We used a rat model in vivo to study the effects of particulate bone cements at the bone-implant interface. A ceramic pin was implanted into the tibiae of 48 rats. Three types of particle of clinically relevant size were produced from one bone-cement base without radio-opacifier, with zirconium dioxide (ZrO₂) and with barium sulphate (BaSO₄). The rats were randomly assigned to four groups to receive one of the three bone cements or normal saline with 2% v/v Sprague-Dawley serum as the control. A total of 10⁹ particles was injected into the knee at 8, 10 and 12 weeks after the original surgery. The animals were killed at 14 weeks and the tibiae processed for histomorphometry. The area of fibrous tissue and the gap between the implant and bone were measured using image analysis.

All three types of particle were associated with a larger area of bone resorption than the control. Only in the case of the BaSO₄-containing cement did this reach statistical significance (p = 0.01). Particles of bone cement appear to promote osteolysis at the bone-implant interface and this effect is most marked when BaSO₄ is used as the radioopaque agent.

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Aseptic loosening is the commonest cause of revision of total joint replacements and is responsible for 79% of failures of total hip replacements. The pathogenesis of the process remains controversial, although migration of the prosthesis, the host response to particulate wear debris and joint fluid pressure have all been suggested as mechanisms. It seems most likely that all three are involved, although the relative contribution of each is yet to be clarified. Central to all theories of aseptic loosening is the development of a ‘synovial-like membrane’ between the implant and bone, first characterised by Goldring et al. This tissue has been shown to stimulate bone resorption when cultured in vitro and macrophages and giant cells present within the membrane are implicated in this process. Cells from the membrane have been shown to produce cytokines such as tumour necrosis factor α, interleukin-1β, interleukin-6 and prostaglandin E₂ which have been linked to bone resorption.

Wear particles of bone cement may be produced by adhesion, abrasion or fatigue. Iwaki et al. characterised the size, shape and number of bone-cement particles in the membrane surrounding total joint replacements which were loose. They found that the size of the particles had a median equivalent circle diameter (ECD) of between 0.65 and 1.51 μm. The ECD is the diameter of a circle having the same area as the measured feature. Most of the particles were less than 10 μm and 67% were submicrometre in size. Their shape was assessed by the aspect ratio (AR), the ratio of the length to the breadth, which had a mean of 1.38. The number of bone-cement particles per gram of tissue taken from around the loose joint replacements ranged from 2 × 10⁷ to 2 × 10⁸/ml. Work from the same group has shown that the size of the osteolytic defect is proportional to the number of wear particles present.

Several proprietary bone cements are available commercially. Data from the Swedish Hip Registry have shown that the type used has an effect on the risk of revision for aseptic loosening. Palacos with gentamicin is the lowest risk, followed by Palacos without gentamicin, Simplex and CMW, the highest risk being the use of Sulfix. Palacos and Sulfix contain ZrO₂ as the radioopaque agent while Simplex and CMW have BaSO₄. It has been suggested by Sabokbar et al. that the type of radio-opacifier contained within bone cements may be responsible for the differences in their ability to stimulate bone resorption in vitro. We have therefore examined the effect of radioopaque agents in bone cement on an in vivo model which we have previously used to demonstrate periprosthetic bone resorption in response to particles of polyethylene, metal alloys and ceramic.
Materials and Methods

Production of particles. The particles were produced from plain Palacos (Schering-Plough, Welwyn, UK) without radio-opacifier, Palacos with 15.6% w/w ZrO₂ and Palacos with 15.6% w/w BaSO₄. The bone cements were polymerised according to manufacturer’s instructions. They were cut into 2 cm blocks, mounted on a Rotoforce-3 specimen mover (Struers, Rodovre, Denmark) and counter-rotated at 300 rpm and 30 N against a 20 µm diamond grinding disc on a Rotopol-21 grinding/polishing machine (Struers) for four hours with constant irrigation of glycerol as lubricant. The particles thus produced, suspended in glycerol, were collected in sterile containers, vortex-mixed with endotoxin-free water and centrifuged at 3500 g for five minutes. This wash process was repeated six times for each sample. The particles were suspended in the smallest volume of endotoxin-free water possible (approximately 3 ml) and dried for one hour at 40°C. Approximately 1 g of particles of each type of bone cement was produced by this method. Contamination from the manufacturing process was investigated using energy-dispersive x-ray analysis (EDXA) and from bacterial colonisation by 72-hour microbiological culture. The Limulus amoebocyte lysate assay (Bio*Whittaker, Wokingham, UK) was performed to determine endotoxin contamination. Particles were sterilised by Isotron plc (Swindon, UK) with gamma irradiation at 25 kG/2.5 Mrad.

Operative technique and injection of particles. We randomly allocated 48 adult, male, Sprague-Dawley rats (Harlan UK Ltd, Bicester, UK) with a mean weight of 421.8 g (SD 25.7 g), to one of four groups (n = 12). Aluminium oxide pins (Precision Ceramics, Birmingham, UK) were implanted into the right tibia of each animal according to the method of Allen et al. Every effort was made to ensure that the pin head lay flush with the tibial plateau at the end of the operation. Postoperative analgesia was provided with an immediate subcutaneous injection of Temgesic (0.15 mg/kg) which was repeated six hours later.

Particles of the three types of bone cement were suspended in 2% v/v male Sprague-Dawley serum in 0.9% w/v saline solution at a concentration of 10¹⁰ particles/ml. The first group of rats received intra-articular injections of 100 µl (10⁹ particles) of the particle suspension of plain Palacos into the operated knee at 8, 10 and 12 weeks after implantation of the pin. The second group received Palacos with BaSO₄ particles and the third group Palacos with ZrO₂. The fourth group was given injections of 100 µl of 2% v/v Sprague-Dawley serum as a control. All the animals were killed two weeks after their last injection and two samples of synovium from the operated knee of each rat were removed under aseptic conditions, placed in separate bottles of brain heart broth (Difco Laboratories, West Moseley, UK) and cultured at 37.5°C for 72 hours.

Tissue preparation. The operated tibiae were removed en bloc and fixed in ice-cold 4% w/v paraformaldehyde (Sigma, Poole, UK) in 0.1 M phosphate buffer (pH 7.4) containing 0.1% w/v sucrose and 0.05% w/v gluteraldehyde for five hours at pH 7.4. They were then washed in ice-cold phosphate-buffered saline and dehydrated through a graded series of solutions of alcohol. Tissues were defatted in xylene (Merck, Poole, UK) and infiltrated in methylmethacrylate (Sigma) resin at 4°C for seven days under vacuum. The resin was polymerised overnight in glass bijoux bottles at 30°C under vacuum. Longitudinal sections, 250 µm thick, were cut through the bone and the length of the pin using a diamond saw (Accutom 5; Struers, Glasgow, UK) and sections were ground on a 40 µm diamond grinding pad and polished using 6 µm and 1 µm diamond paste.

Histomorphometry. The sections were washed in methanol and surface stained using 0.25% w/v Toluidine Blue, at pH 9 at 56°C for 30 minutes, so that structures in the surface of the section were made visible. Histomorphometry was carried out on a single section from each animal using image analysis (PC Image) without previous knowledge of the type of injection which had been given. The total area of the gaps between pin and bone, including any fibrous tissue, was measured around each pin. We have previously shown that this reliably represents serial sections through the tibia.

Statistical methods. The total area of the gaps around the pins in each of the four groups was tested for normality using the Shapiro-Wilk method. A one-way analysis of variance (ANOVA) was performed to ascertain whether there was a difference between the four groups, and post-hoc testing using Tukey’s method was performed to isolate where any differences lay. All statistical analysis was performed using SPSS Base 8.0 for Windows (SPSS Inc, Chicago, Illinois).

Results

Particles. Over 2000 particles of each type were measured. All three groups had mean ECDs between 1.22 and 1.32 µm, almost half were submicrometre in size and none exceeded 6 µm. The results of image analysis of the three types of particle are shown in Table I. Figure 1 shows the distribution of the size of the particles (ECD in µm) for

| Particle | Number | ECD (µm) | AR
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<tbody>
<tr>
<td>Plain</td>
<td>2095</td>
<td>1.30 ± 0.76</td>
<td>1.40 ± 0.37</td>
</tr>
<tr>
<td>BaSO₄</td>
<td>2305</td>
<td>1.32 ± 0.72</td>
<td>1.38 ± 0.30</td>
</tr>
<tr>
<td>ZrO₂</td>
<td>2186</td>
<td>1.22 ± 0.88</td>
<td>1.55 ± 0.53</td>
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Palacos without a radiopaque additive. The distribution of size for the other two types of particle was similar.

All three types of particle were found to be free from contaminants from the manufacturing process by EDXA. There was no evidence of endotoxin contamination of any of the particles on using the *Limulus* amoebocyte lysate assay.

**Operative technique and injection of particles.** There was no significant morbidity and no mortality during or after the operation. All 48 rats were weight-bearing on the operated limb within 48 hours of surgery and within minutes of each injection of particles. There was no clinical evidence of any wound infections.

**Tissue retrieval and culture.** Nine pins were seen to have sunk below the level of the tibial plateau and had been covered by a layer of bone such that they were excluded from the cavity of the joint. Four of these were in the ‘plain’ group, one in the *ZrO₂* group and two each in the control and *BaSO₄* groups. The remaining 39 pins were all well positioned and none was associated with any damage to the femoral condyles.

Synovial tissue from four knees grew bacteria on microbiological culture. Two of the four came from the control group and two from the *ZrO₂* group. In all four cases the growth was from only one of the two samples taken and the bacteria identified (enterococci or diphtheroids) were con-
considered to be contaminants from the retrieval process. They were not associated with histological evidence of infection or gap areas which were above average for their groups.

**Histomorphometry.** We assessed all 48 pins. The sunken pins, regardless of injection group, were associated with complete bony apposition to the pin and the absence of fibrous tissue or a gap at the bone-implant interface. They were therefore excluded from further analysis. The gap areas of all four groups were found to be normally distributed by Shapiro-Wilk testing. The smallest mean area of the gap (Fig. 2) was in the saline control injection group in which there was generally good apposition of bone to the pin (Fig. 3). The BaSO\(_4\) group had the largest mean gap area and some pins from this group were almost completely surrounded by large lesions (Fig. 4). Cells within the fibrous layer were seen to contain vacuoles (Fig. 5) which may represent particles of bone cement that have been dissolved by the organic solvents used in the tissue preparation. This phenomenon has been recognised in human retrieval studies.\(^{16}\)

There was a statistically significant difference between the four groups by ANOVA (\(p = 0.02\)) but only the BaSO\(_4\) group had a significantly larger gap area than the control group on post-hoc testing using Tukey’s method (\(p = 0.01\)). While failing to reach statistical significance, there was a distinct trend of reducing similarity between the ‘plain’ and ZrO\(_2\) group (\(p = 0.887\)), the ZrO\(_2\) and BaSO\(_4\) groups (\(p = 0.357\)) and the BaSO\(_4\) and plain groups (\(p = 0.13\)).

**Discussion**

This study was designed to investigate the effects of particles of bone cement of clinically relevant size on the bone-implant interface using an intra-articular, in vivo model. In a previous study,\(^{14}\) we found that leaving the head of the pin proud of the tibial plateau could result in damage to the femoral condyles, although this did not appear to affect the size of the gap around the pin. In an effort to avoid femoral lesions, we attempted to seat the pins flush with the tibial plateau in this study and as a result, nine pins migrated into the tibial medullary cavity, becoming sealed off from the knee. All of these pins were associated with complete bony apposition showing that in order to function effectively as a model of aseptic loosening, the pin must be intra-articular.

Only particles of bone cement containing BaSO\(_4\) caused a significantly larger gap around the pin than the control. Both plain particles and those containing ZrO\(_2\) produced larger gaps than the control but these did not reach statistical significance.

The bone-cement particles were very similar in size and shape to those retrieved from the membrane around loose joint replacements.\(^9\) To allow the effect of the radio-opacifiers to be assessed, particles were produced from one ‘base’ cement, Palacos. The presence or absence of radio-opaque agents and the type used were the only differences between the three test cements. This is important as proprietary bone cements differ in more than just the type of...
radio-opaque material which they contain. Palacos is a methylmethacrylate copolymer, with chlorophyll added to give it a green colour. CMW is a powdered polymethylmethacrylate (PMMA) polymer, Simplex is a mixture of PMMA and methylmethacrylate-styrene copolymers and Sulfix is a copolymer of PMMA and polybutylmethacrylate. CMW and Simplex have BaSO\(_4\) to make them visible on radiography while Palacos and Sulfix have ZrO\(_2\).

Previous studies in vitro investigating the effects of different radio-opacifiers on bone resorption have used proprietary preparations and while this approach accurately represents the clinical situation, the type of radio-opacifier is not the only variable.\(^1\)\(^2\)\(^13\)

Lazarus et al.\(^18\) compared the effects of particles of bone cement with and without BaSO\(_4\) on a rat subcutaneous pouch model and on the intramedullary PMMA rod originally described by Howie et al.\(^21\) They found an increase in the inflammatory response and more bone resorption with the cement which contained BaSO\(_4\). Sabokbar et al.\(^18\) in their in vitro bone resorption assay, found that CMW caused significantly more bone resorption than Palacos and that both caused more bone resorption than a cement with no radioopaque additives. Our study adds support to their theory that it is the BaSO\(_4\) in CMW which is responsible for the increased bone resorption as compared with Palacos and also accords with the clinical performance of these two bone cements as reported in the Swedish Hip Registry.\(^11\) The situation is complicated by the fact that Sulfix, which contains ZrO\(_2\), has an inferior clinical performance compared with both Palacos and CMW in Sweden.\(^11\)

We suggest that the various material and mechanical differences between the proprietary bone cements contribute to differences in their clinical performances. When the only difference between the cements is the type of radio-opacifier contained, BaSO\(_4\) appears to be disadvantageous. Merely switching the radiopaque agent used in all bone cements to ZrO\(_2\) may not, however, be the solution since Caravia et al.\(^19\) have shown, in their sliding wear tests, that ZrO\(_2\) causes more surface damage than BaSO\(_4\) and can thus potentially cause more abrasive wear. It may be that a return to the use of radiolucent bone cements should be considered. Alternatively, the development of different radiopaque agents could be pursued providing that no secondary disadvantage is introduced.

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References


