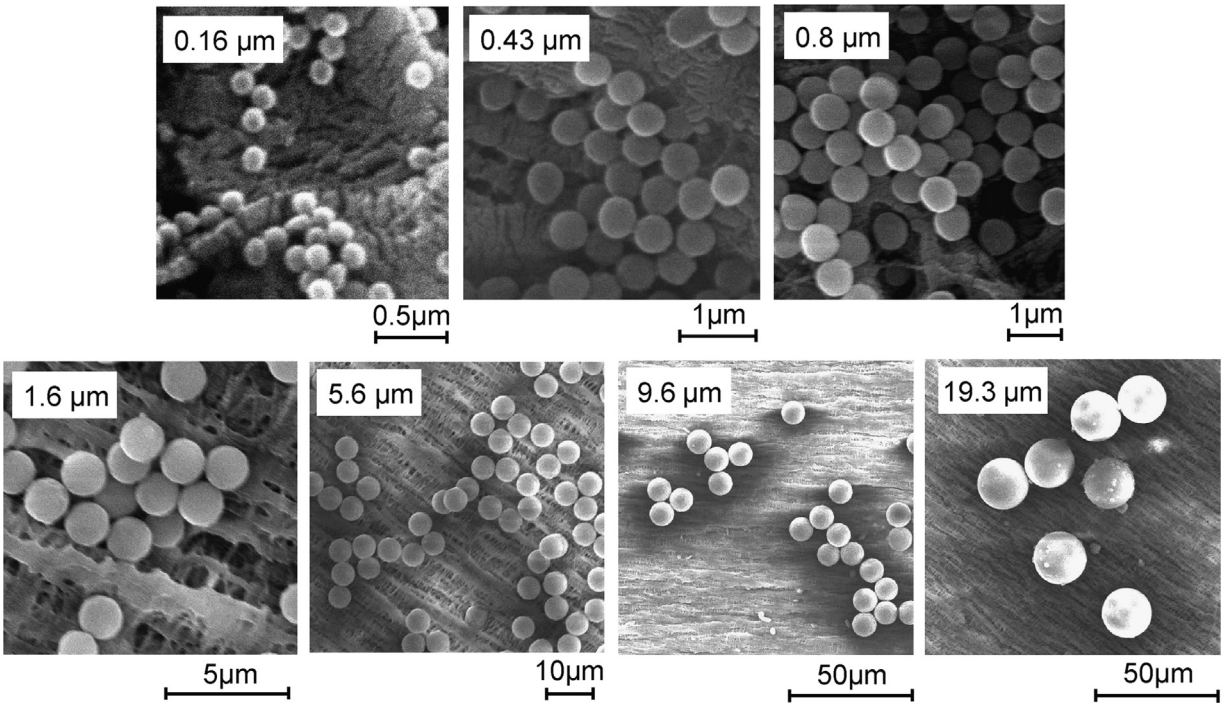

Fig. 1. SEM image of PMMA particles. Particle sizes were 0.16–19.3 μm.