The effect of particle size and electrical charge on macrophage-osteoclast differentiation and bone resorption

A. SABOKBAR[1*], R. PANDEY[1], N. A. ATHANASOU[1,2]
[1]Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Headington, Oxford, OX3 7LD, UK
E-mail: afsie.sabokbar@ndos.ox.ac.uk
[2]Department of Pathology, Nuffield Orthopaedic Center, Headington, Oxford, OX3 7LD, UK

In aseptic loosening, there is commonly periprosthetic bone loss and a heavy macrophage infiltrate in response to biomaterial wear particles generated from the implant materials. Macrophages which have phagocytosed wear particles are known to be capable of differentiation into bone resorbing osteoclasts. In this investigation we determine the role of particle size and particle charge on this process. Mouse monocytes and macrophages were co-cultured with osteoblast-like UMR106 cells and 1,25 dihydroxyvitamin D3 in the presence or the absence of (i) various sizes of latex beads (0.1, 1, 10 and 100 μm) and (ii) uncharged, positively- or negatively-charged sephadex beads of uniform shape and composition. The extent of osteoclast differentiation by monocytes or foreign body macrophages was determined by the expression of the osteoclast-associated enzyme tartrate-resistant acid phosphatase and lacunar bone resorption. No significant difference in the extent of osteoclast formation and bone resorption was noted in response to particle size. Osteoclast formation was also not significantly different in the presence of positively/negatively charged and uncharged particles. These findings indicate that osteoclast formation is not significantly influenced by particle characteristics, such as particle size. They also add support to the hypothesis that macrophage involvement in periprosthetic osteolysis is not dependent on particle phagocytosis and that it may be induced by particle contact.

Introduction

Aseptic loosening is the commonest cause of arthroplasty failure [1, 2]. In the fibrous membrane surrounding a loose prosthesis, there is a heavy foreign body macrophage response to the deposition of polymeric and metallic biomaterial wear particles derived from implant components [3]. Numerous *in vivo* and *in vitro* studies have been carried out to evaluate the role of wear particles in prosthesis loosening. These studies have shown that wear particle-associated macrophages contribute to periprosthetic osteolysis not only through the release of pro-inflammatory factors (e.g. IL-1, IL-6, TNF and PGE2) that enhance osteoclastic bone resorption [4–9] but also through the differentiation of these cells into mature osteoclasts [10–13].

Osteoclasts are formed by the fusion of bone marrow-derived mononuclear phagocyte precursors which circulate in the monocyte fraction [14]. It has been shown that murine macrophages that have phagocytosed implant-derived polymeric or metallic biomaterial wear particles, when co-cultured in the presence of osteoblast-like cells and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), are capable of differentiating into osteoclasts [11–13]. Phagocytosis of biomaterial wear particles thus does not abrogate the ability of these cells to undergo osteoclast differentiation or bone resorption. It was found that PMMA, UHMWPE and titanium particles were more potent in inducing macrophage-osteoclast differentiation than wear particles derived from stainless steel or cobalt chrome, indicating that the extent of osteoclastic differentiation and bone resorption is partly dependent on the composition of the biomaterial particle phagocytosed [11, 12]. This process of macrophage-osteoclast differentiation has subsequently been shown to operate in man using cell cultures of human monocytes containing biomaterial or latex particles and wear particle-associated macrophages isolated directly from the arthroplasty pseudomembrane surrounding failed implants [15, 16]. It has also been shown that the humoral factors required for this to occur are macrophage-colony stimulatory factor (M-CSF) and the ligand for the receptor activator for NF-κB (RANKL) [15, 17].

*Author to whom all correspondence should be addressed.

TABLE I Studies on the optimization of lacunar bone resorption following co-culture of UMR 106 cells and particle-associated macrophages formed in response to subcutaneous implantation of different concentrations of different sizes of latex beads. Values indicate the mean of two separate experiments

mg/capsule	Mean % bone resorption in response to: latex particle size (μm)			
	0.1	1	10	100
10	2.4	2	1.1	0.1
20	6.2	9.5	12.5	8.3
50	2.7	4.2	2.8	3.2

In addition to biomaterial wear particle composition, other factors, such as the size, shape and surface area of wear particles, are thought to influence the extent of osteolysis that occurs in aseptic loosening [18, 19]. Although a wide size range of particles is found in the fibrous membrane adjacent to loose implants, over 90% of these particles are less than 1 μm in size [20, 21]. Submicron wear particles have been shown to induce the release of humoral factors, such as IL-1β, TNFα and PGE2 [22], which indirectly stimulate osteoclastic bone resorption and promote the recruitment of mononuclear phagocytes into these osteolytic lesions [23, 24]. Recent studies have shown that particle phagocytosis by macrophages is not required for cytokine release and that this release can be induced by mononuclear phagocyte contact with particles alone; these findings suggest that large (non-phagocytosable) biomaterial particles may also play a role in promoting inflammation and osteolysis [18]. Macrophage phagocytosis of 1–10 μm wear particles has also been shown to induce the release of matrix metalloproteinases which degrade the organic matrix that covers the surface of mineralized bone [6].

In this study, we have investigated the effect of particle size on osteoclast formation by macrophages which have been exposed to particles that are of different size but of uniform shape and composition. In addition to particle size, we have also determined the influence of surface charge on particle-associated macrophage-osteoclast differentiation. Previous studies have shown that there are marked differences in the pattern of bone remodeling depending on whether particles are positively or negatively charged [25, 26]. It is known that abundant metal ions derived from implant components are released into the circulation [27, 28]. However, it is not known whether ion-containing particles, which may be positively- or negatively-charged, can affect bone remodeling at the bone-implant interface.

Materials and methods

All cell culture work was carried out using alpha minimal essential medium (MEM, Gibco, Paisley, UK) supplemented with 100 IU/ml of penicillin, 10 μg/ml of streptomycin, 100 mM of L-glutamine and 10% fetal calf serum (FCS) (MEM/FCS; Gibco, Paisley, UK). Cloned hormone-responsive rat UMR 106 osteoblast-like cells were kindly provided by Professor T. J. Martin in Melbourne, Australia. The active metabolite of vitamin D_3 ($1,25(OH)_2D_3$) was purchased from Solvay Duphar (The Netherlands) at the stock concentration of 1×10^{-3} M and diluted in MEM/FCS. All cell culture incubations were carried out at 37 °C in 5% CO_2.

Particles

Spherical latex beads (0.1, 1, 10 and 100 μm diameter), charged (positive/negative) and uncharged sephadex beads (particle size 40–120 μm) were purchased from Sigma Chemicals (Poole, Dorset, UK). The various particles were air-dried and weighed prior to sterilization.

Foreign body granuloma formation and isolation of particle-containing macrophages

Gelatin capsules (Size 2, Farillon Ltd, Essex, UK) containing latex particles or sephadex particles were sterilized using ethylene dioxide vapor for 24 h. Each capsule was implanted subcutaneously into the dorsum of an MF1 mouse (Harlan UK Ltd, Bicester, UK). Experiments were first carried out in order to determine the amount of latex particles implanted subcutaneously which yielded the maximal percentage area of lacunar resorption on bone slices in macrophage-UMR 106 co-cultures; three different concentrations of latex particles (10, 20 and 50 mg per capsule) were subcutaneously implanted for the formation of granulomas. It was found that the subcutaneous implantation of 20 mg of particles per capsule was sufficient to induce a moderately-sized granuloma from which a suitable number of particle-containing macrophages could be isolated (Table I). Empty gelatin capsules were implanted in a different mouse, which was used as a negative control.

After 10 days (pre-determined as the optimal implantation period; range of time course investigated 5, 7, 10 and 14 days post-implantation), a particle-associated foreign body granuloma was formed and surgically removed. Imprints of each granuloma were taken on a glass slide for histochemical investigation and part of the lesion was fixed in formalin, routinely processed, and stained with haematoxylin and eosin for histological examination. The remainder of each granuloma was digested using collagenase Type I (Sigma Chemicals, Poole, UK) for 30 min, after which the viability of the resultant cell suspension was determined using the trypan exclusion test and the appropriate cell concentration was layered on bone slices or coverslips previously seeded with UMR 106 cells (see below).

Monocyte isolation and exposure to particles

Blood was collected by heart puncture of MF1 female mice and diluted 1 : 4 in MEM. The monocyte cell suspension was prepared by gradient centrifugation of whole blood using Ficoll-Hypaque (Pharmecia, UK). After centrifugation for 20 min at 693 *g*, the number of mononuclear cells was determined using a haemocytometer. Murine monocytes were then added to wells of a 96 well plate containing human bone slices or glass coverslips (see below).

Murine monocytes were settled on glass coverslips

TABLE II Studies on the optimization of lacunar bone resorption in terms of the concentrations of latex particles added to monocyte/UMR 106 co-cultures. Values indicate the mean of two separate experiments

μg/well	Mean % bone resorption in response to latex particle size (μm)			
	0.1	1	10	100
10	3.1	4.2	1.2	2.1
50	10.7	12	8.1	9.3
100	2.1	3.8	3.9	2.1

and bone slices which had been pre-incubated with UMR106 cells (see below). After 1 h incubation, all glass coverslips and bone slices were transferred to fresh 24 well plates containing 1 ml MEM/FCS with 1×10^{-7} M $1,25(OH)_2D_3$ and 1×10^{-8} M dexamethasone (Sigma Chemicals, UK). At this point of the experiment, latex particles (0.1, 1, 10 and 100 μm) were added to monocyte/UMR-106 co-cultures at varying concentrations (0, 5, 10 and 50 μg/ml). Experiments were carried out to determine the concentration of latex particles added to monocyte-UMR 106 co-cultures, which resulted in maximum bone resorption. Our results showed that the addition of 10 μg/ml of latex particles to monocyte/UMR-106 co-cultures resulted in the maximal percentage area lacunar resorption on bone slices (Table II) and this concentration was used for all subsequent experiments.

Lactate dehydrogenase measurement for determining cytotoxicity

Following 48-h exposure of murine monocyte/UMR106 co-cultures to latex particles and sephadex beads, supernatants were collected, centrifuged to remove wear particles and cell debris, and stored at $-20\,^{\circ}$C. Release of the cytoplasmic enzyme lactate dehydrogenase (LDH), a marker of cell damage, was measured using a commercially available Cytotox 96 kit (Cat no. G1780, Promega, Madison, WI). Each supernatant from each treatment was tested in duplicate. Results indicated that the addition of 10 μg/ml of particles (latex and sephadex) to monocyte cultures did not cause cell cytotoxicity as LDH levels in cultures containing particles were similar to control cultures with no added particles.

Co-cultures on human bone slices and coverslips

Human cortical bone slices (about 10 mm^2), prepared as previously described [11, 12], and glass coverslips (5 mm) were placed in the wells of a 96 well tissue culture plate (Nunc, Denmark). UMR 106 cells were added to each well at a final concentration of 2×10^4 cells per well and cultured for 24 h. Particle-associated granuloma-derived macrophages was then added to each well at 5×10^3 cells per well and settled for 1 h on bone slices and glass coverslips. Similarly, murine monocytes were added to each well at a final concentration of 1×10^5 cells per well and settled on bone slices and coverslips for 1 h. Cell cultures on bone slices and coverslips were then removed from the 96 well plates, washed vigorously in culture media to remove non-adherent cells, and were transferred to 24 well plates (Nunc, Denmark) containing 1 ml MEM/FCS with 1×10^{-7} M $1,25(OH)_2D_3$ and 1×10^{-8} M dexamethasone.

Cultures of particle-containing granuloma-derived macrophages or particle-containing monocytes (incubated in the presence of UMR 106 cells) were maintained for 1, 7 and 14 days with the $1,25(OH)_2D_3$ and dexamethasone being replenished every three days. For all experiments, negative control cultures were set up on bone slices and coverslips in the absence of (i) UMR 106 cells, (ii) monocyte or granuloma-associated macrophages or (iii) $1,25(OH)_2D_3$. Control cultures were also incubated for 1, 7 and 14 days.

Histochemical and immunohistochemical characterization of cultured cells

After 1 and 7 days, coverslips were removed from each well. Cell preparations on glass coverslips were stained for the osteoclast-associated enzyme tartrate-resistant acid phosphatase (TRAP) [29], using a histochemical kit from Sigma Chemicals (Diagnostic Kit number 316A, UK). In addition, coverslips were stained immunohistochemically by an indirect immunoperoxidase method to determine the presence of F4/80 antigen, which is known to be expressed by murine monocytes and macrophages but not osteoclasts [30]. Cultures of UMR 106 cells alone on glass coverslips and imprints from the cell surface of the particle-induced granuloma were similarly stained for TRAP and F4/80.

Scanning electron microscopy (SEM) examination of bone slices to determine resorption pit formation

After 1, 7 and 14 days in culture, bone slices were removed and prepared for SEM examination as described previously [11, 12]. Briefly, the bone slices were immersed in 0.25 M NH_4OH for 24 h to remove the cells and cell debris from the bone surface. After alcohol dehydration, the bone slices were mounted onto Aluminum stubs (Abbot Laboratories, UK), sputter-coated with gold and examined using a Philips SEM 505 scanning electron microscope.

Statistical analysis

In each experiment three or four bone slices were used and each experiment was repeated 4 times for both monocyte and granuloma studies, unless otherwise stated. The results are expressed as mean percentage bone area resorbed; where appropriate, the values are expressed as mean $\pm$ standard error of the mean. Two separate statistical analyzes were carried out. In the first analysis, the mean percentage bone resorption from each experiment was compared using a paired Student's *t*-test. In the second analysis, two factor ANOVA with replication was used; this analysis gives a greater insight into the data as it uses the results from each bone slice.

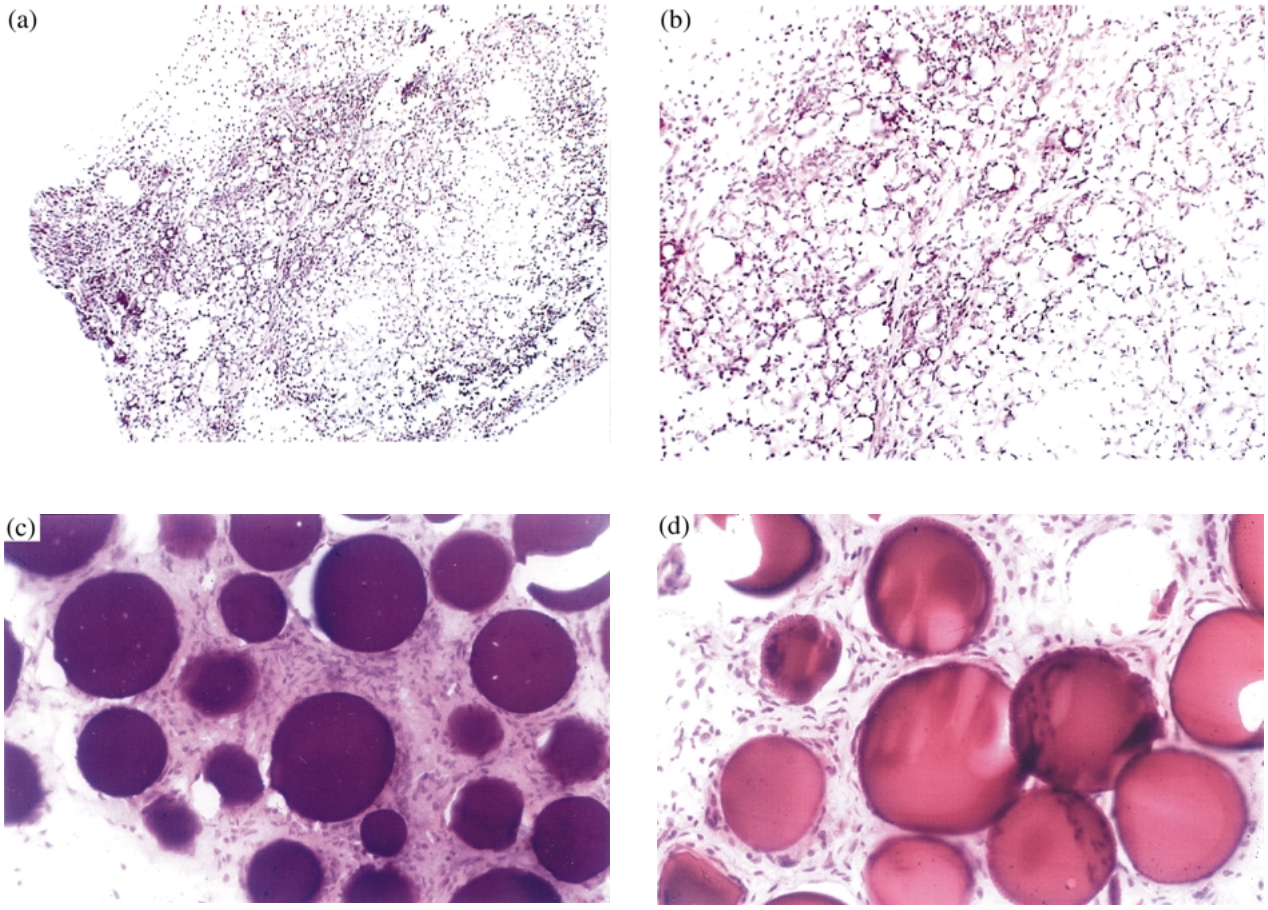

Figure 1 Hematoxylin–eosin-stained histological section of a foreign body granuloma formed in response to subcutaneous implantation of the following materials, showing a macrophage and giant cell response to the implanted particles/beads: (a) 10 μm latex (× 20 magnification), (b) 10 μm latex (higher magnification of (a); × 100 magnification), (c) 40–120 μm negatively-charged sephadex beads (× 100 magnification), (d) 40–120 μm positively-charged sephadex beads (× 250 magnification).

Results

Histology of the subcutaneous granulomas formed in response to latex and charged particles

Subcutaneous lesions formed in response to the various implanted particles showed the formation of a typical foreign body granuloma. Particles of small-size (i.e. 0.1 and 1 μm latex particles) were phagocytosed by macrophages whereas larger particles (i.e. 10 and 100 μm latex particles and 40–120 μm charged or uncharged sephadex particles) were surrounded by macrophages and multinucleated giant cells (Fig. 1(a)–(d)). In some preparations, it was also noted that a few 10-μm latex particles were internalized (i.e. phagocytosed) by inflammatory macrophages. Imprint preparations made on glass slides of the cut surface of the granulomas showed that they contained numerous mononuclear and multinucleated cells which were positive for the macrophage marker F4/80, but negative for the osteoclast-associated enzyme TRAP. No granulomas were formed in response to the subcutaneous implantation of empty gelatin capsules.

Characterization of cells isolated from particle-induced granulomas and particle-containing monocytes

After 24 h in culture, cells isolated from the particle-induced granulomas were characterized as mononuclear phagocytes on the basis that they were strongly F4/80 positive (Fig. 2) and entirely TRAP-negative, both in the presence and absence of UMR 106 cells. Seven day co-cultures of UMR 106 cells and particle granuloma-derived macrophages on glass coverslips, incubated in the presence of $1,25(OH)_2D_3$, contained scattered F4/80-positive cells as well as numerous TRAP-positive cells, many of which were located in clusters (Fig. 3). The number of TRAP-positive multinucleated cells formed in

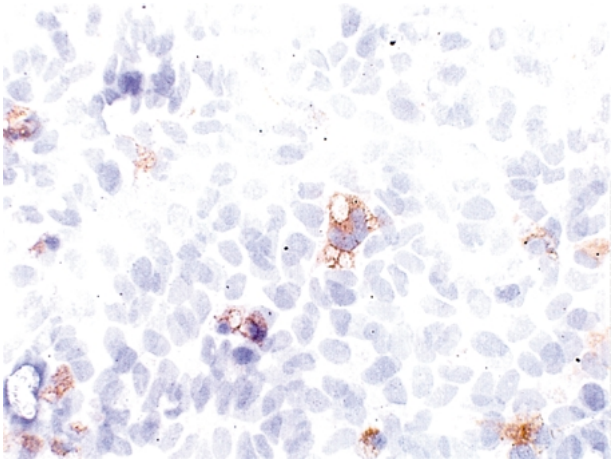

Figure 2 Indirect immunoperoxidase staining of 7-day co-culture of UMR-106 cells and 10-μm latex bead granuloma-derived macrophages showing a positive reaction for F4/80 antigen (× 100).

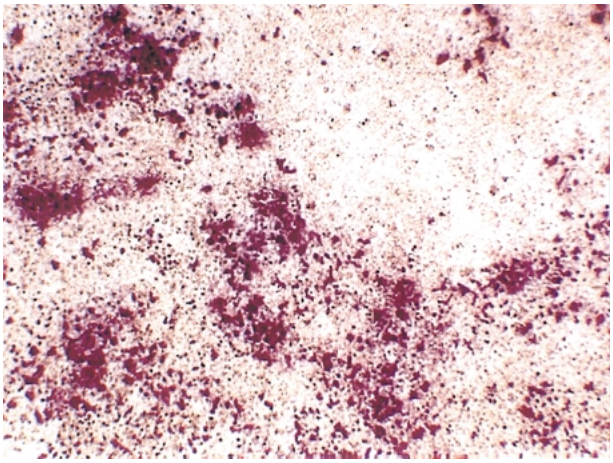

Figure 3 Clusters of TRAP-positive cells formed in 7-day co-cultures of 10 μm latex bead granuloma-derived macrophages and UMR 106 cells (× 100).

these cultures showed no obvious relation to particle size or charge.

After 24 h incubation, cultures of murine monocytes were F4/80-positive and TRAP-negative (both in the presence and absence of UMR 106 cells). Light microscopy showed that particles of small size (i.e. 0.1 and 1 μm beads) were phagocytosed; this was confirmed by scanning electron microscopy (Fig. 4). After 7 days in culture, in the presence of UMR 106 cells and $1,25(OH)_2D_3$, numerous clusters of TRAP-positive cells were noted in monocyte/UMR 106 co-cultures, including those to which particles had been added (Fig. 5); only particles of less than 10 μm in size were phagocytosed by monocytes. The number of TRAP-positive cells was markedly increased in particle-containing monocyte/UMR 106 co-cultures compared with co-cultures to which no particles had been added. The increase in the number of TRAP-positive multinucleated cells was not related to the size or the charge of the added particles.

TRAP-positive multinucleated cells were not seen in control cultures of monocytes or particle granuloma-derived macrophages to which UMR 106 cells or $1,25(OH)_2D_3$ had not been added.

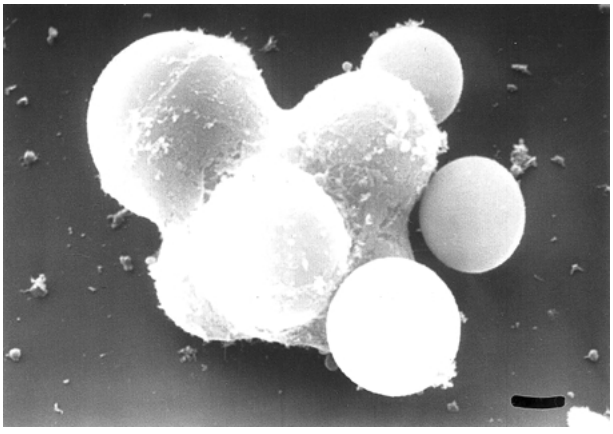

Figure 4 Scanning electron photomicrograph of a monocyte which has phagocytosed several 1-μm latex beads (black Bar = 50 μm).

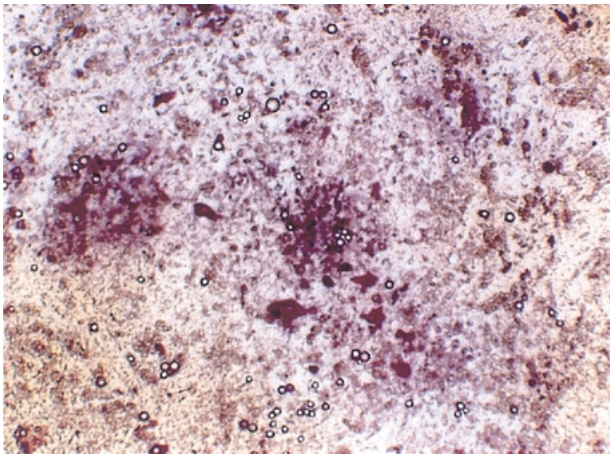

Figure 5 Histochemical staining of co-culture of UMR 106 cells and monocytes to which 10-μm latex beads had been added. There are numerous small and large clusters of TRAP-positive cells (× 100).

Effect on osteoclast formation and bone resorption of granuloma-derived macrophages exposed to particles of different size and charge

SEM examination showed no evidence of lacunar resorption pit formation on bone slices on which macrophages and UMR 106 cells had been co-cultured for 24 h. After 14 days incubation, lacunar resorption, as evidenced by the formation of numerous well-defined resorption pits and confluent areas of bone resorption, was seen on those bone slices upon which granuloma-derived macrophages had been co-cultured with UMR 106 cells in the presence of $1,25(OH)_2D_3$ (Fig. 6).

Lacunar resorption pits were also found on bone slices on which UMR 106 cells were co-cultured with macrophages derived from granulomas containing all sizes of latex particles. There was no significant difference in mean percentage area of bone resorption between each latex particle size (Fig. 7).

Co-cultures of UMR 106 cells and macrophages isolated from granulomas formed in response to the subcutaneous implantation of charged or uncharged beads also showed evidence of lacunar bone resorption on bone slices. There was no significant difference in the

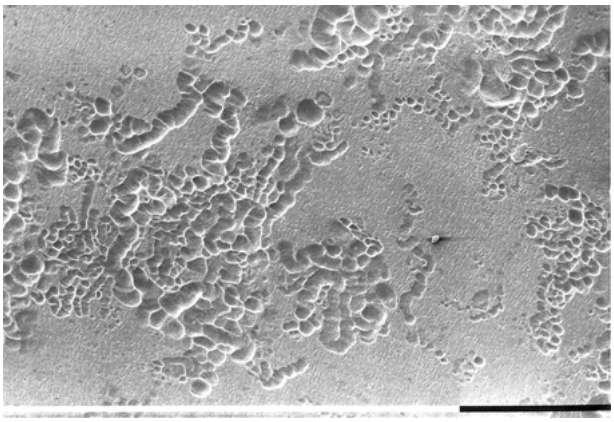

Figure 6 SEM photomicrograph of a human bone slice on which UMR 106 cells were co-cultured with 10 μm latex beads-derived granuloma-derived macrophages, for 14 days in the presence of $1,25(OH)_2D_3$. Removal of these cells showed extensive lacunar resorption of the bone surface with the formation of numerous resorption pits (black Bar = 100 μm).

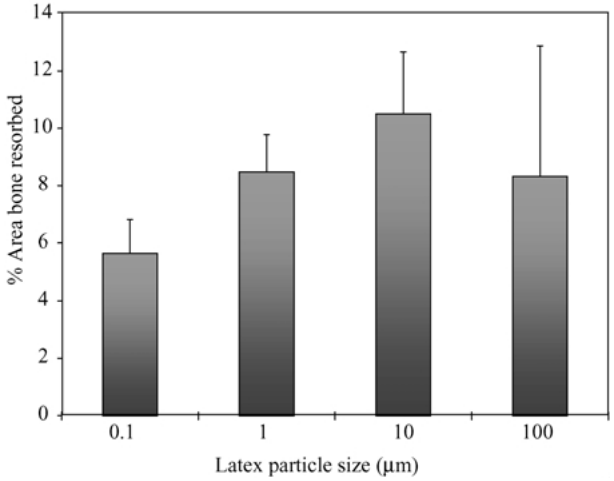

Figure 7 Mean percentage area lacunar bone resorption (± standard error of the mean) seen in 14-day co-cultures of UMR 106 cells and macrophages derived from granulomas formed in response to the subcutaneous implantation of various sizes of latex beads.

mean percentage area lacunar bone resorption between co-cultures of UMR 106 cells and granuloma-derived macrophages containing uncharged or charged beads. Mean percentage lacunar resorption in response to uncharged, negatively or positively charged beads were 0.21% ± 0.02, 0.26% ± 0.04 and 0.25% ± 0.03, respectively.

Effect on osteoclast formation and bone resorption of monocyte exposure to particles of different size and charge

After 24 h incubation, no evidence of lacunar resorption pit formation was found in co-cultures of monocytes and UMR 106 cells on bone slices. After 14 days incubation, in the presence of $1,25(OH)_2D_3$, lacunar bone resorption was noted in monocyte/UMR 106 co-cultures to which no particles had been added. The addition of various sizes of latex particles to monocyte/UMR 106 co-cultures, did not induce a significant increase in mean percentage area resorption as compared to co-cultures

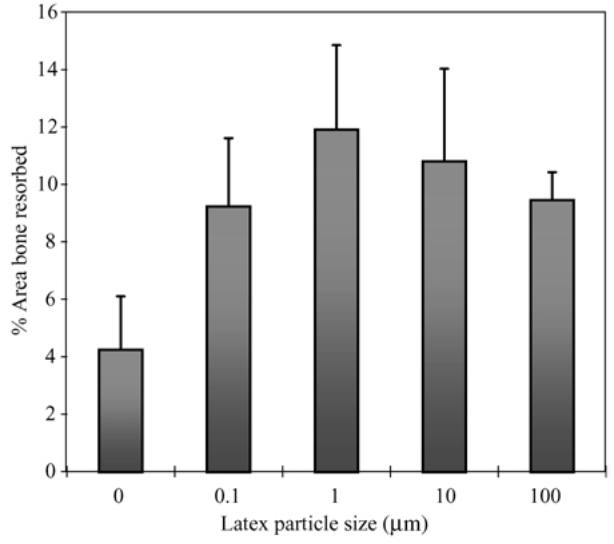

Figure 8 Mean percentage area lacunar bone resorption (± standard error of the mean) seen in 14-day co-cultures of UMR 106 cells and mouse monocytes cultured in the presence or the absence of latex beads of various size.

containing no latex particles (Fig. 8). There was also no significant difference in mean percentage area bone resorption in co-cultures to which different sizes of latex beads were added. Similarly, the mean percentage area bone resorption by osteoclasts formed from macrophage exposure to charged and uncharged beads showed no significant differences when compared to control cultures to which no beads had been added.

In the absence of either UMR 106 cells or particle granuloma-derived macrophages or monocytes, there was no evidence of lacunar bone resorption.

Discussion

A foreign body macrophage response to implant-derived biomaterial wear particles is often found in periprosthetic tissues surrounding loose implant components [2, 3, 31]. The severity and rapidity of onset of aseptic loosening is closely associated with the degree of this foreign body macrophage response induced by wear particles [2, 32]. One means whereby foreign body macrophages contribute to the osteolysis of aseptic loosening is through differentiation of these cells into bone-resorbing osteoclasts. The chemical composition of the generated wear particles, the number and size of these particles as well as their shape, charge and surface area are believed to determine the intensity of the foreign body macrophage response and the extent of periprosthetic osteolysis [18, 19]. In this study, we have shown that two particle-associated factors, i.e. particle size and surface charge, do not significantly influence the extent of macrophage-osteoclast differentiation and bone resorption.

A wide size range of polymeric and metallic biomaterial particles is found in the fibrous membrane surrounding loose arthroplasty components. The majority of these particles are less than 1 μm in size and elicit a predominantly macrophage response which often involves particle phagocytosis [20, 21, 31]. Larger particles are generally associated with a mixed macrophage and macrophage polykaryon foreign body response. The *in vitro* models we have employed in this study induced a macrophage and macrophage polykaryon response essentially similar to that found in the fibrous membrane surrounding loose arthroplasty components. In both the subcutaneous foreign body granulomas and the monocyte cultures to which particles were added, we found that small latex particles (e.g. 0.1 and 1 μm) were phagocytosed by macrophages and macrophage polykaryons whereas larger particles (e.g. 100 μm) were surrounded by these cells.

Murray and Rushton have shown that phagocytosis of any foreign material that is non-toxic will cause macrophage activation [9]. There are numerous reports which have shown that phagocytosis of small particles results in the release of more inflammatory mediators than large (non-phagocytosable) particles. Most of these reports are based on experiments in which monocytes and macrophages were cultured in the presence of various implant-derived wear particles and the supernatant then assayed for the presence of inflammatory mediators which are known to stimulate osteoclast activity [6, 33–35]. Interpretation of these results is difficult due to differences in methodology, the nature of

the mononuclear phagocyte populations studied and the source, composition and concentration of biomaterials employed. Marked differences in the macrophage response to wear particles seen in these studies is highlighted by the work of Glant and Jacobs [36] in which three different murine/macrophage populations (i.e. P388D1, IC21 and peritoneal macrophages) exposed to the same biomaterial wear particles showed considerable variation in mineral resorption and the release of PGE2 and IL-1. Marked intra- and inter-assay variations in the effect of small (0.21 μm) or large (88 μm) polyethylene particles on the release of inflammatory cytokines (e.g. IL-1β, IL-6 and TNFα) has also been noted in studies employing different human macrophage populations [37]. The addition of particles of non-uniform shape, size and composition, as well as the possibility of endotoxin contamination [38], could have contributed to these differences in the release of humoral factors. Increased ^{45}Ca release was observed by Green *et al*. [39] after submicron polyethylene particles were added to organ cultures of newborn mice calvaria; however, ^{45}Ca release is an indirect measure of bone resorption and does not permit distinction between particle effects on osteoclast formation and bone resorbing activity. Our study has specifically focused on the effect of particles of different size and charge on lacunar bone resorption occurring as a consequence of osteoclast formation and has employed beads of uniform shape and composition to ensure that particle size is the only parameter which is varied.

Our findings indicate that both phagocytosable and non-phagocytosable particles should be regarded as having the potential to elicit a macrophage response from which osteoclast formation and bone resorption may occur. Irrespective of particle size, activated macrophages differentiated into bone-resorbing osteoclasts in the presence of osteoblastic UMR 106 cells and $1,25(OH)_2D_3$, and no difference in terms of osteoclast formation and lacunar resorption was seen as a result of macrophages responding to particles in the size range 0.1–100 μm. Our findings would thus indicate that generation of large implant-derived wear particles is also relevant in terms of the production of osteolysis in aseptic loosening. Gelb *et al*. [18] showed that large particles of PMMA (10–126 μm) induced more acute inflammation than small particles (1–10 μm) in a subcutaneous rat air pouch model. They suggested that the inability to clear large particles may be the reason for this increase in the inflammatory response. Macrophage exposure to large (18–20 μm) and small (4–10 μm) particles of UHMWPE are known to induce the release of significant amounts of IL-1β and IL-6 [40]. More recently, Nakashima *et al*. [41] showed that release of pro-inflammatory/resorptive mediators such as TNFα from monocytes and macrophages is not dependent on phagocytosis of particles and that initial signals generated by ligand binding to particles is sufficient to trigger signaling cascades in monocytes and macrophages; this macrophage response to wear particles results in induction of genes for the production of cytokines and is accompanied by an increase in tyrosine kinase activity and activation of NF-κB and NF-IL-6 [42]. These factors are known to be important in the transcriptional activation of the TNFα and IL-6 genes which are known to play a major role not only in osteoclast activation but also osteoclast formation from circulating and marrow precursors.

Previous studies have shown that particle charge may significantly influence bone remodeling. Krukowski *et al*. [25] have shown that negatively-charged beads stimulate extensive bone formation in craniofacial and intramedullary long bones of rats whereas positively charged beads elicit predominantly a marked fibroblastic response and induce the formation of multinucleated foreign body giant cells. It has also been reported that positively charged beads enhance the healing process of cutaneous wounds in rats compared to uncharged or negatively charged beads [26]. In this study we have examined the effect of particle charge on osteoclast formation and found that it does not significantly influence the extent of osteoclast formation. The serum concentration of metal ions in patients with metal on metal total hip replacements is known to be elevated; the local concentration of these ions in the fibrous membrane around implants is also likely to be high [27]. Although our studies do not preclude an effect of particle charge on the functional activity of osteoblasts and osteoclasts, our findings suggest that the effect of particle surface charge on osteoclast formation and bone resorption is not likely to be significant.

It has been shown that a correlation exists between the number of wear particles and the degree of local macrophage response seen in failed joint arthroplasties and that wear particle-associated inflammatory macrophages derived from the arthroplasty membrane are capable of differentiating into osteoclastic bone resorbing cells. These findings indicate that bone resorption and implant loosening may occur as a result of osteoclast differentiation of particle-associated macrophages present in periprosthetic tissues. We have previously shown that the chemical composition of the biomaterial particles to which macrophages are exposed significantly influences the extent of osteoclast formation and bone resorption [11, 12]. Our findings show that there is no significant difference in osteoclast formation and bone resorption when macrophages are exposed to particles of uniform shape and composition but different size, suggesting that it is the presence of macrophages *per se* which is necessary for this process to occur. Phagocytosis of small particles also did not appear to influence significantly either the formation of osteoclasts or their functional bone resorbing activity; this finding is in keeping with those of previous studies which have shown that particle phagocytosis by osteoclasts does not abrogate functional bone resorbing activity [43, 44].

Acknowledgments

This study was supported by the Wellcome Trust and the BOA Wishbone Trust.

References

1. T. M. WRIGHT and S. B. GOODMAN (eds), ''Implant Wear: The Future of Total Joint Replacements'' (Oakbrook, AAOS, 1995).
2. W. J. MALONEY, *J. Bone Joint Surg*. **77A** (1995) 1448.

3. P. A. REVELL, in ''Current Trends in Pathology'', edited by C. Berry (Berlin, Springer-Verlag: 1982) p. 73.
4. S. B. GOODMAN, R. C. CHRIS and S. S. CHIOU, *Clin. Orthop.* **244** (1989) 182.
5. W. A. JIRANEK, M. MACHADO, M. JASTY, D. JERVEVAR, H. J. WOLFE, S. R. GOLDRING, M. J. GOLDBERG and W. H. HARRIS, *J. Bone Joint Surg.* **75A** (1993) 863.
6. T. T. GLANT, J. J. JACOBS, K. MIKECZ, J. YAO, S. CHUBINSKAJA, J. M. WILLIAMS, R. L. URBAN, A. S. SHAHBANG, S. H. LEE and D. R. SUMNER, *Am. J. Ther.* **3** (1996) 27.
7. J. CHIBA, H. E. RUBASH, K. J. KIM and Y. IWAKI, *Clin. Orthop.* **300** (1994) 304.
8. A. S. SHAHBANG, J. J. JACOBS, J. BLACK, J. O. GALANTE and T. T. GLANT, *J. Arthroplasty* **10** (1995) 498.
9. D. W. MURRAY and N. RUSHTON, *J. Bone Joint Surg.* **72B** (1990) 988.
10. J. QUINN, C. J. JOYNER, J. T. TRIFFITT and N. A. ATHANASOU, *ibid.* **74B** (1992) 652.
11. R. PANDEY, J. QUINN, C. JOYNER, J. T. TRIFFITT and N. A. ATHANASOU, *Ann. Rheum. Dis.* **55** (1996) 388.
12. A. SABOKBAR, R. PANDEY, J. QUINN and N. A. ATHANASOU, *Arch. Orthop. Trauma Surg.* **184** (1998) 31.
13. A. SABOKBAR, Y. FUJIKAWA, D. W. MURRAY and N. A. ATHANASOU, *J. Bone Joint Surg.* **79B** (1997) 129.
14. Y. FUJIKAWA, J. M. W. QUINN, A. SABOKBAR, J. O'D. MCGEE and N. A. ATHANASOU, *Endocrinology* **139** (1996) 4058.
15. A. SABOKBAR, Y. FUJIKAWA, S. NEALE, D. MURRAY and N. A. ATHANASOU, *Ann. Rheum. Dis.* **56** (1997) 414.
16. S. D. NEALE, D. R. HAYNES, D. W. HOWIE, D. W. MURRAY and N. A. ATHANASOU, *J. Arthroplasty* **15** (2000) 654.
17. I. ITONAGA, A. SABOKBAR, D. W. MURRAY and N. A. ATHANASOU, *Ann. Rheum. Dis.* **59** (2000) 26.
18. H. GELB, H. R. SCHUMACHER, J. CUCKLER and D. G. BAKER, *J. Orthop. Res.* **12** (1994) 83.
19. A. S. SHANBHANG, J. J. JACOBS, J. BLACK, J. O. GALANTE and T. T. GLANT, *J. Biomed. Mater. Res.* **28** (1994) 81.
20. W. J. MALONEY, R. L. SMITH, D. HVENE, T. P. SCHMALZRIED and H. RUBASH, *J. Bone Joint Surg.* **76A** (1994) 1664.
21. A. S. SHANBHANG, J. J. JACOBS, T. GLANT, J. L. GILBERT, J. BLACK and J. O. GALANTE, *ibid.* **76B** (1994) 60.
22. E. INGHAM and J. FISHER, *Proc. Inst. Mech. Eng.* **214** (2000) 21.
23. T. SUDA, N. UDAGAWA, I. NAKAMURA, C. MIYAURA and N. TAKAHASHI, *Bone* **17** (1995) 87S.
24. N. A. ATHANASOU, *J. Bone Joint Surg.* **78A** (1996) 1096.
25. M. KRUKOWSKI, R. A. SHIVELY, P. OSDOBY and B. L. EPPLEY, *Oral Maxillofac. Surg.* **48** (1990) 468.
26. R. D. GALIANO, R. W. JYUNG, M. KRUKOWSKI and T. A. MUSTOE, *Ann. Plast. Surg.* **36** (1996) 608.
27. W. BRODNER, P. BITZAN, V. MEISINGER, A. KAIDER, F. GOTTSAUNER-WOLF and R. KOTZ, *J. Bone Joint Surg. Br.* **79** (1997) 316.
28. H. G. WILLERT, G. H. H. BUCHHORN, D. GÖBEL, G. KÖSTER, S. SCHAFFNER, R. SCHNK and M. SEMLITSCH, *Clin. Orthop.* **329S** (1996) S160.
29. C. MINKIN, *Calcif. Tissue Int.* **34** (1982) 285.
30. J. M. AUSTYN and S. GORDON, *Eur. J. Immunol.* **11** (1981) 805.
31. H. C. AMSTUTZ, P. CAMPBELL, N. KOSSOVSKY and I. C. CLARKE, *Clin. Orthop. Rel. Res.* **276** (1992) 7.
32. M. JASTY, C. BRAGDON, W. JIRANEK, H. CHANDLER, W. MALONEY and W. H. HARRIS, *ibid.* **308** (1994) 111.
33. J. H. HERMAN, W. G. SOWDER, D. ANDERSON, A. M. APPEL and C. N. HOPSON, *J. Bone Joint Surg.* **71A** (1989) 1530.
34. S. M. HOROWITZ, T. L. GAUTSCH, C. G. FRONDOZA and L. RILEY, *J. Orthop. Res.* **9** (1991) 406.
35. T. R. GREEN, J. FISHER, M. STONE, B. M. WROBLEWSKI and E. INGHAM, *Biomaterials* **19** (1998) 2297.
36. T. T. GLANT and J. JACOBS, *J. Orthop. Res.* **12** (1994) 720.
37. J. B. MATTHEWS, T. R. GREEN, M. H. STONE, B. M. WROBLEWSKI, J. FISHER and E. INGHAM, *Biomaterials* **21** (2000) 2033.
38. A. A. RAGAB, R. VAN DE MOTTER, S. A. LAVISH, V. M. GOLDBERG, J. T. NINOMIYA, C. R. CARLIN and E. M. GREENFIELD, *J. Orthop. Res.* **17** (1999) 803.
39. T. R. GREEN, J. FISHER, J. B. MATTHEWS, M. STONE and E. INGHAM, *J. Biomed. Mater. Res.* **53** (2000) 490.
40. I. VORONOV, J. P. SANTERRE, A. HINEK, J. W. CALLAHAN, J. SANDHU and E. L. BOYNTON, *ibid.* **39** (1998) 40.
41. Y. NAKASHIMA, D. H. SUN, M. C. TRINDALE, W. J. MALONEY, S. B. GOODMAN, D. J. SCHURMAN and R. L. SMITH, *J. Bone Joint Surg.* **81A** (1999) 603.
42. E. M. SCHWARZ, A. P. LU, J. J. GOATER, E. B. BENZ, G. KOLLIAS, R. N. ROSIER, J. E. PUZAS and R. J. O'KEEFE, *J. Orthop. Res.* **18** (2000) 472.
43. W. WANG, D. J. FERGUSON, J. M. QUINN, A. H. SIMPSON and N. A. ATHANASOU, *J. Bone Joint Surg.* **79B** (1997) 849.
44. W. WANG, D. J. FERGUSON, J. M. QUINN, A. H. SIMPSON and N. A. ATHANASOU, *J. Pathol.* **182** (1997) 92.

Received 10 December 2002
and accepted 9 February 2003