NUMBER OF POLYETHYLENE PARTICLES AND OSTEOLYSIS IN TOTAL JOINT REPLACEMENTS
A QUANTITATIVE STUDY USING A TISSUE-DIGESTION METHOD


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Our aim was to analyse the influence of the size, shape and number of particles on the pathogenesis of osteolysis. We obtained peri-implant tissues from 18 patients having revision surgery for aseptically loosened Freeman total knee replacements (10), Charnley total hip replacements (3) and Imperial College/London Hospital double-cup surface hip replacements (5). The size and shape of the polyethylene particles were characterised using SEM and their concentration was calculated. The results were analysed with reference to the presence of radiological osteolysis.

The concentration of polyethylene particles in 6 areas with osteolysis was significantly higher than that in 12 areas without osteolysis. There were no significant differences between the size and shape of the particles in these two groups.

We conclude that the most critical factor in the pathogenesis of osteolysis is the concentration of polyethylene particles accumulated in the tissue.

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MATERIALS AND METHODS

In 18 patients we studied the interface tissues adjacent to the tibial components of ten Freeman-type conforming tibiofemoral total knee replacement prostheses, the acetabular components of three Charnley total hip replacements (THR), and five Imperial College/London Hospital double-cup surface hip replacements (ICLHDC). All were revised for aseptic loosening. Details of the prostheses and the use of cement fixation are shown in Table I.

The polyethylene particles were extracted according to the method of Campbell et al. To avoid contamination by extraneous particles, all the solutions used, including distilled water, had been previously filtered through a 0.1 μm pore size nylon filter (150-0101; Nalge, Rochester, New York).
The weight of the tissues and volume of the applied solutions were accurately measured for the calculation of the concentration of polyethylene particles. Interface tissues were digested by 5N sodium hydroxide at 65°C for one hour. The digested sample was applied to a sucrose-density gradient (5%, 10%, 20% and 50%) and ultracentrifuged at 40 000 rpm (106 000 g) at 2°C for three hours (TL-100, TLS-55 rotor; Beckman Instruments Inc, Palo Alto, California). The top layer was collected and hot distilled water was added to dilute the sucrose. After ultrasonication for ten minutes the solution was applied to two layers of isopropanol-water mixture (density: 0.90 and 0.96 Mg m\(^{-3}\)), and ultracentrifuged again for one hour at 20°C. The particles were visible as a thin white band at the interface of the two layers. They were collected and distilled water added to allow appropriate dilution for homogeneous dispersal on the filter during filtration through a 0.1 μm polycarbonate filter (7060-1301; Whatman Inc, Clifton, New Jersey). The filter was dried, attached to a carbon stub (3347; Agar Scientific Ltd, Stansted, UK), and coated with gold for SEM analysis (JEOL 6300, 6300F, Tokyo, Japan). The SEM photographs were analysed by a computerised image analyser (Quantimet 570, Leica Ltd, Cambridge, UK) (Fig. 1). At least 100 particles were counted in the SEM photographs for each sample. Particle size was defined by the equivalent circle diameter (ECD) which is the diameter of a circle having the same area as the measured feature. The particle shape was determined by the aspect ratio (length/breadth) and roundness (perimeter\(^2\) / \(4\pi \times \text{area}\)).

Table I. Details of the 18 patients with either TKR or THR and particle characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Operation</th>
<th>Prosthesis*</th>
<th>Diagnosis</th>
<th>Fixation†</th>
<th>Proximal</th>
<th>Distal</th>
<th>Osteolysis</th>
<th>Duration (mth)</th>
<th>ECD (μm)</th>
<th>Aspect ratio</th>
<th>Roundness</th>
<th>Number tissue x 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THR</td>
<td>ICLHDC</td>
<td>OA</td>
<td>+C</td>
<td>+C</td>
<td>--</td>
<td>--</td>
<td>216</td>
<td>0.62</td>
<td>1.70</td>
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<td>+C</td>
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<td>--</td>
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<td>+C</td>
<td>--</td>
<td>--</td>
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<td>3.44</td>
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<td>+C</td>
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<td>--</td>
<td>190</td>
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<td>+C</td>
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<td>2.26</td>
<td>2.86</td>
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<td>+C</td>
<td>--</td>
<td>--</td>
<td>234</td>
<td>0.81</td>
<td>2.05</td>
<td>2.96</td>
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<tr>
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<td>OA</td>
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<td>+C</td>
<td>--</td>
<td>+</td>
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<td>0.94</td>
<td>2.13</td>
<td>3.95</td>
<td>7.30</td>
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<td>+C</td>
<td>--</td>
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<td>9</td>
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<td>OA</td>
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<td>-C</td>
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<td>-C</td>
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<td>OA</td>
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<td>+C</td>
<td>--</td>
<td>--</td>
<td>49</td>
<td>0.86</td>
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<td>OA</td>
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<td>-C</td>
<td>+</td>
<td>132</td>
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<td>+C</td>
<td>--</td>
<td>--</td>
<td>159</td>
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<td>1.97</td>
<td>2.56</td>
<td>1.23</td>
</tr>
<tr>
<td>17</td>
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<td>F/S</td>
<td>RA</td>
<td>-C</td>
<td>-C</td>
<td>--</td>
<td>144</td>
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<td>--</td>
<td>160</td>
<td>0.83</td>
<td>1.61</td>
<td>1.50</td>
<td>3.43</td>
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</table>

* ICLHDC, Imperial College/London Hospital double cup surface hip replacements; F/S, Freeman-Samuelson TKR; F/SW, Freeman-Swanson TKR; ICLH, Imperial College/London Hospital THR
† +C = cemented; -C = uncemented
+ equivalent circle diameter (see text)

Fig. 1
SEM of polyethylene particles (case 12 in Table I).

Fig. 2
Particle size distribution for case 18 (Table I); 75% of the particles were less than 1 μm.
The purity of the extracted polyethylene particles was validated by Fourier transform infrared spectrometry (FTIR), energy-dispersive X-ray analysis (EDAX) and a control study using capsule tissue retrieved at a primary TJR.

A control study using commercially-available high-density polyethylene powder of mean particle size 3.5 μm (S-395, Shamrock, New Jersey) and normal capsule tissue allowed us to determine the polyethylene particle retrieval ratio of this extraction method.

The number of particles per gram of wet interface tissue was calculated as follows. The number of particles on the filter face (i.e. the number of particles present in a known volume of the final suspension) was counted by multiplying the average number of particles seen per unit area of the SEM photographs by the area of the filter face. The number of particles extracted could then be related to the wet weight of tissue which originally contained them since all the dilutions and the extraction ratio of the particles were known.

Cases were classified as being ‘osteolysis-positive’ if an osteolytic lesion was seen at the interface preoperatively, or as ‘osteolysis-negative’ when no such lesion was seen. Osteolysis was defined as cystic, progressive bone erosion at the interface, not delimited by a line of sclerotic reactive new bone. In osteolysis-positive cases the interface soft tissue was taken only from the lesion. In osteolysis-negative cases all the retrievable interface soft tissues were pooled for this study.

We evaluated the results in groups of TKRs and THRs, and also in the groups according to the presence or absence of osteolysis using the Mann-Whitney U test and a statistical software package (StatView 4.5, Abacus Concepts Inc, Berkeley, California).

RESULTS

Purity of retrieved polyethylene particles. FTIR showed a typical spectrum of polyethylene and EDAX revealed that there was less than 0.02% contamination with other particles (metal, polymethylmethacrylate or bone). It was also confirmed that in the control study using capsule from a primary TJR less than 0.02% of the particulate debris was contaminated with organic material. We concluded that most of the particles extracted were polyethylene.

Size, shape and number of polyethylene particles (Table I). An example of the distribution of particle size (case 18, Table I) is shown in Figure 2. Although the values of the ECD, aspect ratio and roundness in each patient had skewed distributions, we were able to use the mean as a representative value for each patient because of the central limited theorem. The ECDs for all patients ranged from 0.48 to 1.32 μm (mean ± SEM 0.82 ± 0.06 μm, median 0.82 μm). The aspect ratio ranged from 1.61 to 2.33 (mean 1.96 ± 0.05, median 1.99), and roundness from 1.50 to 3.95 (mean 2.23 ± 0.16, median 2.05). The retrieval ratio of polyethylene particles in the control study was 40.0% (mean of three measurements). Based on this value, the range of concentration of particles was calculated as 0.52 × 10^9 to 91.7 × 10^9/g wet tissue (mean 14.2 × 10^9 ± 5.41 × 10^9, median 7.01 × 10^9).

Comparison between THR and TKR (Table II). No significant difference in size (ECD) (p = 0.79, Fig. 3), aspect ratio or roundness was observed between the particles retrieved from THR and TKR. By contrast, the number of particles accumulated in TKR was significantly greater than that in THR (p = 0.03, Fig. 3).

Comparison between TKRs with an un cemented all-polyethylene tibial component and a cemented tibial component. In six of ten TKR patients with all-polyethylene uncemented tibial components, the number of particles accumulated in the interface tissue was significantly greater than in patients with cemented tibial components (uncemented, mean 34.1 × 10^9 ± 32.4 × 10^9, median 19.7 × 10^9; cemented, 4.57 × 10^9 ± 2.74 × 10^9, median 5.09 × 10^9; p = 0.01).

Comparison between osteolysis-positive and osteolysis-negative groups (Table III). The number of particles in osteolysis-positive lesions was significantly greater than that in osteolysis-negative lesions (p = 0.002, Fig. 4). By contrast, there was no significant difference in the ECD between these two groups (p = 0.81, Fig. 4). The difference in particle shape (aspect ratio and roundness) was also not statistically significant.

DISCUSSION

There have been a number of reports of the deleterious effect of particles and in this study we have focused on the influence of polyethylene particles. Their presence can be detected under polarised light and with Oil Red O staining. Most, however, are too small to be characterised by light microscopy. It was necessary to use higher-resolution methods, such as extraction and characterisation by SEM.

Reported results of the extraction and characterisation of...
Polyethylene particles from interface tissues have identified two technical pitfalls. First, Campbell et al. pointed out the problem of contamination with organic debris during extraction. The method which we used was ultracentrifugation in a sucrose gradient which has been reported to have the least contamination. Secondly, contamination with organic debris may interfere with the accuracy of auto-analysers used to characterise particles. We have therefore characterised particles seen on SEM by the objective parameters of ECD, aspect ratio and roundness.

The most important point in our study is that we have calculated the concentration of polyethylene particles as the number per gram of wet weight of interface tissue. This was possible because the extraction procedure was based on a known wet weight of tissue and all subsequent dilutions were controlled. In addition, we carried out a control study with measured amounts of polyethylene particles and capsule tissues to determine the retrieval ratio of our extraction technique. This ratio may not be absolutely accurate because most of the polyethylene particles in the experimental tissues were much smaller (less than 1 μm) than those used in the control study (3.5 μm). It is possible that these smaller particles may be more adhesive to the surface of the experimental devices (e.g. tubes and pipettes) resulting in incomplete retrieval. Although this represents a criticism of our control procedure, we do not believe that it invalidates our comparisons between osteolysis-positive and osteolysis-negative cases since the tendency to under-extract small particles would have been the same in both. This view is supported by our finding that the distribution of particle size was the same in both groups.

There are some limitations in this retrieval study. First, the number of patients was relatively small and it is necessary to examine more patients to make a proper comparison of wear characteristics in particular prostheses (THR vs. TKR). As far as the difference in macrophage recruitment and osteolysis is concerned, however, we believe that the number of specimens (18) was sufficient to draw conclusions as this is a universal reaction. Secondly, all the specimens were from revision patients who had aseptic loosening. It is necessary to examine wear particles in well-functioning patients to detect the wear characteristics of a particular prosthesis.

In interpreting the results it is important to take the design characteristics into account. We used the tissues around three types of prosthesis: the Charnley hip prosthesis which has a small (22 mm) diameter head and which has been reported to have the least contamination. Secondly, contamination with organic debris may interfere with the accuracy of auto-analysers used to characterise particles. We have therefore characterised particles seen on SEM by the objective parameters of ECD, aspect ratio and roundness.

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In interpreting the results it is important to take the design characteristics into account. We used the tissues around three types of prosthesis: the Charnley hip prosthesis which has a small (22 mm) diameter head and which has been reported to have a low volumetric wear rate, the ICLH resurfacing hip prosthesis which has a larger diameter head (41 to 51 mm) with a relatively high wear rate, and the Freeman-type (ICLH, Freeman/Swanson (F/SW) and Freeman/Samuelson (F/S)) TKR prosthesis. The last prosthesis is peculiar in that both the tibiofemoral and patellofemoral surfaces are fully conforming throughout flexion/extension. The wear rate of the F/S tibiofemoral

![Table III. Comparison of particle characteristics (mean ± sise; median) for osteolysis-positive and osteolysis-negative groups](image)
joint has been measured as 0.025 mm/year on average, volumetrically equivalent to about 22% of that of the Charnley THR.\textsuperscript{27}

The TKRs in our series generated particles similar to those of the ICLH or Charnley hip prostheses since the articulations were fully conforming. By contrast, it has been reported that the particle size from other types of TKR with non-conforming counter-surfaces is 1.24 μm in ECD,\textsuperscript{28} which is larger than our result.

In our study, six of the F-TKR tibial prostheses were fixed without cement and without a metal back; the polyethylene interfaced with bone. This technique has now been abandoned since it provided poor fixation \textsuperscript{29} and when loosening occurred the polyethylene was abraded by the bone, producing more particles.

There was no correlation between the presence or absence of osteolysis and the morphology of the particles present. There was a highly significant (p = 0.002) association between the number of particles and the presence of osteolysis. We were able to relate the number of particles to the wet weight of the interface tissue from which they were extracted, and now report that osteolysis did not occur until the number of particles per gram of wet interface tissue was in excess of 10 billion (Fig. 4). Assuming that the shape of the particles was spherical, this number corresponds to approximately 0.3% by volume of the interface tissue.

We have found that osteolysis may not only be a dose-dependent disease but also one in which there appears to be a threshold dose below which it may not occur. The implication is that osteolysis may be prevented by reducing the particle dose below the threshold concentration. This raises the possibility of being able to eliminate the disease without eliminating wear, an encouraging clinical prospect since some wear is inevitable.

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REFERENCES