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M. von Knoch · G. Buchhorn · F. von Knoch
G. Köster · H.-G. Willert

Intracellular measurement of polyethylene particles

A histomorphometric study

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Abstract A histological and histomorphometric study was carried out on pseudo-capsules retrieved from patients during revision surgery of cementless total hip replacement. Polyethylene loading and areal polyethylene particle size in different cells of the reticuloendothelial cell line were determined within the tissue by histomorphometry. In the reticuloendothelial cell line, foreign-body giant cells are considered to be the result of confluence of mononuclear macrophages caused by large particles or other unknown stimuli. The aim of this study was to investigate whether polyethylene particle size and polyethylene loading are different in mononuclear macrophages and foreign-body giant cells. The specimens were examined under the light-optical microscope with plain and polarized light. The polyethylene loading of 329 macrophages (mononuclear), 81 small foreign-body giant cells (2–5 visible nuclei), and 103 large foreign-body giant cells (6 or more visible nuclei) was determined and the intracellular polyethylene particle size analyzed by histomorphometry. The mean polyethylene loading was 13 ± 8 μm^2, 25 ± 16 μm^2, and 49 ± 42 μm^2, respectively. This difference was statistically significant (Student's *t*-test, $P < 0.05$). The mean polyethylene particle size was 2.34 ± 3.2 μm^2, 3.02 ± 3.46 μm^2, and 4.70 ± 11.25 μm^2, respectively. This difference was statistically significant between macrophages and large foreign-body giant cells (*t*-test, $P < 0.05$). The findings of this light-optical study show that the mean intracellular polyethylene particle size is greater in foreign-body giant cells. In addition, absolute polyethylene loading tends to be higher in foreign-body giant cells than in mononuclear macrophages.

M. von Knoch (✉)
Orthopaedic University Hospital Kiel,
Michaelisstrasse 1, 24105 Kiel, Germany
e-mail: mariusvonknoch@yahoo.com,
Tel.: +49-431-5972457, Fax: +49-431-5972444

G. Buchhorn · G. Köster · H.-G. Willert
Orthopaedic University Hospital, Göttingen, Germany

F. von Knoch
Bone and Biomaterials Research Laboratory,
Institute for Histology and Embryology, University of Vienna,
Vienna, Austria

Introduction

In both cemented and cementless total hip replacement, prosthetic loosening caused by bone resorption is the most significant problem [13, 14]. Implant-derived wear particles are always found in periprosthetic tissues surrounding loose components. In 1977, it was first suggested by Willert and Semlitsch [15] that the macrophage response to particulate wear debris is an important cause of osteolysis and leads eventually to prosthetic loosening. Recently, it was suggested by Kobayashi et al. [8] that the most critical factor in the pathogenesis of osteolysis is the concentration of polyethylene particles accumulated in the tissue. However, little is known about the importance of the size, shape, and concentration of polyethylene particles in the macrophage response. This uncertainty has been reflected in the diversity of composition, size, shape, and dose of particles which have been used in vivo and in vitro [7, 10].

It has been repeatedly observed that small polyethylene particles are phagocytosed by macrophages, whereas large ones are found in foreign-body giant cells [5, 6, 11]. However, to our knowledge, there are no previous reports which have analyzed intracellular polyethylene particle size directly within the tissue and in different types of cells. The aim of this study was therefore to determine the polyethylene loading of both mononuclear macrophages and foreign-body giant cells directly and to investigate the correlation between polyethylene particle size and the type of phagocytic cell line response.

Patients and methods

Nineteen patients with cementless total hip replacement consisting of polyethylene screw-cups, type Endler (AlloPro, Gelsenkirchen, Germany), with cementless titanium-alloy stems, type Zweymüller

(AlloPro), and aluminium-oxide ceramic heads (Feldmühle AG, Plochingen, Germany) were revised for aseptic loosening of the cup between 1989 and 1992 in different hospitals after a mean time in situ of 4.2 years (range 3–7 years). The cup used in this investigation is a threaded all-polyethylene cup which is screwed into the bone after precutting the thread. Apart from wear of the articular surface (articular wear), friction between the bone and polyethylene cup because of micromotion leads to nonarticular polyethylene wear, which induces a granulomatous foreign-body reaction with osteolysis and, finally, cup loosening [16]. Once the cup is loose, macromotion between the cup and the acetabular bone generates greater amounts of particles which are also of greater size – some measuring more than 200 μm in length [9].

Intraoperatively, the stems were all found to be firmly in position. Specimens taken from the pseudo-capsules were sent to our laboratory (Orthopaedic University Hospital, Göttingen) for histologic examination. Seven of them were suitable for histomorphometric examination according to the criteria given below. The mean age of the patients, who were all women, was 64.4 ± 3.7 years on the day of the revision procedure. Primary implantation of the prostheses had been performed on average 4.2 ± 1.8 years before revision surgery because of osteoarthritis of the hip in all cases.

The specimens were fixed in formalin, decalcified in ethylene diamine tetra-acetic acid (EDTA), embedded in paraffin, cut into 2–3 μm sections, stained with hematoxylin and eosin, and examined under both plain and polarized light. Multiple sections of each tissue were analyzed. Because of the small size of many of the particles, a magnification of as much as 1:1500 and oil immersion and very high-quality optics were used. Since particulate polyethylene transmits light and is not stained by hematoxylin and eosin, it is usually not visible with plain light illumination. The identification of these particles, however, is possible because of its birefringent property: they shine in polarized light and emit a whitish colour.

For histomorphometric analysis, areas within the tissue were chosen where phagocytosing cells (macrophages and foreign-body giant cells) with clearly demarcated outlines could be found, i.e., each cell which could be clearly outlined was analyzed. According to the number of visible nuclei, the cells were classified into macrophages (one visible nucleus, cell class I), small foreign-body giant cells (2–5 visible nuclei, cell class II), and large foreign-body giant cells (6 or more visible nuclei, cell class III).

The specimens were photographed with a highly sensitive black/white film (Kodak T-Max 3200 ISO, Stuttgart, Germany) to keep the exposure time in the polarized light below 15 min. First, each cell was photographed under plain light to determine the extent of the cell (cell size). Next, the intracellular polyethylene particles were photographed under polarized light without changing the position of the specimens. The negatives were used as slides and were evaluated by histomorphometry. For this purpose, a personal computer, a digital table, a digital pen, and a custom-made computer program were used. The slides of the cells were projected on the digital table, the circumference of each cell was outlined on the slide taken under plain light, while the polyethylene particles were encircled on the slide taken under polarized light. This was performed manually with a digital pen. Calibration of this system was achieved and controlled by repetitive determinations of a standard measure. Comparison to a fully automated histomorphometric system showed that manual measurements were more exact with respect to distinction between large particles and agglomerations of smaller particles. The computer program calculated the areal size of the cell (cut cell surface), polyethylene loading of a cell (sum of intracellular cut particles), and the relative polyethylene loading of a cell, i.e., polyethylene loading related to cell size (quotient of absolute polyethylene loading and cell size).

Student's *t*-test was used to compare the polyethylene loading and the size of polyethylene particles in the different cell classes.

Results

Microscopic examination of the specimens showed granulomatous tissue dominated by macrophages and foreign-body giant cells. Under polarized light, an abundant number of polyethylene particles was found, either intracellularly within macrophages and foreign-body giant cells or extracellularly in the interstitial tissue. Particles from the ceramic head or the titanium-alloy stem were rarely seen, probably because the stems were all still firmly fixed at revision without friction between the bone and the femoral implant. In addition, ceramic particles were not expected to be generated by the articulation between the ceramic femoral head and the polyethylene acetabular cup.

The areal size of intracellular polyethylene particles was between 0.5 μm^2 and 43 μm^2 in cell class I (macrophages). Particles which were bigger than 43 μm^2 usually lay extracellularly except for four large intracellular particles in cell class III. The areal size of these particles was 84, 142, 198, and 237 μm^2, respectively. Whether or not these particles consisted of agglomerations of many small particles could not be clearly determined. Indeed, under plain and polarized light, the signal emitted by these particles was not totally homogeneous. Particles of less than 0.5 μm^2 (submicron range) are usually not visible under the light microscope.

The total number of clearly demarcated cells within the tissues was 541. There were 329 (62%) macrophages (cell class I), 81 (17%) small foreign-body giant cells (cell class II), and 103 (21%) big foreign-body giant cells (cell class III). The total number of polyethylene particles within these cells visible under the light microscope was 3539.

The mean areal cell size was 191 ± 66 μm^2 (range 66–402 μm^2) in class I, 607 ± 203 μm^2 (range 288–1371 μm^2) in class II, and 1417 ± 487 μm^2 (range 591–3534 μm^2) in class III (Fig. 1). The mean absolute polyethylene loading was 13 ± 8 μm^2 (range 1 to 49 μm^2) in Class I, 25

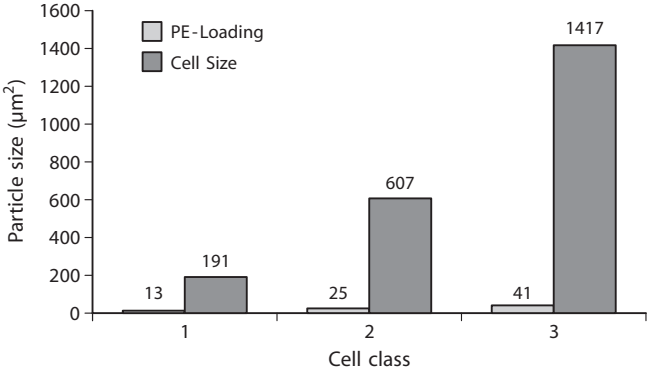

Fig. 1 Mean areal cell size and mean polyethylene loading of cells (in μm^2) in cell classes I, II, and III. Multinucleated foreign-body giant cells (cell classes II and III) are larger than mononuclear macrophages (cell class I). Polyethylene loading of foreign-body giant cells is higher than polyethylene loading of macrophages

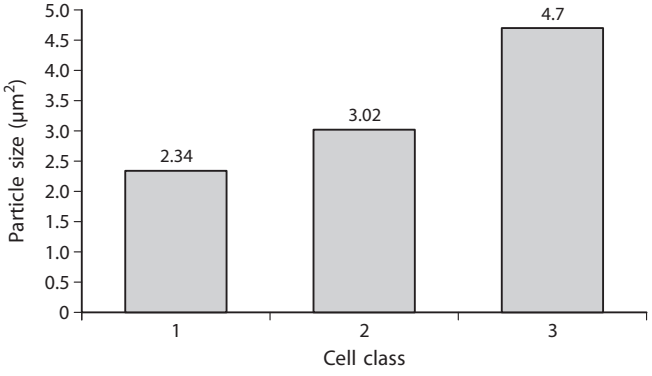

Fig. 2 Mean intracellular polyethylene particle areal size in cell classes I, II, and III (in μm^2). The mean polyethylene particle size is larger in multinuclear foreign-body giant cells (cell classes II and III) than in mononuclear macrophages (cell class I)

$\pm$ 16 μm^2 (range 1 to 94 μm^2) in Class II, and 49 $\pm$ 42 μm^2 (range 1 to 254 μm^2) in Class III (Fig. 1). The absolute polyethylene loading was different in all cell classes, and the difference was statistically significan ($P < 0.05$). The relative polyethylene loading was 6.7% $\pm$ 3.6% in cell class I. The minimum relative polyethylene loading of macrophages was 1%, the maximum 21%. The mean relative polyethylene loading was 4.1% $\pm$ 2.4% in cell class II (range 0.8%–14.4%) and 3.5 $\pm$ 2.9% in cell class III (range 0.2%–19%). The average areal particle size was 2.34 $\pm$ 3.2 μm^2 in cell class I, 3.02 $\pm$ 3.46 μm^2 in cell class II, and 4.70 $\pm$ 11.25 μm^2 in cell class III (Fig. 2).

The measurements show clearly that particles in giant cells tend to be larger than those within macrophages. The difference in particle size between cell class I and cell class III was statistically significant ($P < 0.05$). Since four large particles in cell class III could not be identified as one-piece particles with complete certainty, the average particle size was calculated after exclusion of these four particles. The corrected average particle size was 4.5 μm^2, i.e., 0.2 μm^2 less than the uncorrected particle size. The difference in particle size was still significantly different between cell classes I and III ($P < 0.05$).

Discussion

While the findings of this study confirm previous statements [6, 15], this is – to the best of our knowledge – the first investigation with direct intracellular measurement of polyethylene particle size and polyethylene loading and with comparison of particle size and polyethylene loading in different cells of the reticuloendothelial cell line. In previous works on the size of implant-derived particles, the tissues were fractionated chemically to isolate particles and determine their size in a second step [11]. By that method, it is problematic to distinguish whether a particle lies intra- or extracellularly; the determination of polyethylene loading and particle size of different cell types is almost impossible. In this investigation, intracellular particles were measured in the intact tissue within the cells. The method of this study was histologic and histomorphometric by light microscopy.Therefore, there were some limitations which have to be kept in mind. Due to the histological technique of cutting the tissue, the cut of the microtome may not always have been through the widest or longest part of the cell or particle. Cell size, number of nuclei (and classification), polyethylene particle size, and polyethylene particle number may have been underestimated. To overcome this limitation, the number of analyzed cells and particles was maximized (541 cells, 3539 particles). Another limitation of the study was that the resolution of light microscopy prevents the identification and measurement of the smaller polyethylene particles including submicron particles. Nevertheless, the starting point was that foreign-body giant cells contain larger particles than mononuclear macrophages. Inclusion of submicroscopic particles would probably have led to average values far below the presented ones. Nevertheless, it has been proven by our findings that the generation of giant cells is closely connected with the occurrence of large particles.

Histological specimens from cases with the cementless all-polyethylene Endler cup (AlloPro, Gelsenkirchen, Germany) were chosen because this cup has a characteristic loosening pattern creating all sizes of particles [9]. Because the polyethylene is screwed directly into the bone bed, micro- and macromotion create larger particles from the anchoring surface of the polyethylene by nonarticular wear than would be expected from mere articular wear between the acetabular cup and the femoral head. Therefore, comparison of polyethylene particle size to other studies with different cup designs producing mere articular wear might be misleading. Despite this, the loosening pattern of the Endler cup served unintendedly as an in vivo model of the generation of different sizes of polyethylene particles which made it possible to study the correlation between particle size and cell size. Although large particles of more than 200 μm long were found in the tissue, the areal size of intracellular particles (in mononuclear macrophages) did not exceed 43 μm^2. Other authors have suggested that only particles that are small enough to be phagocytosed by macrophages can start the osteolytic cascade [3]. In contrast, in our investigation, the borderline areal size of intracellular polyethylene particles was 43 μm^2 in macrophages. Larger particles were only seen extracellularly or in giant cells.

Particle-containing multinucleated giant cells that line the bone surface at the bone-implant interface are generally regarded as foreign-body giant cells or macrophage polykaryons [12]. Giant cell formation is known to occur after the simultaneous attachment of two phagocytes to the same material by the fusion of endosome margins [1, 2, 4]. It has been discussed that one stimulus for fusion is the ingestion of large particles. In this light-microscopical study, the mean polyethylene particle size was different between macrophages and larger foreign-body giant cells, and the difference was statistically significant (*t*-test, $P < 0.05$). Particles within foreign-body giant cells tended to

be bigger than those within mononuclear macrophages. Furthermore, in this investigation, the maximum relative polyethylene loading of macrophages – i.e., polyethylene loading related to cell size – was 21%. This limitation of relative polyethylene loading shows that there may be a saturation point of polyethylene loading in relation to the cell size of macrophages.

In conclusion, in aseptic loosening of cementless total hip replacement, polyethylene loading of different cells of the reticuloendothelial cell line is not uniform. Foreign-body giant cells tend to contain larger particles than mononuclear macrophages. In addition, polyethylene loading tends to be higher in foreign-body giant cells, whereas relative polyethylene loading – i.e., polyethylene loading related to cell size – tends to be higher in mononuclear macrophages.

This investigation was performed at the Orthopaedic University Hospital, Göttingen, Germany.

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