Polyethylene and titanium alloy particles reduce bone formation

Dose-dependence in bone harvest chamber experiments in rabbits

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Particles similar to those generated from joint replacements affect net bone formation within the Bone Harvest Chamber in rabbits. Whether these effects depend on the concentration of particulate materials is unknown. In this study, we performed a histomorphologic and morphometric analysis of net bone formation in the Bone Harvest Chamber in the presence of different concentrations of phagocytosable particles of high density polyethylene and titanium 6-aluminum 4-vanadium alloy. Chambers were implanted in 9 mature New Zealand white rabbits bilaterally. Concentrations of 10^6, 10^7 and 10^8 polyethylene particles/mL, and 10^8 and 10^9 particles/mL of titanium alloy in 1% sodium hyaluronate carrier were implanted for 3-week periods in sequence in each of the chambers. 3-week control periods in which nothing was implanted in the chamber were included between the treatments. Increasing concentrations of polyethylene particles were associated with a more marked foreign body response and fibrosis. Net bone formation for the three polyethylene doses was reduced by 11%, 21% and 33% of controls, respectively. For titanium alloy, net bone formation was reduced by 8% and 56% of controls, for concentrations of 10^8 and 10^9 particles/mL, respectively. Our findings suggest possible adverse effects of wear debris on net bone formation and bony remodeling in the prosthetic bed, when concentrations of specific particles reach critical local levels.

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The generation of wear debris, and the processes of prosthetic loosening and osteolysis appear to be related (Goldring et al. 1983, Maloney et al. 1990, Ohlin et al. 1990, Santavirta et al. 1990, 1992, Amstutz et al. 1992, Schmalzried et al. 1992, Wright and Goodman 1996). Phagocytosis of polymeric and metallic particles by macrophages stimulates the release of substances associated with the resorption of bone (Howie et al. 1988, Murray and Rushton 1990, Haynes et al. 1993, Glant et al. 1993, Horowitz et al. 1993, 1994). In previous studies using the Bone Harvest Chamber implanted in the rabbit tibia, small, phagocytosable particles of bone cement, high density polyethylene and cobalt chrome alloy, but not titanium alloy, inhibited bone ingrowth into a 1 mm pore in a titanium implant (Goodman et al. 1994, 1995). Given that the particles were approximately the same size, and a similar concentration of particles was used (10^8 particles/mL), these studies demonstrated that net bone formation in the presence of particulate materials is modulated, in part, by the properties of the materials themselves. Whether the concentration of particulate materials has a dose-response effect on net bone formation in vivo is unknown. We have now performed a histomorphologic and morphometric analysis of net bone formation in the Bone Harvest Chamber in the presence of different concentrations of phagocytosable particles of high density polyethylene and titanium 6-aluminum 4-vanadium alloy. We chose these two materials for testing because they are similar to those commonly used in total joint replacement and, in a previous study, at a concentration of 10^8 particles/mL, one (polyethylene) depressed net bone formation, whereas the other (titanium alloy) had no effect (Goodman et al. 1994,1995).

Material and methods

The Bone Harvest Chamber

The Bone Harvest Chamber, a device made of commercially pure titanium, is implanted in the proximal medial tibial metaphysis of mature rabbits (Albrektsson et al. 1984, Goodman et al. 1994, 1995). The

chamber consists of a fixed outer cylinder and an inner removable core, which are held together by two threaded screws. After perforating the tibial cortex with a 6 mm drill, the outer cylinder is screwed into bone so that the two 1 mm round holes at opposite ends of the cylinder are at the level of the cortex. The cylinder undergoes osseointegration with the surrounding bone over a 6-week period. The inner, removable core fits into the cylinder and contains a 1×1×5 mm groove that is continuous, with the holes in the outer cylinder. When assembled together, this groove and the floor and holes of the cylinder provide a continuous canal for tissue ingrowth.

Particulate materials

For these studies, we attempted to procure particles as similar as possible to those observed in retrieved membranes. The particles chosen were less than 10 μm in diameter and therefore could undergo phagocytosis by macrophages. The size of the particles was measured on a scanning electron microscope interfaced with a computer morphometric image analysis system.

We obtained small phagocytosable particles of high density polyethylene; this material is similar to ultra-high molecular weight polyethylene, but the former is slightly more dense and has a lower molecular weight. Indeed, some of the polyethylene wear particles generated in vivo by the process of chain scission may be high density polyethylene (Bostrom et al. 1994).

The high density polyethylene particles were a gift from Smith and Nephew Richards, Memphis, TN, USA. These particles were produced by Shamrock Technologies, Newark, NJ, USA, using a proprietary method, and were reported to be 100% pure and highly crystalline, with specific gravity 0.95. The particles averaged 4.7 (SD 2.1) μm. The particles of titanium 6-aluminum 4-vanadium alloy (also a gift from Smith and Nephew Richards) were produced by cyclic loading and resultant fretting of titanium 6-aluminum 4-vanadium alloy modular components in saline. The fretting conditions were chosen to produce sufficient quantities of metallic particles for the study. The particles of titanium alloy averaged 4.0 (4.4) μm.

The particle concentrations chosen for this experiment were based on previous studies using the same model, in which 10^8 particles/mL of high density polyethylene depressed net bone formation, whereas the same concentration of titanium alloy particles had no effect (Goodman et al. 1994, 1995). For each material, the highest concentration of particles was first used. This was aliquoted and serially diluted after vigorous shaking to ensure that the particles were evenly dispersed. Particle dispersions of 10^6, 10^7, and 10^8 particles/mL of high density polyethylene, and 10^8 and 10^9 particles/mL of titanium alloy in saline were thus prepared. The saline was removed by careful pipetting and by evaporation in vacuum. The particles were then sterilized with ethylene oxide and next reconstituted to the appropriate concentration with the carrier, 1% sodium hyaluronate solution (Healon, Pharmacia AB, Uppsala, Sweden) at the time of surgery. This substance has been previously shown neither to facilitate nor depress ingrowth of bone into the Bone Harvest Chamber (Goodman et al. 1994, 1995). The dispersions of titanium alloy were grey-black; the polyethylene dispersions were colorless-milky white. The 1×1×5 mm groove in each chamber core was completely filled with the dispersed particles. In each case, a volume of 0.005 mL of test solution was implanted.

Surgical procedure

Institutional guidelines for the care and use of laboratory animals were strictly followed. Chambers were implanted bilaterally in 9 mature, male, New Zealand white rabbits, as previously described (Goodman et al. 1994, 1995). With the animals under general endotracheal anesthesia, the lower extremities were shaved from the hip to the hock, and were prepared using a sterile technique. A 3 cm incision was made exposing the proximal, medial tibia subperiosteally, adjacent to the medial collateral ligament. A 6 mm hollowed drill was used to excise a round, cortical window. The cylinder of the chamber was screwed into bone with a special wrench, so that the holes in the cylinder were at the level of the cortex of the tibia. The core was then positioned in the cylinder, forming a continuous canal through the chamber for tissue ingrowth. The two screws connecting the components were tightened.

After a 6-week period for osseointegration, the contents of the chamber were harvested for the first time and discarded. Healon, the carrier, was then placed in the groove of the chamber core and the chamber was reassembled. The tissue in the chamber was harvested 3 weeks later. Different concentrations of polyethylene or titanium alloy particles were then placed in the chambers bilaterally, in sequential order. The chambers were harvested at 3-week intervals, cleaned meticulously with saline-soaked cotton swabs, and then a new treatment (a higher concentration or a different material) was instituted. Intermittent 3-week "rest" periods without any material implanted in the chambers were incorporated in the study to monitor sequencing effects and the possible effects of any residual material.

Table 1. Morphometric analysis. Mean percentage of bone in sections containing increasing concentrations of particles. The values for the right and left chambers are averaged for each of the 9 rabbits

No.	Healon	PE6	PE7	PE8	rest1	Ti8	Ti9	rest2
1	24	22	36	16	32	37	13	28
2	31	14	18	10	16	33	15	32
3	37	30	18	16	35	22	6	35
4	16	26	13	21	25	27	7	34
5	28	24	22	21	30	24	12	32
6	30	26	26	26	31	19	20	32
7	24	21	23	16	27	23	9	25
8	23	22	9	16	25	17	11	19
9	27	28	25	21	37	20	NA	NA
mean	27	24	21	18	29	25	12	30
S.D.	6	5	8	5	6	7	5	5

Healon sodium hyaluronate (the carrier),
PE6 10^6 high density polyethylene (HDPE) particles/mL,
PE7 10^7 HDPE particles/mL, PE8 10^8 HDPE particles/mL,
rest1 first treatment with no particles or Healon implanted,
Ti8 10^8 Ti 6-Al 4-V particles/mL, Ti9 10^9 Ti 6-Al 4-V particles/mL,
rest2 second treatment with no particles or Healon implanted,
NA not available.

Specimen processing and evaluation

The harvested tissue was fixed in 10% buffered formalin, decalcified, embedded in paraffin and cut into 5 µm sections stained with hematoxylin and eosin. A histomorphologic examination using transmitted and polarized light was performed on 5–8 serial, longitudinal sections that enabled the entire length of the specimen to be visualized on one slide. A computerized image analysis system for planar morphometry measurements (Microcomp-PM, Southern Micro Instruments, Inc., Montgomeryville, PA, USA) interfaced with a Computer Deskpro 386/20 computer (Compaq Computer Corp., Houston, TX, USA) and a real-time video light microscope system was used to perform a morphometric analysis of the percentage of trabecular bone in one longitudinal section taken from the center of the specimen. Each fifth section was re-evaluated twice; in each case, the values for percentage of bone within the section was within 5% of the first measurement.

Statistics

The ratio of the total area of trabecular bone within a section divided by the total section area was determined for each histological section, and this value was multiplied by 100 and therefore expressed as a percentage. The values for the 2 bilateral chambers in each animal with the same treatment were averaged (Table 1). The mean and standard deviation were then calculated for each of the treatments. An analysis of variance was then performed on the entire data set, which was found to be statistically significant. During further analysis, it was assumed that the dose-response relationship was approximately linear in the investigated dose range. Individual dose-response relationships for polyethylene and titanium alloy particles were estimated by the mean difference in percentage bone area between consecutive harvests containing the carrier only and the carrier with increasing concentrations of particles. For each animal, the mean value from the 2 chambers was used. The exact binomial test was used when considering the existence of a dose-response relationship, and Wilcoxon's signed rank test for matched pairs was used when comparing polyethylene and titanium alloy particles with respect to the slope of their dose-response relationship. The percentages of bone ingrowth in the three control treatments (the carrier solution alone and the two rest periods) were compared, using an analysis of variance.

Results

Histology

The carrier solution alone and rest treatments both produced an extensive amount of woven, trabecular bone, aligned parallel to the longitudinal axis of the pore in a fibrovascular stroma (Figure 1). Towards the ends of each specimen, near the holes in the chamber and along the walls of the implant the woven bone had a more compact, lamellar appearance. There was no evidence of foreign body or inflammatory reac-

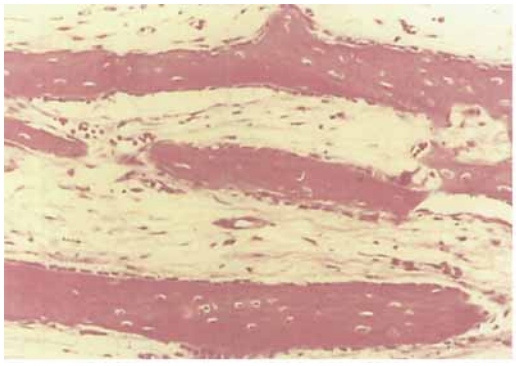

Figure 1. Specimen from a control "rest" chamber in which no particles were implanted for 3 weeks. Longitudinally oriented woven, trabecular bone is seen in a fibrovascular stroma. HE, 200×.

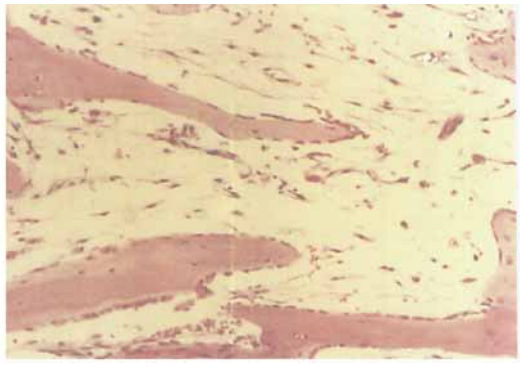

Figure 2. Specimen from a chamber in which 10^6 polyethylene particles/mL were implanted for 3 weeks. The section resembles control sections, except for the presence of occasional positively birefringent polyethylene particles. HE, polarized light. 200×.

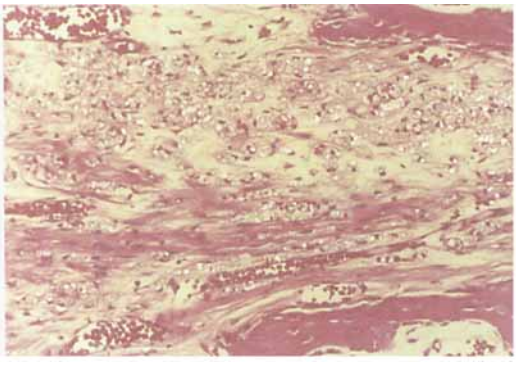

Figure 3. Specimen from a chamber in which 10^8 polyethylene particles/mL were implanted for 3 weeks. A moderate foreign body response and chronic inflammatory reaction are noted; trabecular bone is less prominent in this section than in Figure 2. HE, polarized light, 200×.

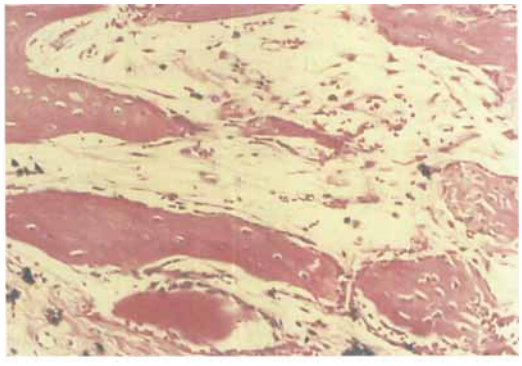

Figure 4. Specimen from a chamber in which 10^8 Ti 6-Al 4-V particles/mL were implanted for 3 weeks. Black granules are seen in scattered macrophages, often in small groups in the interstitium. The fibrovascular stroma contains extensive trabecular and compact woven bone, similar to the control sections. HE, 200×.

tion. Sections from rest periods between particle treatments were indistinguishable from sections in which the carrier, 1% sodium hyaluronate solution, alone had been implanted.

The polyethylene particles were globular-shaped and positively birefringent, using polarized light. In the 10^6 particle/mL treatment, these particles were very scarce and were occasionally phagocytosed by scattered macrophages (Figure 2). The surrounding stroma and bone were similar to the control sections. The polyethylene particles were more conspicuous in the 10^7 and 10^8 particle/mL sections, and were surrounded by groups of mono- and multi-nucleated macrophages and chronic inflammatory cells in a more fibrous stroma (Figure 3). Intracellular particles were noticeable and they displaced the nucleus to one side. Longitudinally aligned trabecular bone was less prominent in sections containing the two highest polyethylene concentrations than it was in the controls.

Sections from the 10^8 particle/mL titanium alloy treatment contained black granules dispersed throughout, with little evidence of a foreign body or inflammatory reaction (Figure 4). Some black granules of titanium alloy were seen in scattered macrophages, often in small groups in the interstitium. The stroma was fibrovascular, with extensive trabecular and compact woven bone, similar to the control sections. Sections from the 10^9 particle/mL titanium alloy treatment contained large areas of dispersed and clumped black granules, and a more fibrous background stroma (Figure 5). Phagocytosed particles were a more conspicuous finding. Trabecular and compact bone was less prominent with increasing concentrations of titanium alloy particles. There was no evidence of cell necrosis or hyaline degeneration,

Figure 5. Specimen from a chamber in which 10^9 Ti 6-Al 4-V particles/mL were implanted for 3 weeks. Phagocytosed particles are more conspicuous and the stroma is more fibrous. Trabecular and compact bone is less prominent and is located adjacent to the chamber walls. HE, 200×.

previously seen after implantation of similar-sized cobalt chrome alloy particles in the same model (Goodman et al. 1994, 1995).

Polyethylene and titanium alloy particles were distributed throughout the sections, in the interstitium and adjacent to bone. There did not appear to be a difference in the location of the two types of particles or in the characteristics of the underlying woven bone.

Histomorphometric analysis (Table 1)

With implantation of 10^6, 10^7, and 10^8 particles/mL of polyethylene, bone ingrowth was decreased by 11%, 21% and 33% of the carrier solution alone, respectively. Titanium alloy particles reduced bone ingrowth by 8% and 56% of the carrier solution alone, respectively. The 3 control treatments (the carrier and the 2 rest periods without particles) did not differ. A dose-response relationship was found for polyethylene particles ($p = 0.04$) and titanium alloy particles ($p = 0.008$), with respect to bone ingrowth. The curve was steeper for titanium alloy particles than for polyethylene particles ($p = 0.01$).

Discussion

Our results point to a concentration-dependence for each of the materials tested in this study. For polyethylene particles, net bone formation was depressed at concentrations of 10^6–10^8 particles/mL. The foreign body and chronic inflammatory histological reactions were less prominent when the lowest concentration was used. Using a concentration of 10^8 particles/mL titanium alloy particles, the foreign body reaction was far less than that seen with a similar concentration of polyethylene. However, when the concentration of titanium alloy was increased to 10^9 particles/mL, net bone formation was depressed and a more marked foreign body and fibrous reaction was noticeable. As the particles of polyethylene and titanium alloy were approximately the same size, it would appear that the intrinsic physical and chemical properties of the particulate materials and the mode of presentation modulated tissue differentiation and net bone formation in the chamber (Nagura et al. 1977, Kawaguchi et al. 1986, Tabata and Ikada 1988, Glant et al. 1993, Goodman et al. 1994, 1995). Our findings are consistent with those of Shanbhag et al. (1994) using human monocyte cultures; these authors also demonstrated a steeper dose-response relationship for titanium alloy than for polyethylene particles.

We chose these 2 materials for initial testing because one (polyethylene) depressed net bone formation in previous studies, whereas the other (titanium alloy) had no effect (Goodman et al. 1994). Furthermore, titanium alloy and ultra-high molecular weight polyethylene have been the focus of much recent controversy with regard to their long-term suitability as bearing surfaces for total joint replacement (Agins et al. 1988, Amstutz et al. 1992, Campbell and Amstutz 1992, Wright and Goodman 1996). We recognize that ultra-high molecular weight polyethylene and high density polyethylene are different materials; however, many of the polyethylene particles found in the periprosthetic tissues may indeed be of the high density rather than the ultra-high molecular weight type because of their small size (Personal communication, Richard Landingham, Lawrence Livermore Laboratory, Livermore, CA, USA). This hypothesis may also be substantiated by the studies by Bostrom and coworkers (1994), who noted that chain scission of ultra-high molecular weight polyethylene in vivo might produce smaller particles, with increased ionic density similar to high density polyethylene. On a practical note, high density polyethylene particles are readily available commercially, whereas ultra-high molecular weight particles are extremely difficult to manufacture and are not for general distribution to orthopedic research laboratories (Leigh et al. 1992).

The decrease in net bone formation with increasing concentrations of polyethylene and titanium alloy particles was not simply a "space-occupying effect" of the material itself. Calculations have shown that concentrations of 10^9 particles/mL would constitute less than 5% of the total volume of the canal in the chamber. At the higher concentrations of polyethylene and titanium alloy particles, net bone formation was probably offset by the space occupied by the cellular response associated with the particles and by the liberation of factors that reduce the formation and/or

enhance the degradation of bone (Glant et al. 1993, Horowitz et al 1993, 1994, Frøkjaer et al. 1995). In a previous study, a concentration of 10^8 high density polyethylene particles/mL was associated with decreased net bone formation and increased numbers of tartrate-resistant, acid phosphatase positive osteoclasts compared to controls (Goodman et al. 1995). It would appear that wear particles might modulate both the formative and resorptive processes of mesenchymal tissue.

The steeper dose-response curve for titanium alloy particles than for polyethylene may be due to the effects of one of the constituents of titanium alloy when a threshold level is exceeded. Compared to similar-sized particles of cobalt chrome alloy at a concentration of 10^8 particles/mL, titanium alloy particles depressed net bone formation to a lesser degree, and demonstrated less toxicity (Goodman et al. 1994, 1995). This in vivo finding, using the Bone Harvest Chamber model in rabbits, is consistent with in vitro studies by others (Rae 1975, 1981, Glant et al. 1993, Haynes et al. 1993, Shanbhag et al. 1994).

The generation of wear debris is an ongoing process that occurs with every total joint replacement. Phagocytosis and distant transport of particles by macrophages are mechanisms by which the biological effects of inorganic, nonbiodegradable wear debris are mitigated. We recognize that the particles used in this study may not parallel the clinical situation exactly, and caution is advised in applying these data directly to humans. Nevertheless, our studies suggest possible adverse effects of wear debris on net bone formation and bony remodeling in the prosthetic bed, when concentrations of specific particles reach critical local levels.

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References

Agins H S, Alcock N W, Bansal M, Salvatti E A, Wilson P D, Pellici P M, Bullough P G. Metallic wear in failed titanium-alloy total hip replacements. J Bone Joint Surg (Am) 1988; 70: 347-56.

Albrektsson T, Jacobsson M, Kälebo P. The harvest chamber—a newly developed implant for analysis of bone remodelling in situ. In: Biomaterials and biomechanics. (Eds. Ducheyne P, Van der Perre G, Ubert A). Elsevier Science Publishers, Amsterdam 1984; 283-8.

Amstutz H C, Campbell P, Kossovsky N, Clarke I C. Mechanism and clinical significance of wear-induced osteolysis. Clin Orthop 1992; 276: 7-18.

Bostrom M P, Bennett A P, Rimnac C M, Wright T M. The natural history of ultra-high molecular weight polyethylene. Clin Orthop 1994; 309: 20-8.

Campbell P, Amstutz H C. The need for improved orthopaedic bearing surfaces: A clinical perspective. In: Surface modification technologies V. (Eds. Sundarshan T S, Braza J F). Cambridge University Press, 1992: 15-24 .

Frøkjær J, Deleuran B, Lind M, Overgaard S, Søballe K, Bünger C. Polyethylene particles stimulate monocyte chemotactic and activating factor production in synovial mononuclear cells in vivo. An immunohistochemical study in rabbits. Acta Orthop Scand 1995; 66 (4): 303-7.

Glant T T, Jacobs J J, Molnar G, Shanbhag A S, Valyon M, Galante J O. Bone resorption activity of stimulated macrophages. J Bone Miner Res 1993; 8:9: 1071-9.

Goldring S R, Schiller A L, Roelke M, Rourke C M, O'Neill D A, Harris W H. The synovial-like membrane at the bone cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg (Am) 1983; 65: 575-84.

Goodman S B. The effects of micromotion and particulate materials on tissue differentiation. Bone chamber studies in rabbits. Acta Orthop Scand (Suppl 258) 1994; 65.

Goodman S B, Aspenberg P, Song Y, Knoblich G, Huie P, Regula D, Lidgren L. Tissue ingrowth and differentiation in the bone harvest chamber in the presence of cobalt chrome alloy and high density polyethylene particles. J Bone Joint Surg (Am) 1995; 77: 1025-35.

Haynes D R, Rogers S D, Hay S, Pearey M J, Howie D W. The differences in toxicity and release of bone-resorbing mediators induced by titanium and cobalt-chromium-alloy wear particles. J Bone Joint Surg (Am) 1993; 75: 825-34.

Horowitz S M, Doty S B, Lane J M, Burstein A H. Studies of the mechanism by which the mechanical failure of polymethylmethacrylate leads to bone resorption. J Bone Joint Surg (Am) 1993; 75: 802-13.

Horowitz S M, Rapuano B P, Lane J M, Burstein A H. The interaction of the macrophage and the osteoblast in the pathophysiology of aseptic loosening of joint replacements. Calcif Tissue Int 1994; 54: 320-4.

Howie D W, Vernon-Roberts B, Oakesholt R, Mantney B. A rat model of resorption of bone at the cemented bone interface in the presence of polyethylene wear particles. J Bone Joint Surg (Am) 1988; 70: 257-63.

Kawaguchi H, Koiwai N, Ohtsuka Y, Miyamoto M, Sasakawa S. Phagocytosis of latex particles by leukocytes. Dependence of phagocytosis on the size and surface potential of particles. Biomaterials 1986; 7: 61-6.

Leigh H D, Taylor P, Swaney A, Black J. Research and development report: Production of fine particulate ultra high molecular weight poly (ethylene) for biological response studies. J Appl Biomat 1992; 3: 77-80.

Maloney W J, Jasty M, Harris W H, Galante J O, Callaghan J J. Endosteal erosion in association with stable uncemented femoral components. J Bone Joint Surg (Am) 1990; 72: 1026-34.

Murray D W, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg (Br) 1990; 72: 988-92.

Nagura H, Asai J, Kojima K. Studies on the mechanisms of phagocytosis. I. Effect of electrical surface charge on phagocytic activity of macrophage for fixed red cells. Cell Struct Funct 1977; 2: 21-8.

Ohlin A, Johnell O, Lerner U H. The pathogenesis of loosening of total hip arthroplasties. The production of factors by periprosthetic tissues that stimulate in vitro bone resorption. Clin Orthop 1990; 253: 87-96.

Rae T. A study on the effects of particulate metals of orthopaedic interest on murine macrophages in vitro. J Bone Joint Surg (Br) 1975; 57: 444-50.

Rae T. The toxicity of metals used in orthopaedic prostheses. An experimental study using cultured human synovial fibroblasts. J Bone Joint Surg (Br) 1981; 63: 435-40.

Santavirta S, Konttinen Y T, Begroth V, Eskola A, Tallroth K, Lindholm S. Aggressive granulomatous lesions associated with hip arthroplasty. Immunopathological studies. J Bone Joint Surg (Am) 1990; 72: 252-8.

Santavirta S, Gristina A, Kontinnen Y T. Cemented versus cementless hip arthroplasty. A review of prosthetic biocompatibility. Acta Orthop Scand 1992; 63: 225-32.

Schmalzried T P, Kwong L M, Jasty M, Sedlacek R C, Haire T C, O'Connor D O, Bragdon C R, Kabo J M, Malcolm A J, Harris W H. The mechanism of loosening of cemented acetabular components in total hip arthroplasty. Analysis of specimens retrieved at autopsy. Clin Orthop 1992; 279: 60-78.

Shanbhag A S, Black J, Jacobs J J, Galante J O, Glant T T. Human monocyte response to submicron fabricated and retrieved polyethylene, Ti-6Al-4V and Ti particles. Trans Orthop Res Soc 1994; 19: 849.

Tabata Y, Ikada Y. Effect of size and surface charge of polymer microspheres on their phagocytosis by macrophage. Biomaterials 1988; 9: 356-62.

Wright T M, Goodman S B (Eds). Implant wear: The future of total joint replacement. American Academy of Orthopaedic Surgery, Oakbrook, IL, USA 1996.