Differential effects of oxidised and non-oxidised polyethylene particles on human monocyte/macrophages in vitro

K. Kamikawa, Y. Harada, K. Nagata, H. Moriya
From Chiba University, Chiba, Japan

Sterilisation by gamma irradiation in the presence of air causes free radicals generated in polyethylene (PE) to react with oxygen, which could lead to loss of physical properties and reduction in fatigue strength. Tissue retrieved from failed total hip replacements often has large quantities of particulate PE and most particles associated with peri-implant osteolysis are oxidised. Consequently, an understanding of the cellular responses of oxidised PE particles may lead to clarification of the pathogenesis of osteolysis and aseptic loosening.

We have used the agarose system to demonstrate the differential effects of oxidised and non-oxidised PE particles on the release of proinflammatory products such as interleukin-1β (IL-1β), IL-6, and tumour necrosis factor-α (TNF-α) from monocytes/macrophages (M/M). Oxidised PE particles were shown to stimulate human M/M to phagocytose and to release cytokines. Oxidation may alter the surface chemistry of the particles and enhance the response to specific membrane receptors on macrophages, such as scavenger-type receptors.

Materials and Methods

Isolation of cells. We prepared human M/M from freshly drawn, heparinised, peripheral blood by Ficoll-diatrizoate (Histopaque #1077; Sigma, St Louis, Missouri) gradient separation after dilution with two volumes of Dulbecco’s phosphate-buffered saline. The blood cells were washed and seeded onto Petri dishes with RPMI 1640 medium (Gibco BRL, Grand Island, New York) containing 10% fetal calf serum (FCS; Sigma). After incubation at 37°C for one hour in a humidified incubator with 5% CO₂, the cells were washed three times with phosphate-buffered saline and a population of more than 95% M/M remained as adherent cells. After isolation, M/M were harvested by treatment with trypsin-EDTA. The cells were then washed thoroughly and resuspended in fresh medium.

Preparation of particles. Oxidised and non-oxidised particles of high-molecular-weight polyethylene (HMWPE) were purchased from Daniel Products Company Inc (Jersey City, New Jersey). Silica (SiO₂) particles of 0.5 to 10 μm in size and latex particles of 3 μm were purchased from Sigma. The shape and surface topography of the particles were assessed using field-emission SEM (Hitachi S-4500). The distribution of size was analysed by a laser-based...
particle size analyser (CIS-1; Galai Production Ltd). The oxidation index was calculated from Fourier transform infrared spectroscopy scans (FTIR), (System 2000R Perkin Elmer) and is defined as the ratio of the carbonyl peak area (1650 to 1750 cm^(-1)) to the methyl/methylene peak area (1468 cm^(-1)).

**Preparation of PE particles for culture.** We prepared 1.5 × RPMI and 1% agarose (Sigma) separately. PE particles were added to the 1.5×RPMI at predetermined concentrations followed by ultrasonification for five minutes. One volume of 1% agarose, two volumes of the PE particles-RPMI mixture and 0.3 volumes of FCS were mixed quickly and added to a 24-well tray. Before completion of the gelling reaction, the plates were centrifuged at 2000 rpm (approximately 800 g). This resulted in a uniform superficial layer of PE with a minimal number of floating particles.

**Cell culture.** M/M in a concentration of 5 × 10^5 cells/well were cocultured with particles diluted at a concentration of 0.1 v/v% and 0.3 v/v%. After incubation for 48 hours at 37°C in a humidifier incubator with 5% CO₂, the supernatant was harvested and stored at -20°C for subsequent assays. To assess the phagocytosis of particles, cells were harvested and plated in a four-well chamber slide (Nunc Inc, Naperville, Illinois) followed by examination by light microscopy after Giemsa staining.

**Assays.** Cell viability was determined by direct cell counting using a haemocytometer and Trypan Blue exclusion. Interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) were assayed by an enzyme-linked immunosorbent assay (ELISA; Amersham, Buckinghamshire, UK). The results were expressed as the mean ± s.d of four measurements. Statistical significance was determined using Student’s t-test.

**Results**

**Characterisation of PE particles.** The shape and surface topography of the particles were assessed by SEM. The oxidised and non-oxidised PE particles were shown to be irregularly-shaped with rough surfaces (Fig. 1). Analysis of the distribution of size indicated that there were no remarkable differences between oxidised and non-oxidised PE particles. Their mean sizes were 5.63 μm and 6.66 μm (0 to 20), respectively (Fig. 2). The oxidation index calculated from FTIR scans was 0.86 for oxidised PE compared with less than 0.1 for non-oxidised PE.

**Phagocytosis of particles by M/M.** Examination of cells using phase-contrast optics showed that the percentage of cells which phagocytosed oxidised PE particles at 48 hours was 51.5 ± 5.2% compared with 32.4 ± 3.7% for non-oxidised PE-treated cells (Fig. 3). The mean number of particles phagocytosed was 13.3 particles/cell (4 to 22) in M/M cocultured with latex beads (3 μm), 4.6 particles/cell (2 to 6) for oxidised PE particles and 1.2 particles/cell (1 to 2) for non-oxidised PE particles. Silica particles tend to become solid and are invisible to phase-contrast optics; consequently the number of silica particles is uncountable.

**Effects of particles on the release of proinflammatory products.** The release of proinflammatory cytokines from M/M cocultured with particles of varying composition was assessed by ELISA. Analysis of the effects of the particles on the release of cytokines showed the levels to be almost maximum by 24 to 48 hours and to have remained elevated at 48 hours. Therefore only the values at 48 hours are shown. Figure 4a shows the effects of particles of different composition on the release of IL-1β from human M/M. Exposure to the silica particles resulted in a marked increase in the level of IL-1β which was 585.5 ± 27 pg/ml compared with the control level of 152.5 ± 22 pg/ml or 175.5 ± 23 pg/ml in M/M incubated with latex particles. The values were statistically significant (Student’s t-test, p < 0.001). Incubation with oxidised and non-oxidised PE particles also produced a significant increase in the level of IL-1β (p < 0.01) compared with control or latex-treated cells. At a concentration of 0.1% (v/v) incubation with non-oxidised PE particles produced 259.5 ± 39 pg/ml of IL-1β and at 0.3% (v/v) 267.5 ± 13 pg/ml. At 0.1% (v/v) incubation with oxidised PE particles gave 297.5 ± 50 pg/ml and at 0.3% (v/v) 308.5 ± 29 pg/ml. At 0.3% (v/v), incubation with oxidised PE particles produced a significant increase in the level of IL-1β compared with non-oxidised PE particles (p < 0.05).

The effect of particles of different composition on the release of TNF-α is shown in Figure 4b. Exposure to particles of different composition, except for latex, resulted in a marked increase (p < 0.001) in the level of TNF-α compared with the control level of 705.5 ± 91 pg/ml (p < 0.001). Silica particles gave 2498.5 ± 210 pg/ml, non-oxidised PE particles at 0.1% (v/v) 1711.5 ± 85 pg/ml, non-oxidised PE particles at 0.3% (v/v) 1741.5 ± 108 pg/ml, oxidised PE particles at 0.1% (v/v) 1782.5 ± 81 pg/ml and oxidised PE particles at 0.3% (v/v) 1924.5 ± 83 pg/ml. At 0.3% (v/v), incubation with oxidised PE particles produced a significant increase in the level of TNF-α level compared with M/M incubated with non-oxidised PE particles (p < 0.05). In addition, oxidised PE particles at a concentration of 0.3% (v/v) produced a significant increase in the level of TNF-α compared with oxidised PE particles at a concentration of 0.1% (v/v) (p < 0.05).

The effects of particles of different composition on the release of IL-6 from human M/M were similar to that of TNF-α (Fig. 4c). Except for latex, there was a marked increase in the level of IL-6 compared with the control level of 1409.5 ± 117 pg/ml, for silica particles (3490.5 ± 250 pg/ml), non-oxidised PE particles of 0.1% (v/v)
and exposure to oxidised PE particles at 0.3% (v/v) produced a significant increase (p < 0.05) compared with oxidised PE particles 0.1% (v/v) (p < 0.05).

Discussion

Wear damage to PE is a serious clinical problem which limits the survival of total joint replacement. It can lead to loosening caused by a biological reaction to particulate PE debris. Histological studies of interface membranes have confirmed that there is a foreign-body granulomatous reaction involving macrophages and phagocytosis by macrophages leads to the synthesis and secretion of proinflammatory mediators. Several studies have demonstrated a positive correlation between the resorption of the proximal femur and wear and in vitro tests revealed that the macrophage response to particulate wear debris depends on the number and size of particles. Cells were shown to be more reactive to small phagocytosable (submicron) and irregularly shaped particles than to those which were larger and more regularly shaped. In our study, the differential effects of oxidised and non-oxidised PE particles on M/M

Fig. 1a
Scanning electron micrographs showing a) oxidised and b) non-oxidised PE particles.

Fig. 1b

Fig. 2a
Graphs showing the distribution of size for a) oxidised and b) non-oxidised PE particles.

Fig. 2b

Fig. 3
Graph showing the percentage of cells which phagocytosed oxidised (OX) and non-oxidised (NOX) particles.
were revealed. Recently, there has been considerable interest in the negative effects of the use of gamma irradiation for sterilisation, particularly in the presence of oxygen. Free radicals generated in PE after gamma irradiation could react with oxygen resulting in a reduction of fatigue strength and liberated wear debris contains many oxidised PE particles.

Although there have been studies of the effects of PE particles using several types of experimental model in vivo\textsuperscript{13,14} direct evaluation of these particles on individual cell types has not been performed. To overcome the limitation of these in vivo systems, in vitro cell culture models were developed to study the effects of PE particles on individual cells.\textsuperscript{8,9,15} These models have continued to be problematical since many PE particles remain in suspension and do not achieve direct contact with adherent monolayer cells in culture. In our study, the PE particles were stabilised on the top layer of the agarose gel, which allowed M/M to attach directly and to phagocytose the particles. Murray and Rushton\textsuperscript{15} estimated that the percentage of cells phagocytosing PE particles had to exceed 60% in order to stimulate a cellular response. It is, however, clear that phagocytosis is not the only factor which determines the capacity of a particle to modulate cell activity. For example, phagocytosis of >90% of latex particles fails to elicit a cellular response.

In our study, the percentage of cells which phagocytosed the oxidised PE particles was 51.5% compared with 32.4% in non-oxidised PE-treated cells at the higher concentration of 0.3% v/v. Furthermore, the oxidised PE particles were shown to stimulate the release of several different products which have been implicated in the pathogenesis of peri-implant osteolysis, including IL-1\(\beta\), IL-6 and TNF-\(\alpha\). These products are most likely to be responsible for the granulomatous inflammation and the disturbance in bone remodelling which leads to the osteolytic process.\textsuperscript{8,12} Oxidised PE particles have many carbonyls, which may play a role in surface charge and opsonisation. The surface charge of particles has some relevance to hydrophilia which may increase the tendency to phagocytosis. Maloney et al\textsuperscript{16} showed that macrophages responded to serum protein opsonised Ti-alloy particles by releasing cytokines. Other studies have revealed that antialbumin IgG opsonised latex particles stimulated alveolar macrophages to release more TNF-\(\alpha\) than non-opsonised particles.\textsuperscript{17} Scavenger-type receptors have also been demonstrated to be involved in the

Graphs showing the effect of particles of different composition on the release of a) IL-1\(\beta\), b) TNF-\(\alpha\) and c) IL-6. The values are shown as the mean and s.d. of four measurements. Statistical significance was determined using Student’s \(t\)-test and the differences between the treated and the control values (no particles) were statistically significant (\(p < 0.01\)) (OX, oxidised PE particles; NOX, non-oxidised PE particles; and Si, silica particles).
reaction between macrophages and oxidised low-density lipid. Consequently, it is possible that carbonyls may also redirect the particles to a specific membrane receptor on macrophages.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


