Wear particles and surface topographies are modulators of osteoclastogenesis *in vitro*

Beatrice Sommer,[1] Rolf Felix,[1] Christoph Sprecher,[2] Michael Leunig,[3] Reinhold Ganz,[3] Willy Hofstetter[1]
[1]*Group for Bone Biology, Department of Clinical Research, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland*
[2]*AO-Research Institute, CH-7270 Davos, Switzerland*
[3]*Clinic for Orthopaedic Surgery, Inselspital, University of Bern, CH-3010 Bern, Switzerland*

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Abstract: Prosthetic and osteosynthetic implants from metal alloys will be indispensable in orthopedic surgery, as long as tissue engineering and biodegradable bone substitutes do not lead to products that will be applied in clinical routine for the repair of bone, cartilage, and joint defects. Therefore, the elucidation of the interactions between the periprosthetic tissues and the implant remains of clinical relevance and several factors are known to affect the longevity of implants. Within this study, the effects of metal particles and surface topography on the recruitment of osteoclasts was investigated *in vitro* in a coculture of osteoblasts and bone marrow cells. The cells were grown in the presence of particles of different sizes and chemical composition or on metal discs with polished or sandblasted surfaces, respectively. At the end of the culture, newly formed osteoclasts were counted. Osteoclastogenesis was reduced when particles were added directly to the coculture. The effect depended on the size of the particles, small particles exerting stronger effects than larger ones. The chemical composition of the particles, however, did not affect the development of osteoclasts. In cocultures grown on sandblasted surfaces, osteoclasts developed at higher rates than they did in cultures on polished surfaces. The data demonstrate that wear particles and implant surfaces affect osteoclastogenesis and thus may be involved in the induction of local bone resorption and the formation of osteolytic lesions, leading eventually to the loosening of orthopedic implants. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 72A: 67–76, 2005

Key words: wear particles; surface topography; bone resorption; osteoclast; osteoblast

INTRODUCTION

The success of orthopedic implants, as assessed by their longevity, depends on a number of contributing factors. Primary stability,[1] the bonding of the periimplant tissue to the implant,[2] and the development of chronic inflammations caused by the shedding of wear particles from the implant[3] all affect the final outcome of the surgical procedure.

Chemistry and topography of the implant surface determine to a large extent the interactions with the periprosthetic tissues. Implants, which are surrounded by body fluids, develop a surface oxide layer due to natural passivation. Differences in the crystallinity of the metal and the segregation of alloy components may strongly affect the nature of the oxides formed on the implant surface. These differences in surface chemistry and crystallinity may in turn affect binding of proteins to the surface.[4] As a consequence, minor changes and heterogeneities in the composition and the topography of the implant may cause significant differences in the microenvironment encountered by the cells of the periimplant tissues.

Although surface chemistry and topography are important factors in implant–tissue interactions, wear particles have been suggested for several years to be involved in aseptic loosening of prosthetic implants.[3,5] The two parameters, however, are strongly interdependent. A structured surface may enable tighter bonding between implant and periprosthetic bone, but may also be prone to shed particles. Vice versa, polished surfaces may form a less tight implant–tissue interface, but may shed less particles. The accumulation of wear particles in the periprosthetic tissue causes a chronic aseptic inflammation, including the invasion of immune cells and the formation of foreign body giant cells. These cells release inflammatory cy-

Correspondence to: W. Hofstetter; e-mail: willy.hofstetter@dkf.unibe.ch
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tokines that have the capacity to activate bone resorption such as interleukin (IL)-1α and -1β, IL-6, and tumor necrosis factor (TNF)α.[6]

The role of TNFα in osteolytic bone resorption has been particularly well studied. Application of polymethylmethacrylate particles onto the external periosteum of murine calvariae induced osteolysis at the site of implantation. These animals were protected against the osteolytic effects of implanted polymethylmethacrylate particles when they were deficient either in both the p55 and p75 TNFα receptors or in the p55 TNFα receptor alone.[7] The important nature of the p55-mediated effects of TNFα on osteoclastogenesis and bone resorption was further corroborated by the finding that in cell cultures a functional p55 receptor was required to maintain the TNFα effect on the formation of osteoclasts.[8] Because TNFα is released by cells of the monocyte/macrophage lineages upon exposure to particles, the crucial role of the cytokine in the loosening of orthopedic implants is further underlined.[9,10]

Wear particles shed from the implant are not the only modulators of cell biology in the periprosthetic tissues. The topography, chemistry, and the size of the implant surface exert strong effects on the biology of the cells which get into contact with it as well. Chemistry and surface structure affect the adhering cells and the capacity of protein binding. In the past it has been shown that cells of different origin, such as cells of the osteoblast[11–13] and monocyte/macrophage[14] lineages, become activated when in contact with rough, topographically complex surfaces, as compared with smooth and polished surfaces. However, the size of the particles determines whether they are phagocytosed, or whether they elicit a foreign body reaction. Monocytes phagocytosing small wear particles release inflammatory modulators generating a microenvironment favorable for the development of osteoclasts,[15] whereas larger particles elicit foreign body granulomas that recruit osteoclast progenitor cells to the site of inflammation.[16] Particles, however, not only affect the cells of the monocyte/macrophage lineages in dependence of their size, because also osteoblast lineage cells can ingest small-size particles. As a consequence, the functions of osteoblasts are altered, eventually decreasing their bone-forming capacity while inducing the release of osteoclastogenic cytokines.[17]

Within the present study, the differentiation behavior of cells of the osteoclast lineage was investigated. Because osteoclast precursors require cells of the osteoblast lineage to generate a suitable microenvironment for their development, in these studies a coculture system of primary osteoblasts and bone marrow cells was used.[18] This culture system allows the investigation of effects on either the mesenchymal or the hematopoietic compartments during the culture period. Therefore, it may serve as a suitable model to investigate the formation of osteoclasts *in vitro* in dependence of wear particles or of surface structures.

MATERIALS AND METHODS

Metal discs and particles

Metal discs from prosthetic materials [commercially pure (cp) titanium (Ti), CoCr28Mo6, TiAl6Nb7, FeCrNi] were kindly provided by M. Windler (Centerpulse AG, Winterthur, Switzerland). The test discs were 1.4 cm in diameter and fitted into the wells of 24-well plates. The surfaces of the discs were either polished or sandblasted. Before use, the discs were washed in ethanol, rinsed in water, and steam sterilized.

For the studies with particles, mainly commercially available particles were used (Aldrich Chemicals, Buchs, CH). The particles were Ti, namely Ti100 with a size of <150 μm and Ti325 with a size of <45 μm, copper (Cu) with a size of <1 μm and aluminium oxide (AlOx) with a size of <10 μm. In addition, sub-μm particles of cp Ti were kindly provided by Dr. M. Wimmer and Ch. Sprecher (AORI, Davos, CH). Before use, the particles were washed in acetone and ethanol, extensively rinsed with distilled water, dried, and resuspended in culture medium.

Coculture experiments

To investigate the formation of osteoclasts *in vitro*, a coculture system of primary murine calvarial osteoblasts and bone marrow cells was used.[15] Osteoblasts were seeded at densities of 1×10^4 and 2×10^4, respectively, per 2 cm^2 together with 1.5×10^5 bone marrow cells and grown in α Eagle minimum essential medium (αMEM) supplemented with 10% fetal bovine serum (FBS) and $10^{-8}M$ $1,25(OH)_2D_3$. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. After 3 days, the medium was changed and after 6 days, osteoclasts had formed.

Staining for tartrate-resistant acid phosphatase (TRAP)

For the identification of osteoclasts, the cell cultures were stained for TRAP. Depending on culture conditions, a colorimetric or a fluorescent staining protocol was applied. For TRAP staining of the cocultures in plastic dishes, a commercially available leukocyte acid phosphatase assay was used according to the recommendations of the manufacturer (SIGMA, Buchs, CH). Osteoclasts were counted as TRAP-positive cells containing three or more nuclei.

In cocultures on metal discs, the cells were visualized with the nuclear stain 4′,6-diamidine-2′-phenylindol-dihydrochloride (DAPI; Roche Diagnostics, Rotkreuz, CH). Osteoclasts were stained using the phosphatase substrate ELF{R}-97 (Molecular Probes, JURO Supply AG, Lucerne,

CH), which yields a fluorescent product. For this purpose, the same kit as above was used. The cells were fixed with 4% buffered paraformaldehyde for 10 min. Thereafter, the cell cultures were incubated at 37°C in 100 m*M* Na-acetate/50 m*M* Na-tartrate, pH 5.2, containing 2.5 m*M* ELF{R}-97. The reaction was allowed to run for 10 min. Subsequently, the substrate was removed by washing the cells with water and the formation of the reaction product was visualized under UV, using a fluorescence microscope.

To determine total TRAP activity in the cell cultures, the cells were lysed in 1*M* NaCl/0.2% Triton X100. Aliquots of the cell lysate were incubated with 5 m*M* *p*-nitrophenol phosphate (SIGMA) in 25 m*M* Na-acetate/20 m*M* Na-tartrate, pH 4.8 at 37°C. The reaction was stopped by the addition of 0.1*M* NaOH and the OD was determined at 405 nm.

Growth of bone marrow-derived macrophages (BMDM)

BMDM were grown from bone marrow cells in Teflon bags in the presence of colony-stimulating factor-1.[19] For this purpose, 25×10^6 nucleated bone marrow cells were cultured in 20 × 20 cm Teflon bags in 100 mL of Dulbecco's modified Eagle medium supplemented with 15% horse serum, 30% conditioned medium from L929 cells as the source for colony-stimulating factor-1,[20] 25 ng/mL vitamin B_{12}, and 30 ng/mL biotin. After 10 days at 37°C in a humidified atmosphere with 5% CO_2, the cells were harvested and the proportion of BMDM was determined by staining the cells with the pan-macrophage marker F4/80.[21]

Determination of cellular proliferation rate

To assess the proliferation rates of osteoblasts in the presence of particles, [^{3}H]thymidine incorporation into DNA was determined. For this purpose, the cells were grown in normal culture medium, containing the particles, to partial confluence. Four hours before harvesting, [^{3}H]thymidine (Amersham Biosciences, UK) was added to the cultures at a concentration of 1 μCi/200 μL.

Production of conditioned media (CM)

To investigate the release of osteoclastogenic growth factors by osteoblasts and monocytes/macrophages upon exposure to wear particles, CM were produced after exposing the cell cultures to particles. For this purpose, 2×10^4 osteoblasts or 5×10^5 and 10^6 BMDM in αMEM/10% FBS were seeded into 24-well plates (2 cm^2/well) and particles were added at various concentrations. After an incubation period of 24 h, the media were collected, filtered through a 0.22-μm filter, and used immediately. Freezing of the CM was avoided.

Quantitation of TNFα in CM

To investigate the stimulation of BMDM by wear particles, the release of TNFα into the cell supernatant was measured. For this purpose, a mouse TNFα optEIA enzyme-linked immunosorbent assay set was used according to the recommendations of the manufacturer (BD Pharmingen, Basle, CH).

RESULTS

Formation of osteoclasts *in vitro* on biomaterials

To assess the effect of surface topography on the formation of osteoclasts *in vitro*, cocultures were performed, seeding the cells directly onto metal discs placed in bacterial dishes. To quantify the formation of osteoclasts, either the total activity of TRAP in the culture or the number of TRAP-positive, multinucleated cells was determined. After seeding, osteoblasts and bone marrow cells adhered to the metal discs independently of the surface topography and the chemical composition and osteoclasts developed in the presence of $1,25(OH)_2D_3$. Osteoclasts were visualized using the phosphatase substrate ELF{R}-97 (Fig. 1).

Determination of total TRAP activity and of the number of osteoclasts revealed that in each individual experiment, osteoclastogenesis was more efficient on sandblasted surfaces than on polished surfaces (Table I). Although the number of osteoclasts differed between the experiments, TRAP activity and osteoclast number always moved in parallel. Although the topography of the surface exerted a positive effect, the chemical composition of the metal discs did not affect significantly the development of the cells of osteoclast lineage.

Direct effects of particles on the formation of osteoclasts *in vitro*

To determine the direct effect of particles on the formation of osteoclasts, metal particles were added to cocultures at various concentrations, ranging from 1 to 0.001 mg/mL. Cu particles fully prevented cell growth and differentiation in this culture system because of the toxic effect of the metal (not shown). The AlOx particles exerted a strong inhibitory effect on the formation of osteoclasts at the two highest concentrations (1 and 0.1 mg/mL), whereas osteoclast formation was not different from the control cultures at particle concentrations of 0.01 and 0.001 mg/mL (Fig. 2). The three lots of Ti particles exerted an effect on the formation of

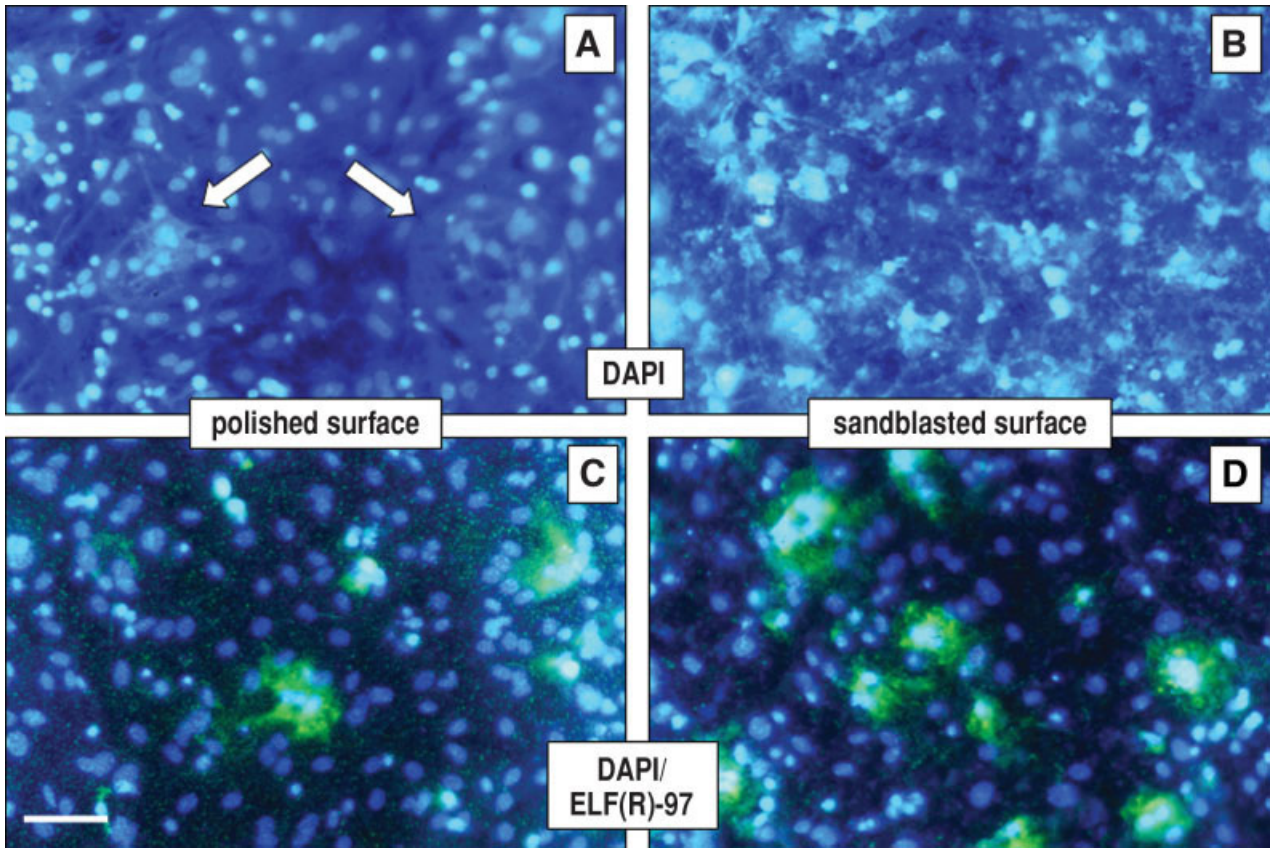

Figure 1. Formation of osteoclasts on metal discs. Cocultures with 10^4 osteoblasts and 1.5×10^5 bone marrow cells were performed on metal discs. To visualize the cells, the cultures were stained with DAPI (A,B). Staining of osteoclasts for TRAP was performed using the phosphatase substrate ELF{R}-97 (C,D). In cultures on polished surfaces, osteoclasts were visible also without a specific stain (arrows in A), but could be identified easily after staining for TRAP (B). In cultures grown on sandblasted surfaces, no osteoclasts could be seen without staining for TRAP (C), but numerous TRAP-positive cells were detected upon staining with ELF{R}-97 (D). Bar represents 200 μm.

osteoclasts that was dependent on the size of the particles. The formation of osteoclasts *in vitro* was decreased by cp Ti >1 μm at concentrations of 1 and 0.1 mg/mL, whereas with Ti325 and Ti100 an inhibition was observed at a particle concentration of 1 mg/mL only.

TABLE I
Formation of Osteoclasts on Surfaces With Different Topographies

	Sandblasted/Polished	
Metal	TRAP Activity	No. of Osteoclasts
TiAl6Nb7	1.85 ± 0.20*	1.70 ± 0.05*
CoCr28Mo6	3.43 ± 0.13*	2.57 ± 0.19*
FeCrNi	1.88 ± 0.37	1.90 ± 0.30*
cp Ti	2.31 ± 0.61*	3.41 ± 1.29*

Determination of total TRAP activity of the culture and the number of osteoclasts are shown (cumulative data from five individual experiments, each experiment consisting of three independent cultures). The values are given as the ratio between TRAP activity and osteoclast number from cultures grown on sandblasted versus cultures grown on polished surfaces. In each experiment, total TRAP activity and the number of osteoclasts were significantly increased (except for TRAP activity on FeCrNi surfaces), when the cells were cultured on sandblasted as compared with polished surfaces (*$p > 0.05$).

Effect of particles on the proliferation of osteoblasts

To assess whether the observed effects of particles on the formation of osteoclasts are at least partially caused by a decrease in cell number, the proliferation of osteoblasts in the presence of particles was determined (Fig. 3). Cu particles inhibited cell proliferation at all concentrations (1, 0.1, and 0.01 mg/mL) because of the toxic effects of Cu released into the cell supernatant. AlOx particles, with a size of <10 μm, inhibited cell proliferation at a concentration of 1 mg/mL, whereas no effect was observed at concentrations of 0.1 and 0.01 mg/mL. The size seemed again to be the determining factor for the inhibitory activity of Ti particles. Thus, cp Ti (<1 μm) exerted stronger effects than Ti325 (<45 μm) and Ti100 (<150 μm).

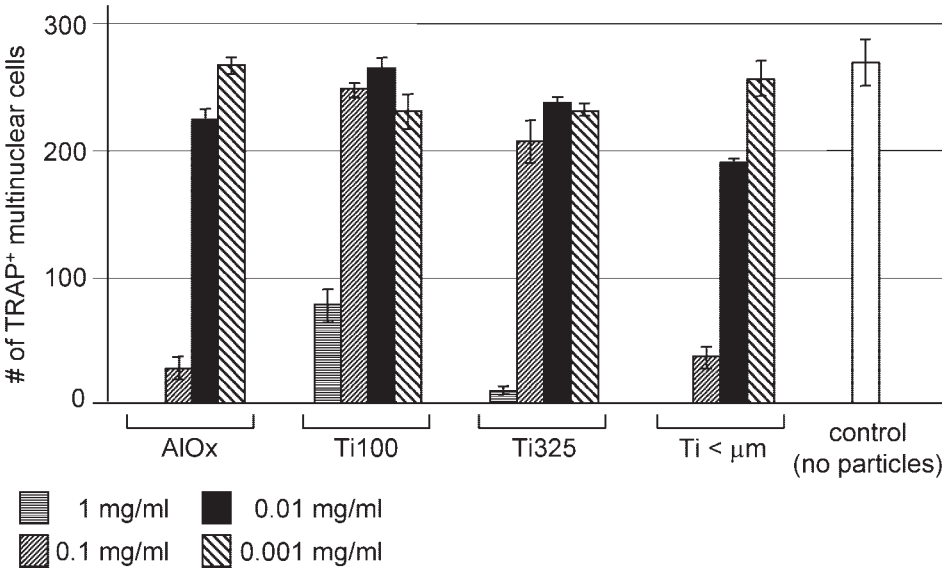

Figure 2. Formation of osteoclasts in cocultures in the presence of metal particles. Cocultures of osteoblasts and marrow cells were performed in the presence of different amounts of particles. With each of the different particles, the formation of osteoclasts was greatly reduced at the concentration of 1 mg/mL. At lower concentrations, particularly 0.01 and 0.001 mg/mL, the effect of the particles on osteoclastogenesis disappeared. The inhibitory effect of the particles on the formation of osteoclasts decreased with the increase in particle size, but no differences were seen, whether particles of AlOx or Ti were added to the culture.

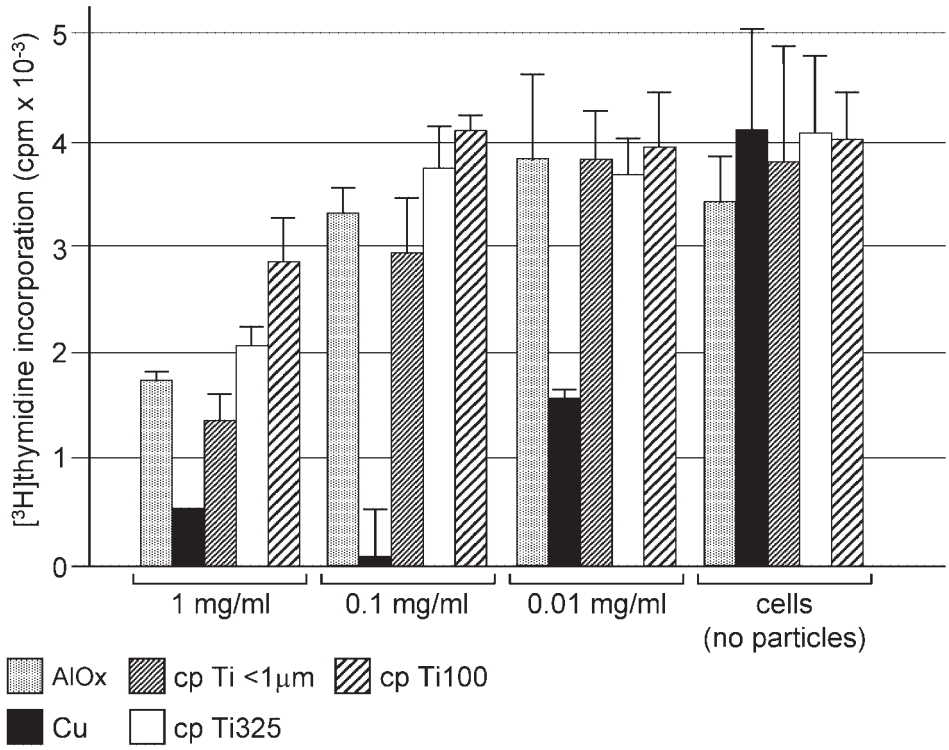

Figure 3. Proliferation of osteoblastic cells in the presence of particles. AlOx, Ti, and Cu particles were added to cultures of primary osteoblasts and the incorporation of [^{3}H]thymidine was determined as a parameter for cell proliferation. Cu particles exerted an inhibitory effect on cell proliferation at all three concentrations tested because of their toxicity. AlOx and the three different size Ti particles were inhibitory to varying degrees at 1 mg/mL, but the effect was lost at 0.1 and 0.01 mg/mL, respectively. Smaller size particles were more efficient in inhibiting osteoblastic cell proliferation than were larger size particles.

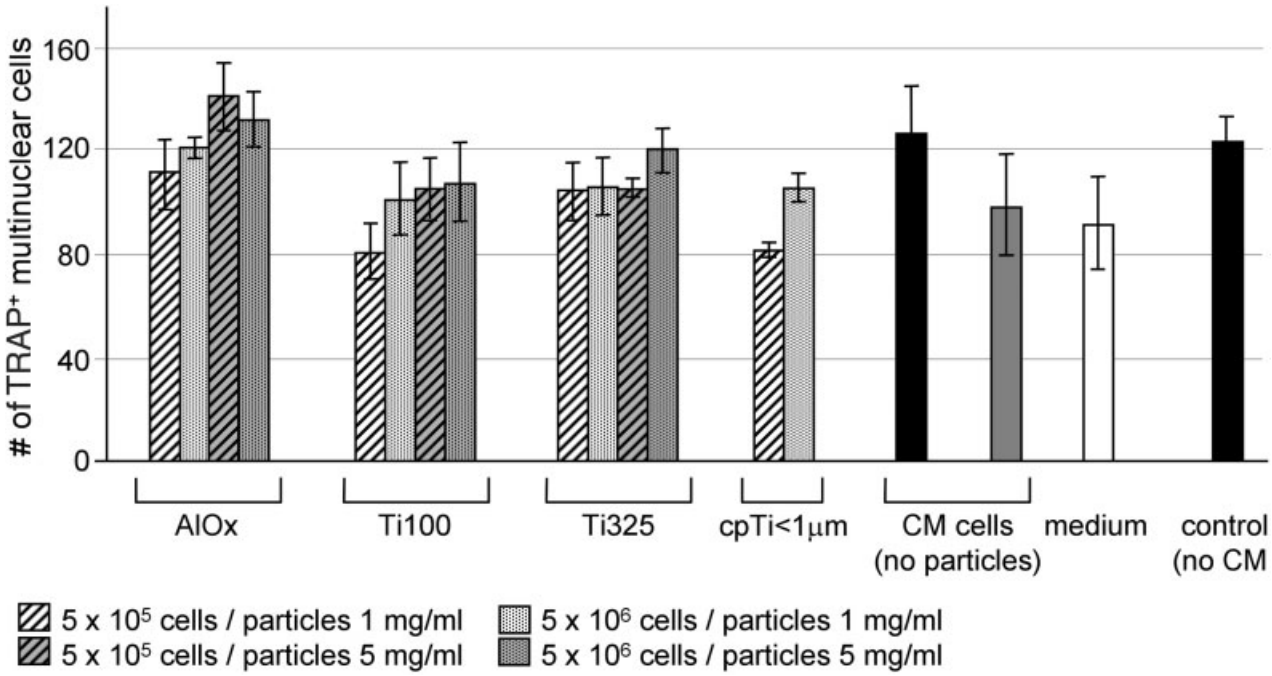

Figure 4. Effects of CM from BMDM on the formation of osteoclasts *in vitro*. BMDM were treated for 24 h with particles and the CM was subsequently added to the cocultures at a concentration of 50%. Independently of the particles used in these experiments, no significant effects on the formation of osteoclasts were observed. As controls, osteoclasts were grown in cocultures with CM from cells that were not exposed to particles, with aged medium (medium) and without addition of a conditioned or aged medium (control), respectively.

The effect of CM from cells exposed to particles

To investigate whether osteoblasts or BMDM upon exposure to particles release soluble factors affecting the formation of osteoclasts, CM from cells exposed to particles were tested on the cocultures.

CM from BMDM was added to the cocultures at a concentration of 50% during the complete culture period. In normal control cocultures (without CM), in cocultures containing medium conditioned without particles and cells, and in cocultures with CM from untreated cells, approximately the same numbers of osteoclasts were counted (Fig. 4). Furthermore, in CM from BMDM (5×10^5 and 5×10^6 cells/2 cm^2) treated with cp Ti and AlOx particles (1 or 5 mg/mL), respectively, the development of osteoclasts *in vitro* was as in the control cultures. For the Ti <1 μm particles, the experiment could be performed with only 1 mg/mL because of the limited availability of the particles.

Similarly, CM from osteoblastic cells treated with Ti and AlOx particles also did not affect the formation of osteoclasts *in vitro* (Fig. 5). Cell culture medium that was incubated together with Cu particles without cells, however, completely inhibited osteoclastogenesis because of the toxic effect exerted by solubilized Cu. Although the variation was considerable, no effects on the formation of osteoclasts were seen after addition of 10, 20, and 30% of CM from osteoblasts exposed to particles.

Release of TNFα by BMDM upon exposure to particles

TNFα is one of the major cytokines released by activated macrophages. Therefore, the levels of TNFα in the culture supernatant of BMDM exposed to particles were determined by enzyme-linked immunosorbent assay. Ti and AlOx particles did induce the release of TNFα by BMDM (Fig. 6). For all particles, except for the cp Ti <1 μm due to a lack of particles, 5 mg/mL induced a higher release of the cytokine than 1 mg/mL. It is particularly noteworthy that with Ti100, Ti325, and AlOx, the levels of TNFα released into the cell supernatant are inverse proportional to the size of the particles.

DISCUSSION

The longevity of prosthetic implants depends on a number of independent factors that are determined by the surgeon, by the implant, and by the host's reaction to the implant. A central event in the loosening of implants is the formation of osteolytic lesions impairing the maintenance of a stable interface between the implant and the patient's bone.[22,23] It is hypothesized that focal bone resorption of the periprosthetic bone is induced by changes in the composition of the hema-

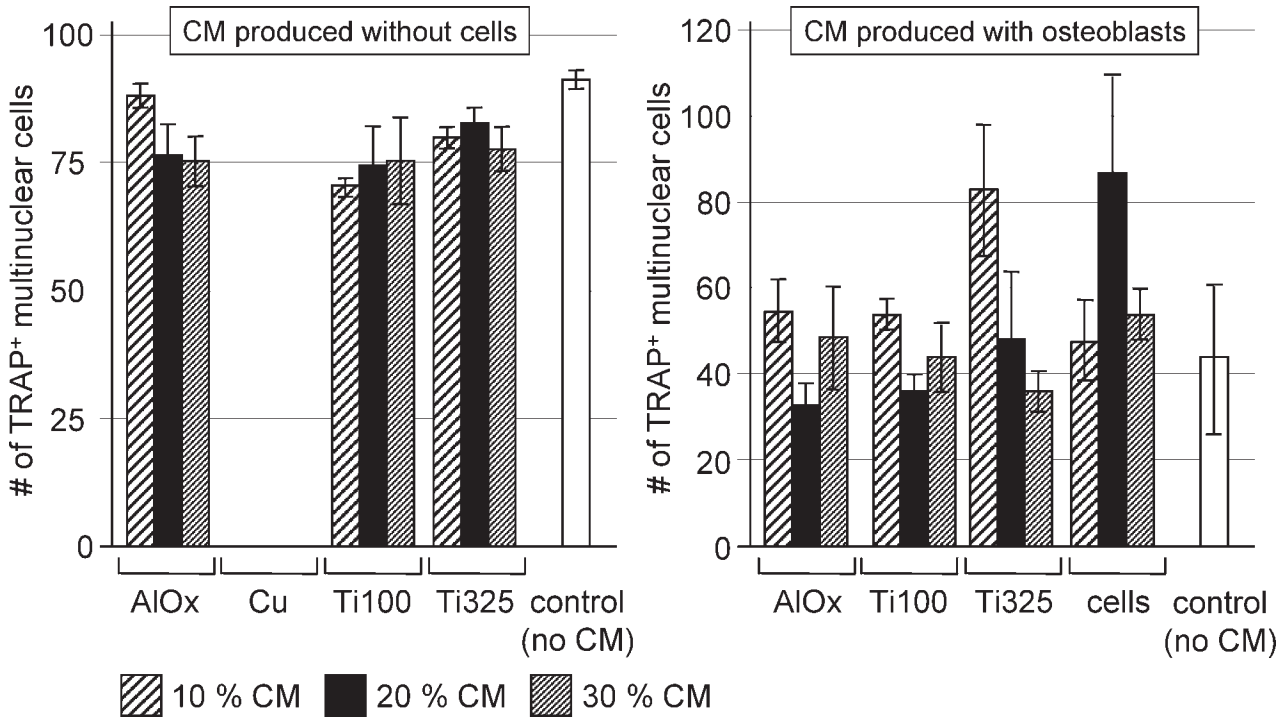

Figure 5. Effects of CM from osteoblasts on the formation of osteoclasts *in vitro*. Osteoblasts were treated for 24 h with particles and this CM was added to the cocultures at concentrations ranging from 10 to 30%. Controls were performed with CM that were produced without cells, but that did contain particles (CM produced without cells). CM from medium containing Cu particles induced cell death in the coculture, whereas media incubated with the other particles did not affect osteoclastogenesis. When CM were obtained from cells treated with particles, the CM were not found to cause a formation of osteoclasts different from control cultures at the three concentrations of CM used in the experiment.

topoietic microenvironment.[6] Therefore, data are presented herein describing the formation of osteoclasts in a coculture system of osteoblasts and hemopoietic precursors in response to implant topography and to metal particles. Both of these parameters have been suggested to contribute to the modulation of local bone resorption and thus to mediate aseptic prosthetic loosening.

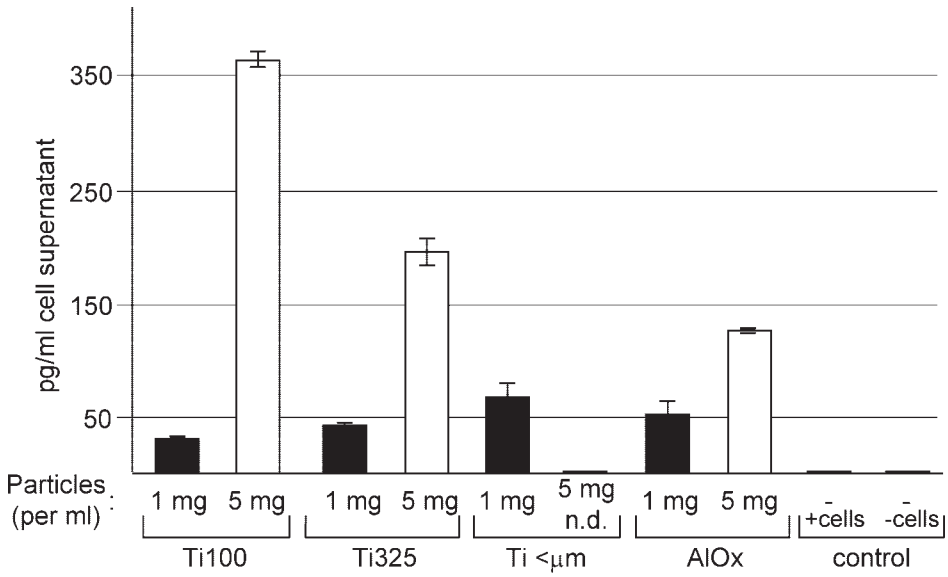

Figure 6. Release of TNFα by BMDM. To assess the activation of BMDM by metal particles, TNFα in the cell supernatant was quantified after exposure to particles. Treatment with 1 or 5 mg/mL caused the cells to release TNFα at significantly higher levels than did untreated control cells. The response was dose dependent, the particles at 5 mg/mL inducing a stronger response than did particles at 1 mg/mL.

The data presented in this study suggest that the chemistry of the metal surface is of little importance, rather it is the structure of the surface or the size of the particles that affects osteoclastogenesis. Using discs from TiAl6Nb7, CoCr28Mo6, FeCrNi, and cp Ti, it was demonstrated that bone cells are able to grow on these materials, both on polished and on sandblasted surfaces. This may be expected, because surfaces exposed to body fluids or culture media become rapidly oxidized and will be coated with proteins.[24] Cells attaching to the metal surface, therefore, may not be in direct contact with the metal itself, but rather with the biofilm deposited on the surface.

Although the material of the metal substratum did not affect osteoclastogenesis, consistently higher numbers of osteoclasts were counted in cultures grown on sandblasted than in cultures grown on polished surfaces. Cell function thus seems to be affected more profoundly by the topography, rather than by the chemical composition, of the surface. Activation of cells of osteoblast and macrophage lineages has been reported to depend on the topography of the implant surface, although the contributions of surface chemistry and topography to the modulation of cell behavior cannot always be clearly distinguished. Bone marrow stromal cells were found to decrease proliferation and to increase differentiated functions such as the synthesis of alkaline phosphatase or the formation of nodules and mineral deposition *in vitro* when cultured on metal surfaces with increasing roughness.[11,25] Furthermore, the macrophage cell line J774A.1 was found to be stimulated to release bone morphogenetic protein-2 upon culture on sandblasted surfaces, which was not the case on polished surfaces or on tissue culture plastic.[26] An increase in the differentiated functions of osteoblasts may well be associated with an increase in the capacity of these cells to support the formation of osteoclasts, as was observed in the present study. Differentiation is also associated with the ability of osteoblasts to support osteoclastogenesis in cocultures with bone marrow cells,[27] because $1,25(OH)_2D_3$, which is required for the development of osteoclasts from hematopoietic precursors, supports the expression of an osteoblastic phenotype.[28]

Orthopedic implants may affect the development of bone cells not only through the structure of their surface, but also through the release of wear particles that are shed from the implant during mechanical load. Surface topography and shedding of wear particles, however, are interdependent, because rough surfaces are more prone to shed wear particles than are polished surfaces. In the investigations of the reactions of the periprosthetic tissue to wear particles, much interest has been focused on the cells of the mononuclear phagocytic system. This cell lineage comprises the precursor cells eventually giving rise to osteoclasts.[28] Furthermore, the cells of the monocyte/macrophage lineages upon activation release as their main products the inflammatory cytokines IL-1 and TNFα, both potent stimulators of bone resorption *in vitro*[29,30] and *in vivo*.[31] Indeed, the present studies have confirmed data published previously by other laboratories[9,32] that monocytes upon exposure to wear particles release increased levels of TNFα. In agreement with previously published data,[33] however, wear particles added directly to the coculture did not increase the number of osteoclasts derived from bone marrow cells. They rather inhibited osteoclastogenesis in dependence of the respective dose and size of the particles added to the cell cultures. The smallest particles (cp Ti <1 μm) were most efficient in inhibiting osteoclastogenesis, followed by the slightly larger AlOx particles (<10 μm) and the even larger cp Ti325 and cp Ti100 particles. The effects of particles from stainless steel (<10 μm) fitted well into the described scheme (data not shown).

Whereas the effect of wear particles on the cells of the monocyte/macrophage lineages has been studied extensively, and the effect on the release of proinflammatory cytokines is well established, the effect of particles on the cells of the stromal/osteoblast lineages is less clear. The overall effects of particles on these cells, however, seem to be antiproliferative and antidifferentiating.[17] In the present study, a decrease in cell proliferation was found for high concentrations of particles and it was previously shown that particles may exert a cytotoxic effect on osteoblasts.[34,35] Gene expression and functional analysis on osteoblasts[36,37] and stromal cells[38] demonstrate changes in gene expression favoring bone resorption over bone formation upon treatment of the cells with metal particles.

Whereas small particles were most efficient in suppressing the formation of osteoclasts, the release of TNFα by bone marrow cells was less pronounced in the presence of small particles as compared with larger ones. A possible explanation may be that phagocytosing monocytes/macrophages release lower amounts of the cytokine than do macrophages that are encountering larger particles that they are not able to phagocytose. Because the main portion of wear particles shed *in vivo* from an implant are of the sub-μm range,[39] it can be assumed that there is a continuous release of low levels of TNFα. The local synthesis of TNFα in response to the exposure of macrophages to wear particles is of relevance, because in the recent past, the significant role of the cytokine factor in the regulation of bone resorption has been elucidated. TNFα stimulates bone resorption not only by itself, but it modulates the process also in synergism with the receptor activator of NF-κB ligand (RANKL), another member of the TNF family of growth factors. Through synergistic actions, low concentrations of TNFα, in the appropriate hematopoietic environment, may exert deleterious effects on local

bone metabolism.[40] The data thus suggest that different cellular events take place in monocytes/macrophages exposed to small (sub-μm) or large (>10 μm) wear particles. Small particles are phagocytosed by monocytes/macrophages, inhibiting their differentiation to osteoclasts.[15,33] In addition, the simultaneous inhibitory effects on the development of osteoblasts further mediate antiosteoclastogenic effects.[17] Larger particles that cannot be phagocytosed, however, induce the development of foreign body granulomas that lead to a chronic inflammation, recruiting activated inflammatory cells and potential osteoclast progenitors to the respective periprosthetic site.[16,41]

In conclusion, within the present study it was shown that wear particles shed from an implant or the topography of implant surfaces can induce changes in the local hematopoietic microenvironment regulating the recruitment and activation of osteoclasts. These local conditions may be causing the formation of osteolytic lesions in the periprosthetic bone, contributing to the loosening of the implant, which will eventually make revision surgery a necessity. Control of the hematopoietic environment, therefore, may be a means to delay or prevent the formation of osteolytic lesions and as a consequence prosthetic loosening.

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