In vitro and *in vivo* biological responses to a novel radiopacifying agent for bone cement

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Iodixanol (IDX) and iohexol (IHX) have been investigated as possible radiopacification agents for polymethylmethacrylate (PMMA) bone cement, to replace the currently used barium sulphate and zirconia. IDX and IHX are both water-soluble iodine-based contrast media and for the last 20 years have been used extensively in clinical diagnostic procedures such as contrast media enhanced computed tomography, angiography and urography. One of the major reasons to remove the current radiopacifying agents is their well-documented cytotoxicity and their potential to increase bone resorption.

Using *in vitro* bone resorption assays, the effect of PMMA particles plus IDX or IHX to induce osteoclast formation and lacunar resorption on dentine slices has been investigated. These responses have been compared with the *in vitro* response to PMMA particles containing the conventional radiopacifying agents, that is, barium sulphate and zirconia. In parallel, the *in vivo* reaction, in terms of new bone formation, to particles of these materials has been tested using a bone harvest chamber in rabbit tibiae.

In vitro cell culture showed that PMMA containing IHX resulted in significantly less bone resorption than PMMA containing the conventional opacifiers. *In vivo* testing, however, showed no significant differences between the amounts of new bone formed around cement samples containing the two iodine-based opacifying agents in particulate form, although both led to fewer inflammatory cells than particles of PMMA containing zirconia. Our results suggest that a non-ionic radiopacifier could be considered as an alternative to the conventional radiopacifying agents used in biomaterials in orthopaedic surgery.

Keywords: bone cement; radiopacifying agent; particles; biocompatibility; bone resorption

1. INTRODUCTION

Total joint replacement is the most successful method of treating end-stage arthritis. It significantly improves the quality of life and the functional capability of patients suffering from arthritic disease. Currently an estimated 1 million total joint arthroplasties are performed each year worldwide. In Sweden, more than 22% are performed in patients less than 65 years old (Malchau *et al.* 2002), and in the UK over 42% are in this younger age group (Department of Health Research and Development Directorate, Biomaterials and Implants Advisory Group 1995; Royal College of Surgeons 2000). Despite the attractions of non-cemented fixation, the vast majority of joint arthroplasties still involve some polymethylmethacrylate (PMMA)-based cement for fixation. In Sweden, over 96% of hip arthroplasties (Malchau *et al.* 2002) and over 98% of knee arthroplasties (Swedish Knee Register 2003) use

bone cement for the fixation of at least one component. Similarly, a recent UK audit report has indicated that bone cement is used in over 90% of total joint replacements (Royal College of Surgeons 2000). The use of cemented fixation is particularly high in both Sweden and the UK, but is also popular elsewhere, particularly for more elderly patients.

Bone cement consists of a polymer powder, usually PMMA or a PMMA co-polymer, which is polymerized in the operating theatre with a liquid monomer. Contrast agents, notably barium sulphate (BaSO₄) and zirconium dioxide (ZrO₂), are commonly added to the bone cement in order to confer radiopacity and thus aid in the radiographic assessment of the implants. The addition of radiopacifying agents to PMMA is, however, not achieved without altering the properties of the bone cement. Although changes in the mechanical strength of the bone cement may be insignificant (Chan & Ahmed 1991; Ginebra *et al.* 2002), radiopacifying agents are harder than metallic femoral heads and, if they enter the joint space, may cause third body

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wear, damaging both the articulating surfaces. Damage to the metal component is more serious as plastic deformation of the surface leads to the production of ridges which consequently scratch, and continue to scratch, the opposing, usually polyethylene surface, leading to the accelerated production of wear debris (Issac *et al.* 1987; Caravia *et al.* 1990).

Conventional radiopacifying agents used in bone cements have been shown to evoke a significant pathological response *in vivo* and *in vitro*. BaSO₄ injected intradermally into experimental animals is known to cause a foreign body inflammatory reaction (Adams 1976); barium has also been shown to intensify the release of inflammatory mediators in response to PMMA particles (Lazarus *et al.* 1994). Very few reports are available with regards to ZrO₂, although the development of epithelioid granulomas after the injection of elemental zirconium has been reported (Adams 1976). It has been shown by various groups that polymerized PMMA particles containing barium or zirconium can induce a greater osteolytic response, both *in vitro* (Sabokbar *et al.* 1997, 1998) and *in vivo* (Wimhurst *et al.* 2001), than PMMA particles with no added radiopacifying agents. As there is an increase in the number of joint replacements (notably with the use of PMMA bone cement), it is imperative to find alternative radio-contrast media in order to reduce the failure of prosthetic implants or aseptic loosening caused by the biological responses evoked by PMMA particles containing BaSO₄ or ZrO₂. Some groups have attempted to use iodine as an opacifier, in the form of methacrylates containing iodine that are used to replace the monomer phase while retaining the conventional PMMA powder phase (Davy *et al.* 1997; Artola *et al.* 2004; van Hooy-Corstjens *et al.* 2004). These cements are prepared in the same way as the conventional MMA/PMMA cements.

A different approach has been used in this paper. Iodixanol (IDX) and iohexol (IHX) are non-ionic monomers and dimers, respectively, containing iodine, and were originally developed as water-soluble contrast media to be used in radiographic investigations such as angiography (Almén 1995). Since then, they have been used in millions of investigations annually worldwide with minimal side effects (Davenport *et al.* 1999; Nakamura 2003). Both these contrast media have now been produced as fine ground powder, which is mixed in with the powder phase of bone cement prior to cement polymerization. As radiopacifiers, they have been shown not to reduce the mechanical properties of the resulting cement compared with cements manufactured with conventional radiopacifiers (Kjellson *et al.* 2001). Additionally, IDX and IHX provide suitable levels of radiopacification for clinical applications (Kjellson *et al.* 2004). Prior to their clinical application, the effect they have when combined with bone cement on both osteoclasts and osteoblasts needs to be investigated. The biologically based clinical requirements for novel opacifiers are that they produce less effect on osteoclasts and do not reduce osteoblast activity compared with PMMA bone cement containing the conventional barium and zirconium. The aim of the current study, is therefore, to evaluate the *in vitro*

and *in vivo* effects of PMMA bone cement containing IDX and IHX, and compare their cellular response to PMMA bone cement containing conventional radiopacifiers.

In vitro cell co-culture with PMMA particles has been used to assess the level of resorption produced on dentine slices by murine osteoclasts. Osteoclasts are multinucleated bone-resorbing cells formed from phagocytes (Fujikawa *et al.* 1996). Reproducible models have been produced using both human and mouse precursor peripheral blood monocytes (Sabokbar *et al.* 1998; Neale *et al.* 2000) and used to assess the response to a range of different biomaterials. It is now known that for the pre-osteoclastic monocytes to differentiate into active bone resorbing osteoclasts the cooperation of osteoblasts with monocytes is essential (Lacey *et al.* 1998). Osteoblasts are known to express a novel membrane-bound factor, RANKL. The interaction of RANKL with RANK, a receptor specific to osteoclast precursor cells, and macrophage colony stimulating factor (M-CSF), is essential (Lacey *et al.* 1998). The transformation of pre-osteoclasts into osteoclasts can be followed during *in vitro* cell culture by the expression of various factors. Early on, pre-osteoclasts are able to express tartrate resistant acid phosphatase (TRAP). Once mature, osteoclasts are able to resorb bone or any similar substrate on which they are being cultured. In the current investigation, we aim to study the ability of PMMA particles containing IDX or IHX to induce osteoclast formation in monocytes/osteoblast co-culture bone resorption assay systems. The extent of their osteoclastic reaction will be assessed in terms of TRAP expression on glass coverslips and lacunar resorption on dentine slices.

A rabbit model utilizing a bone harvest chamber has been used to assess the biocompatibility of the materials and degree of bone regrowth around the materials. This model has been used to assess the effects of biomaterials as solids and particles, including bone cement, polyethylene, titanium alloy, cobalt-chrome and hydroxyapatite particles on bone formation (Goodman *et al.* 1993, 1996, 1999; Goodman 1994; Wang *et al.* 1994).

2. MATERIALS AND METHODS

2.1. Material preparation

The particles were produced from five different samples based on Palacos bone cement (Schering-Plough AB, Stockholm, Sweden), prepared as instructed by the manufacturer. These samples were:

- (i) PMMA with no radio-contrast agent (Palacos-based PMMA; as negative control);
- (ii) PMMA with 15% (w/w) ZrO₂, which is reported (Buchhorn & Willert 2001) to be particles of 8 µm mean diameter which agglomerate to produce up to 50 µm diameter clusters (Palacos R; as positive control);
- (iii) PMMA with 10% (w/w) BaSO₄, which is reported (Buchhorn & Willert 2001) to be particles of 2 µm mean diameter which agglomerate to produce up

- to 100 μm diameter clusters (as positive control; from Stryker-Howmedica);
- (iv) PMMA with 10% (w/w) IDX mean particle size <20 μm (from Nycomed Amersham, Norway); and
- (v) PMMA with 10% (w/w) IHX mean particle size <20 μm (from Nycomed Amersham, Norway).

The cement was ground down using an ultracentrifuge mill with liquid nitrogen to embrittle the cement. Size distribution was determined by scanning electron microscopy and by laser light particle size analysis (Malvern Instruments, MS20, UK). These results demonstrated that there was a limited range of particle sizes and that 99% were less than 10 μm , with the majority (>80%) of the particles between 1 and 5 μm . To ensure that the particles were sterile and endotoxin-free they were washed three times in absolute alcohol and rinsed in MilliQ water. The absence of endotoxins in all samples was checked using E-Toxate limulus assay (Sigma-Aldrich Chemicals, UK).

2.2. In vitro studies

For cell culture, alpha minimal essential medium (MEM; Gibco, UK) was supplemented with 100 IU ml^{-1} penicillin, 10 $\mu\text{g ml}^{-1}$ streptomycin and 10 mmol L-glutamine (Gibco, UK), and 10% foetal calf serum (FCS; Gibco, UK). Recombinant mouse soluble RANKL and M-CSF were purchased from Peprotech (London, UK) and R&D Systems Europe (Abingdon, UK), respectively. All incubations were carried out at 37 °C in 5% CO_2 .

2.2.1. Preparation of monocyte cultures. The whole blood of mice was collected and diluted in culture media, and peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation technique. The PBMCs were used either to investigate the cytotoxic effect of the particles or to determine the *in vitro* effect of PMMA particles on the extent of osteoclast formation and subsequent lacunar resorption.

2.2.2. Cytotoxicity tests. For the cytotoxic assays, 10 μg of particles per millilitre of MEM/FBS were exposed to 1.5×10^5 PBMCs for 24–48 h, and then centrifuged to remove the particles and cell debris. Once prepared, the solutions were stored at –20 °C until ready for testing. Cell damage was measured by the release of the cytoplasmic enzyme lactate dehydrogenase (LDH), using a commercially available Cytotox 96 kit (Catalogue no. G1780, Promega, Madison, WI).

2.2.3. Preparation of plates for bone resorption assay. Isolated PBMCs were seeded at 1.5×10^5 cells well^{-1} onto 4 mm diameter dentine slices or 6 mm diameter glass coverslips and placed in the 7 mm diameter wells of a 96-well tissue culture plate. The cells were allowed to settle on their substrates for 2 h and then removed, washed and placed on their substrate in 24-well culture

Figure 1. Schematic of the Bone harvest chamber used. (Reproduced with permission from Aspenberg *et al.* 1988.)

plates. The MEM/FBS was further supplemented by soluble RANKL (30 ng ml^{-1}) and M-CSF (25 ng ml^{-1}). To exaggerate the cellular responses and hence facilitate the comparison between the samples, cultures were also supplemented with 10^{-8} M dexamethasone (Sigma-Aldrich Chemicals). Particle samples were re-suspended in MEM and sonicated for 10 min prior to addition to 24 well plates (at 10 $\mu\text{g ml}^{-1}$ well^{-1}). Monocyte cultures $\pm$ particles were maintained for 7 or 14 days with the supplemented media replaced every 3–4 days.

2.2.4. Detection of osteoclasts on coverslips and dentine slices. The cells on coverslips were cultured for 7 days and then were fixed in a citrate/acetone solution and stained for acid phosphatase, using naphthol AS-BI phosphate as a substrate. The product was reacted with fast garnet GBC salt in the presence of 1.0 M tartrate, and the multinucleated osteoclasts appeared red.

After 10 days in culture, dentine slices were treated with NH_4OH (1 N) for 30 min and cleaned by ultrasonication to remove adherent cells. The slices were then washed with distilled water and stained with 0.5% (w/v) toluidine blue for 3 min, before being air-dried. The surface of each dentine slice was examined for lacunar pit formation by light microscopy. The resorption was determined by calculating the percentage surface area of lacunar resorption on each dentine slice, using image analysis software (PHOTOSHOP 5.5, Adobe). Student's *t*-test was used to compare the different groups. For each treatment four dentine slices were used and each experiment was repeated at least three times in total.

2.3. In vivo studies

2.3.1. Experimental set-up. For the *in vivo* testing, the particles were sterilized with gamma irradiation. New Zealand White rabbits, over six months old, weight 3.3–4.3 kg and of mixed gender, were used for the study. Bone harvest chambers (figure 1) were implanted in both tibiae of each rabbit. Each chamber contains an inner core with a 1 mm diameter $\times$ 5 mm groove that is coaxial with the holes in the outer cylinder, providing

Figure 2. Amounts of dentine resorbed by peripheral blood mononuclear cells (PBMCs) co-cultured with no particles (control) or particles of bone cements containing different opacifiers.

a continuous canal through the chamber for tissue ingrowth. The chambers were implanted in the proximal tibiae and allowed to osseointegrate for six weeks.

Following osseointegration, 10 mg of particles of one of the four cement types (PMMA with barium sulphate was omitted from this section of the study) were mixed in a carrier of 0.4 ml hyaluronate gel (Healon, Kabi Pharmacia, Uppsala, Sweden). This dilution yielded an approximate concentration of 1×10^8 particles ml^{-1} (Goodman 1994). One cement type was added to the left tibia chamber of each rabbit and a different type added to the contralateral tibia chamber. After three weeks, tissue from both chambers was harvested and prepared for histology. The chambers were then cleaned and the test was repeated with the left-right assignment of the cement types reversed. These repeat tests allowed test reproducibility to be assessed. These series of tests were repeated for the other cement types in different combinations. Each material was implanted repeatedly four times in the left or right sides of six rabbits, giving a total of 20–22 implantations per material. In addition, 12 implantations of the carrier without cement particles were performed as controls.

2.3.2. Histological analysis. A total of 96 specimens were studied histologically. The specimens were decalcified, embedded in paraffin, cut into 6 μm sections and stained with haematoxylin and eosin. A foreign body reaction between ingrown tissue and cement particles was indicated by the presence of macrophages, giant cells, polymorphonuclear leucocytes and lymphocytes.

The amount of new bone formation was measured blindly using an imaging system (analysIS, Soft Imaging System, Munster, Germany). The percentage of bone formation was calculated as the area of

trabecular bone divided by the total area of tissue. These values were statistically analysed by ANOVA.

3. RESULTS

3.1. In vitro studies

There was no significant change in LDH release by monocytes cultured in the presence of various PMMA particles as compared with cultures with no added particles; thus suggesting that the particle concentration used for the current study was not toxic to the monocyte cultures.

Osteoclast differentiation, evidenced by the expression of TRAP and lacunar resorption, was noted in all co-cultures (figure 2). Although the number of TRAP positive multinucleated cells was not significantly different in treated wells, significant differences in the mean percentage area lacunar resorption were observed between treatments. Relative to controls (i.e. those with no added particles and those containing pure Palacos bone cement with no additives), cultures containing cement particles with added BaSO_4 and 10% IDX showed a significant increase in osteoclast formation. Similarly, PMMA particles with added ZrO_2 resulted in a significant osteoclast formation as compared with cultures with no added particles, and those cultured in the presence of PMMA particles with no additive. However, the extent of osteoclast formation in response to PMMA + ZrO_2 was markedly less than that observed in cultures incubated with PMMA + BaSO_4 .

In contrast, there were no significant differences between control cultures (PMMA alone) and cultures containing cement particles with 10% IHX. Moreover, cultures containing cement particles with added IHX showed considerably less osteoclast formation and bone resorption than cultures containing cement particles with added BaSO_4 , ZrO_2 or 10% IDX. Additionally,

Figure 3. Pictures showing normal bone formation in the Bone Harvest Chamber in the control and experimental materials at 3 weeks: (a,b) carrier alone control; (c,d) plain Palacos without radio-contrast; (e,f) Palacos R with zirconium dioxide; (g,h) Palacos R with IDX; (i,j) Palacos with IHX. (a,c,e,g,i: magnification 4 $\times$, bar=200 μ m; b,d,f,h,j: magnification 10 $\times$, bar=100 μ m).

there was no significant difference between the PMMA particles with added BaSO₄ or IDX.

3.2. In vivo studies

In the *in vivo* testing there was no infection with any of the materials. Bone was well ingrown into the channel in the bone harvest chamber (figure 3). The specimens from chambers with carrier only showed extensive woven trabecular bone, and osteoblasts without

inflammatory cells. In the other sections, some areas were occupied by the cement particles. The tissue with plain PMMA particles showed normal fibrous tissue and a few mononuclear histiocytic cells surrounding the larger particles (over 20 μ m). New trabecular bone was formed near the particles, but did not surround the particles (figure 3c,d). The newly formed trabeculae appeared similar in quality to that seen in the control sections (figure 3a,b). In most of the bone cement samples, macrophages and giant cells were

Figure 4. Macrophages and multinuclear cells associated with the particles: (a) Palacos with IDX. Macrophages surround small PMMA particles less than 10 µm (thick arrows show macrophages, thin arrows show small particles of PMMA, magnification 40×, bar=20 µm); (b) Palacos R with zirconium dioxide. The microphages surround small PMMA particles less than 10 µm (thick arrows show macrophages, thin arrows show small PMMA particles, magnification 32×, bar=20 µm); (c) Many small black particles less than 1 µm (arrows) were found in the macrophages (magnification 80×, bar=10 µm).

found surrounding the PMMA particles that were smaller than 10 µm (figure 4*a,b*). In the samples of PMMA with ZrO₂, many small black particles, less than 1 µm in diameter, could be identified; these are zirconium dioxide, which unlike PMMA, is not dissolved during processing (figure 3*e,f*). Some of these particles were located within macrophages and giant cells (figure 4*c*). A similar histological picture was noted in the sections with IDX and IHX containing cement (figure 3*g-j*). In these pictures, only a few histiocytic and lymphocytic cells were associated with small particles. Most areas show a normal tissue ingrowth. The new ingrown bone appeared to be similar in quality to that containing carrier alone. In none of the sections, with any of the materials, was there evidence of acute inflammation (leucocyte infiltrates).

In the histomorphometric analysis, there were no statistically significant differences between the left and right legs for any material. Therefore, the right and left leg data for all materials were combined. There were no significant differences ($p=0.28$) in the amount of new bone ingrowth around any of the materials (table 1).

Table 1. New bone formation with different materials in the bone harvest chambers (mean $\pm$ s.d.)

implant	number of specimens	percentage bone ingrowth (mean $\pm$ s.d.)
control	12	28.4 $\pm$ 7.1
PMMA	20	27.2 $\pm$ 7.9
PMMA + 15% ZrO ₂	22	26.6 $\pm$ 6.9
PMMA + 10% IDX	22	31.3 $\pm$ 8.4
PMMA + 10% IHX	20	26.8 $\pm$ 8.8

4. DISCUSSION

PMMA particles are thought to evoke the osteolysis which leads to joint prosthesis loosening in several ways, such as activate macrophages, which in turn stimulate osteoclastic bone resorption (Jiranek *et al.* 1993; Kadoya *et al.* 1997) and provide a precursor cell population for osteoclast formation (Udagawa *et al.* 1990; Quinn *et al.* 1992). Inflammatory macrophages associated with PMMA wear particles have been shown to be capable of osteoclast differentiation (Wimhurst *et al.* 2001).

The *in vitro* study results indicate that radiopacifying agents in bone cement may contribute to the pathological bone resorption of aseptic loosening by enhancing macrophage–osteoclast differentiation. The present findings show that BaSO₄- and IDX-containing Palacos particles are probably associated with more osteoclast formation and lacunar resorption *in vitro*, than particles of Palacos cement without any additives. Our results also suggest that particles of PMMA with 10% IHX appear to be less osteolytic than particles of PMMA containing either BaSO₄, IDX or ZrO₂. There were no significant differences between control cultures (PMMA alone) and cultures containing cement particles with 10% IHX ($p=0.42$). Moreover, cultures containing cement particles with added IHX showed considerably less osteoclast formation and bone resorption than cultures containing cement particles with added BaSO₄, ZrO₂ or 10% IDX. Additionally, there was no significant difference between the PMMA particles with added BaSO₄ or IDX ($p=0.65$ and 0.32, respectively). We are unable to explain the difference in responses to IHX and IDX seen *in vitro*, which is not repeated in the *in vivo* study. Previous clinical studies of both IHX and IDX either have not shown significant differences between the two or have shown a slightly greater adverse reactions to IHX (Sundgren *et al.* 1996; Jones & Goodall 2003).

Several *in vitro* studies indicate that PMMA particles containing BaSO₄ or ZrO₂ produce significantly greater releases of tumour necrosis factor alpha, interleukin-6 and interleukin-1 beta, and pro-osteolytic cytokine, which cause a highly significant increase in bone resorption (Sabokbar *et al.* 1996; Wimhurst *et al.* 2001; Shardlow *et al.* 2003). The mechanism of bone resorption is by pro-inflammatory cytokines inducing osteoclast differentiation from macrophages, as well the cytokines directly stimulating bone resorption (Sabokbar *et al.* 2003). Using the same preparation of

cement particles for the *in vivo* study, the histology shows foreign body reaction with macrophages around the small cement particles with or without radiopacifier. Even though the cells are around the particles, they do not stop bone ingrowth and do not influence the quality of new bone formation. The study by Lohmann *et al.* (2002) found that ZrO₂ and PMMA particles increased osteoblast proliferation and alkaline phosphatase specific activity in cell culture. This result may suggest that the different cells have different responses depending on the type of radiopacifiers.

The particles from PMMA with IDX or IHX showed better biocompatibility compared with cement with conventional radiopacifiers. The *in vitro* study shows that the particles with IDX slightly increase bone resorption compared with the response to particles with IHX. The results from the *in vivo* study indicate that PMMA particles with IDX show a trend of increasing bone formation. The histology shows no difference between the two non-ionic radiopacifying agents.

In order to compare with a clinically available bone cement, we selected Palacos R containing ZrO₂ as the positive control in the *in vivo* study. In this model, the particles from Palacos R do not influence bone formation when compared with Palacos-based PMMA without a radiopacifying agent. Furthermore, there are no significant differences in bone formation between the carrier alone and particles with or without ZrO₂ in the chamber. This result is not in agreement with a previous study that shows bone cement particles decrease the formation of new bone in a bone harvest chamber (Goodman *et al.* 1993). However, in that study, Simplex P containing BaSO₄ was used. *In vitro* studies have shown that particles containing BaSO₄ were associated with 50% more bone resorption than those containing ZrO₂ (Sabokbar *et al.* 1996). The studies by Aspenberg and colleagues (Aspenberg & van der Vis 1998; Skoglund & Aspenberg 2003) also suggest that cement particles alone are not the main reason for the bone resorption, but that the combination of particles and fluid pressure is important for the induction of osteolysis.

The inflammatory cells surrounding small PMMA particles (less than 10 µm, especially with ZrO₂) are probably associated with phagocytosed particles (figure 4b,c). However, this reaction did not influence bone formation, which is the response measured by the bone chamber model. If the macrophages could not remove the particles once the bone had matured, these particles may have attracted more macrophages and released inflammatory mediators, or differentiated the particle-associated macrophages into bone-resorbing osteoclasts, and this situation may have caused later bone resorption (Keen *et al.* 1992; Tomazie-Jezic *et al.* 2001). Thus, the difference between the *in vitro* and *in vivo* studies reported here is that the *in vitro* study measured bone resorption initiated by the particles, while the *in vivo* study measured whether new bone formation was affected by the presence of the particles. In the clinical situation, these two effects will be occurring in parallel, although on different levels.

These results are encouraging, as they indicate that alternative radiopacifying agents could be employed.

The lack of adverse cellular reaction would also support a potential use not only in clinical diagnosis, such as angiography and CT enhancements, but also that these iodine-based compounds could be considered for use in other biomaterials for orthopaedic re-constructive surgery.

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REFERENCES

- Adams, D. O. 1976 The granulomatous inflammatory response: a review. *Am. J. Pathol.* **84**, 164–191.
- Almén, T. 1995 Visipaque—a step forward. A historical review. *Acta Radiol. Suppl.* **399**, 2–18.
- Artola, A., Gurruchaga, M., Vázquez, B., San Roman, J. & Goñi, I. 2004 Elimination of barium sulphate from acrylic bone cements. Use of two iodine containing monomers. *Biomaterials* **24**, 4071–4080.
- Aspenberg, P. & van der Vis, H. 1998 Migration, particles, and fluid pressure. A discussion of causes of prosthetic loosening. *Clin. Orthop. Rel. Res.* **352**, 75–80.
- Aspenberg, P., Albrektsson, T., Lohmander, L. S. & Thorngren, K. G. 1988 Drug test chamber: a titanium implant for administration of biochemical agents to a standardized bone callus *in situ*. *J. Biomed. Eng.* **10**, 70–73.
- Buchhorn, G. H. & Willert, G.-H. 2001 Wear and osteolysis. *Bone cement and cementing techniques* (ed. G. H. I. M. Gallenkamp & D. W. Murray), pp. 163–179. New York: Springer.
- Cavaria, L., Dowson, D., Fisher, J. & Jobbins, B. 1990 The influence of bone and bone cement debris on counterface roughness in sliding wear tests of ultra-high molecular weight polyethylene on stainless steel. *Proc. Inst. Mech. Eng. H* **204**, 65–70.
- Chan, K. H. & Ahmed, A. M. 1991 Polymethylmethacrylate. *Joint replacement arthroplasty* (ed. B. F. Morrey), pp. 23–37. New York: Churchill Livingstone.
- Davenport, D., Cohen, M. D., Hanna, M. P., Bugaieski, E. & Heifetz, S. A. 1999 Studies of iodixanol in the rabbit lung and peritoneum. *Pediatr. Radiol.* **29**, 724–730.
- Davy, K. M. W., Anseau, M. R., Odlyha, M. & Foster, G. M. 1997 X-ray opaque methacrylate polymers for biomedical applications. *Polym. Int.* **43**, 143–154.
- Department of Health Research and Development Directorate, Biomaterials and Implants Advisory Group 1995 R&D priorities for biomaterial and implants report, p. 7. London: Department of Health.
- Fujikawa, Y., Quinn, J. M. W., Sabokbar, A. S., McGee, J. O'D. & Athanasou, N. A. 1996 The human mononuclear osteoclast precursor circulates in the monocyte fraction. *Endocrinology* **139**, 4058–4060.
- Ginebra, M. P., Albuixech, L., Fernandez-Barragan, E., Aparicio, C., Gil, F. J., San Roman, J., Vazquez, B. & Planell, J. A. 2002 Mechanical performance of acrylic bone cements containing different radiopacifying agents. *Biomaterials* **23**, 1873–1882.
- Goodman, S. B. 1994 The effects of micromotion and particulate materials on tissue differentiation. Bone chamber studies in rabbits. *Acta Orthop. Scand. Suppl.* **258**, 65.

- Goodman, S. B., Aspenberg, P., Wang, J.-S., Song, Y., Doshi, A., Regula, D., Emmanual, J. & Lidgren, L. 1993 Cement particles inhibit bone-growth into titanium chambers implanted in the rabbit. *Acta Orthop. Scand.* **64**, 627–633.
- Goodman, S. B., Aspenberg, P., Song, Y., Regula, D. & Lidgren, L. 1996 Polyethylene and titanium alloy particles reduce bone formation. Dose-dependence in bone harvest chamber experiments in rabbits. *Acta Orthop. Scand.* **67**, 599–605.
- Goodman, S. B., Song, Y., Chun, L., Regula, D. & Aspenberg, P. 1999 Effects of TGFbeta on bone ingrowth in the presence of polyethylene particles. *J. Bone Joint Surg.* **81-B**, 1069–1075.
- Isaac, G. H., Atkinson, J. R., Dowson, D., Kennedy, P. D. & Smith, M. R. 1987 The cause of femoral head roughening in explanted Charnley hip prostheses. *Eng. Med.* **16**, 167–173.
- Jiranek, W. A., Machado, M., Jasty, M., Jevsevar, D., Wolfe, H. J., Goldring, S. R., Goldberg, M. J. & Harris, W. H. 1993 Production of cytokines around loosened cemented acetabular components: analysis with immunohistochemical techniques and *in situ* hybridization. *J. Bone Joint Surg.* **75-A**, 863–879.
- Jones, C. I. & Goodall, A. H. 2003 Differential effects of the iodinated contrast agents ioxaglate, iohexol and iodexanol on thrombus formation and fibrinolysis. *Thromb. Res.* **112**, 65–71.
- Kadoya, Y., Revell, P. A., Kobayashi, A., Al Saffar, N., Scott, G. & Freeman, M. A. R. 1997 Wear particulate species and bone loss in failed total joint arthroplasties. *Clin. Orthop. Rel. Res.* **340**, 118–129.
- Keen, C. E., Philip, G., Brady, K., Spencer, J. D. & Levison, D. A. 1992 Histopathological and microanalytical study of zirconium dioxide and barium sulphate in bone cement. *J. Clin. Pathol.* **45**, 984–989.
- Kjellson, F., Wang, J.-S., Almén, T., Mattson, A., Klaveness, J., Tanner, K. E. & Lidgren, L. 2001 Tensile mechanical properties of a bone cement containing non-ionic contrast media. *J. Mater. Sci. Mater. Med.* **12**, 889–894.
- Kjellson, F., Almén, T., Tanner, K. E., McCarthy, I. D. & Lidgren, L. 2004 Bone cement X-ray contrast media: a clinically relevant method of measuring their efficacy. *J. Biomed. Mater. Res B* **70**, 354–361.
- Lacey, D. L. *et al.* 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* **93**, 165–176.
- Lazarus, M. D., Cuckler, J. M., Schumacher, H. R., Ducheyne, P. & Baker, D. G. 1994 Comparison of the inflammatory response to particulate polymethylmethacrylate debris with and without barium sulfate. *J. Orthop. Res.* **12**, 532–541.
- Lohmann, C. H., Dean, D. D., Koster, G., Casasola, D., Buchhorn, G. H., Fink, U., Schwartz, Z. & Boyan, B. D. 2002 Ceramic and PMMA particles differentially affect osteoblast phenotype. *Biomaterials* **23**, 1855–1863.
- Malchau H., Herberts P., Garellick G., Söderman P., Eisler T. 2002 Prognosis of total hip replacement. Report of the Swedish Hip Register, Scientific Exhibit at the 69th meeting of the American Academy of Orthopaedic Surgery, Dallas. See www.jru.orthop.gu.se.
- Nakamura, M., Uozumi, J., Soejima, K., Kanou, T., Tokuda, N. & Naito, S. 2003 Inhibition of gluconeogenesis and *p*-aminohipuric acid accumulation in rat renal cortical slices by ionic and nonionic contrast media. *Clin. Exp. Nephrol.* **7**, 98–103.
- Neale, S. D., Haynes, D. R., Howie, D. W., Murray, D. W. & Athanasou, N. A. 2000 The effect of particle phagocytosis and metallic wear particles on osteoclast formation and bone resorption *in vitro*. *J. Arthroplasty* **15**, 654–662.
- Quinn, J., Joyner, C., Triffitt, J. T. & Athanasou, N. A. 1992 Polymethylmethacrylate-induced inflammatory macrophages resorb bone. *J. Bone Joint Surg.* **74**, 652–658.
- Royal College of Surgeons 2000 Audit Commission Report on 'National hip replacement outcome study'. See www.rcseng.ac.uk.
- Sabokbar, A., Fujikawa, Y., Brett, J., Murray, D. W. & Athanasou, N. A. 1996 Increased osteoclastic differentiation by PMMA particle-associated macrophages— inhibitory effect by interleukin 4 and leukemia inhibitory factor. *Acta Orthop. Scand.* **67**, 593–598.
- Sabokbar, A., Fujikawa, Y., Murray, D. W. & Athanasou, N. A. 1997 Radio-opaque agents in bone cement increase bone resorption. *J. Bone Joint Surg.* **79**, 129–134.
- Sabokbar, A., Pandey, R., Quinn, J. & Athanasou, N. A. 1998 Osteoclastic differentiation by mononuclear phagocytes containing biomaterial particles. *Arch. Orthop. Traum. Surg.* **184**, 31–36.
- Sabokbar, A., Kudo, O. & Athanasou, N. A. 2003 Two distinct cellular mechanisms of osteoclast formation and bone resorption in periprosthetic osteolysis. *J. Orthop. Res.* **21**, 73–80.
- Shardlow, D. L., Stone, M. H., Ingham, E. & Fisher, J. 2003 Cement particles containing radio-opacifiers stimulate pro-osteolytic cytokine production to form a human monocytic cell line. *J. Bone Joint Surg.* **85**, 900–905.
- Skoglund, B. & Aspenberg, P. 2003 PMMA particles and pressure—a study of the osteolytic properties of two agents proposed to cause prosthetic loosening. *J. Orthop. Res.* **21**, 196–201.
- Sundgren, P. C., Baath, L., Tornquist, C., Hougens Grynne, B., Kjaersgaard, P. & Almen, T. 1996 Image quality and safety after iodixanol in intravenous urography; a comparison with iohexol. *Br. J. Radiol.* **69**, 699–703.
- Swedish Knee Arthroplasty Register, Annual Report 2003. See www.ort.lu.se/knee.
- Tomazic-Jezic, V. J., Merritt, K. & Umbreit, T. H. 2001 Significance of the type and the size of biomaterial particles on phagocytosis and tissue distribution. *J. Biomed. Mater. Res.* **55**, 523–529.
- Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Nishihara, Koga, T., Martin, T. J. & Suda, T. 1990 Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proc. Natl Acad. Sci. USA* **87**, 7620–7624.
- van Hooy-Corsjens, C. S. J., Govaert, L. E., Spoelstra, A. B., Bulstra, S. K., Wetzels, G. M. R. & Koole, L. H. 2004 Mechanical behaviour of a new acrylic radiopaque iodine-containing bone cement. *Biomaterials* **25**, 2657–2667.
- Wang, J.-S., Goodman, S. B. & Aspenberg, P. 1994 Bone formation in the presence of phagocytosable hydroxyapatite particles. *Clin. Orthop. Rel. Res.* **304**, 272–279.
- Wimhurst, J., Brooks, R. & Rushton, N. 2001 The effects of particulate bone cement at the bone-implant interface. *J. Bone Joint Surg.* **83**, 588–592.