

Exploration of the size, shape and abundance of UHMWPE wear particles using atomic force microscopy

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ABSTRACT

Atomic force microscopy is proposed and explored as a technique for the characterisation of ultra-high molecular weight polyethylene (UHMWPE) wear particles from knee prostheses. This new approach may be an alternative to other techniques such as filtration and scanning electron microscopy. Atomic force microscopy can measure the size and geometry of polyethylene wear particles extracted from the joint lubricant with a precision on the nanometre-scale. This can further the understanding of wear processes at this length-scale. However, information about the absolute and the fractionated abundances of the particles in the lubricant is lost, since the particles precipitate non-uniformly on substrates suitable for atomic force microscopy. It is demonstrated that conventional filtration of polyethylene particles can cause similar non-uniform precipitation on a filter medium. This result gives support to the view that uniformity across a substrate or filter medium needs to be established in detail and quantitatively using an appropriate experimental protocol so that abundance distributions may be obtained reliably.

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1. Introduction

The wear dynamics of ultra-high molecular weight polyethylene (UHMWPE) and especially the effects of particulate wear debris from this material pose limits to some applications, such as knee prostheses. UHMWPE is the currently preferred material for tibial inserts in total knee arthroplasty, due to its low wear rate, excellent abrasion resistance and high mechanical stability [1]. In spite of its favourable properties, UHMWPE wears out due to the high dynamic stresses experienced in a knee joint. Different types of wear modes have been distinguished to be relevant during the lifetime of prostheses. These wear modes cause surface degradation of the UHMWPE tibial insert which may be characterised as delamination, pitting, scratching, deformation, abrasion, burnishing and debris embedding [2,3]. All wear modes generate UHMWPE wear particles which can affect the clinical performance of the joint. Particles released from the joint are typically phagocytosed by macrophages, which then interact with fibroblasts, osteoblasts, osteoclasts and other cells [4]. These interactions provoke the release of substances that induce periprosthetic bone resorption, that leads to eventual prosthesis loosening, and subsequent revision surgery. It is clear

that a reduction in wear of the UHMWPE bearing material or some control over the properties of the polyethylene wear particles would improve the clinical performance of knee prostheses.

Since the size and geometry of the polyethylene wear particles may be expected to influence their biological activity, a large number of authors have characterised the diameter, shape and abundance (quantity) of particles generated in knee prostheses by extracting these particles *in vitro* and *in vivo* [5–10]. The bioactivity of different particle sizes has also been studied. Different authors give varying size ranges for the most biologically active particles (0.1–10 μm [5,6], 0.3–10 μm [7], 0.24–0.45 μm [8]). Irregularly shaped particles have been found to be more biological reactive than regularly shaped ones [9,10].

In most published studies the particle shapes and sizes are determined indirectly via size fractionation using filtration and imaging of the filter surface with scanning electron microscopy (SEM) [9–14]. Fractionated abundance distributions are often obtained by counting particles in characteristic SEM images. It may be noted that particle diameters span six orders of magnitude (1 nm to 1 mm) and that this straightforward approach relies upon the fundamental assumption that the filtration process maintains the original abundance distribution of the particles as it is present in the lubricant. Furthermore, it is assumed that particles distribute and precipitate relatively uniformly over the filter medium during filtration. Only some authors, e.g. Refs. [9,10], have verified such uniformity by comparing SEM images from different locations of the filter medium. It appears, however, that quantitative verifications that

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the precipitation on the filter medium is uniform are, in general, not being attempted.

With SEM a particle is characterised via its two-dimensional projection in the image. Three-dimensional size information, such as the particle shape, has to be inferred from such two-dimensional projections. The question arises whether the three-dimensional shape and size of polyethylene wear particles can be characterised and measured more directly. Such measurement would reduce uncertainty in regard to abundance distributions, since any undesired bias during filtration, or SEM sample preparation and imaging, may be excluded. Using different sample preparation, atomic force microscopy (AFM) has the potential to provide such a technique. With AFM the dimensions of wear particles can be measured with nanometre accuracy as long as the particles are microscopic ($<10\ \mu\text{m}$). Any larger particles are evident from optical microscopy. By characterising a statistically significant number of particles with AFM, reliable abundance information for all particle size fractions in a lubricant may be obtained.

This work aims to explore the potential and limitations of AFM as a new means to characterise the size, shape and abundance of UHMWPE wear particles generated in knee prostheses. Results should be relevant to other types of artificial articulations which involve a polyethylene component.

2. Materials and methods

In order to obtain a variable spectrum of sizes of UHMWPE debris particles, a new knee-prosthesis (a Low Contact Stress Mobile Bearing Knee system, cobalt–chrome, standard size) was actuated in a constant load machine designed and built for this project. Axial load and flexion were similar to those experienced during ‘walking’, however, a realistic simulation was not attempted. Mass loss of the UHMWPE tibial component due to a combination of rolling- and sliding-wear was measured gravimetrically. Samples of the water lubricant containing suspended polyethylene wear particles were taken regularly. The wear particles were extracted from the lubricant samples and prepared for characterisation with AFM.

2.1. Constant-load actuator

Fig. 1(a) shows a photo of the constant-load knee prosthesis actuator used in this work. The axial load is applied to the joint with a spring and can be set over a range of 0–900 N. An electric

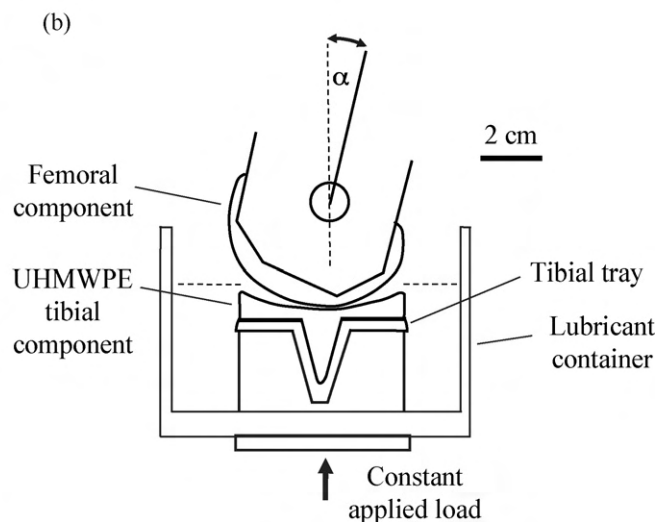
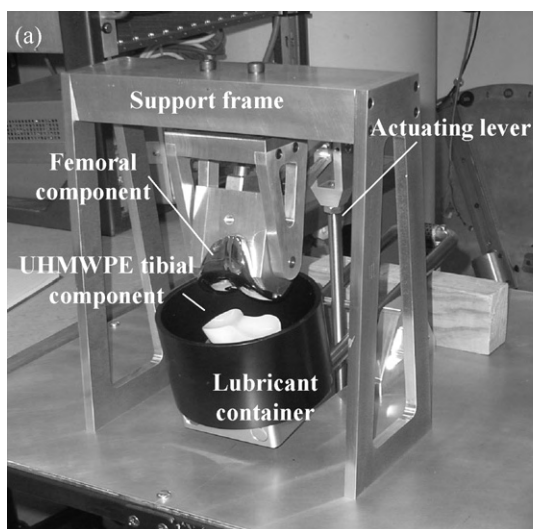


Fig. 1. (a) Photo of the constant-load knee prosthesis actuator before a load is being applied to a knee prosthesis. (b) Schematic cross-sectional view of the prosthesis in the actuator.

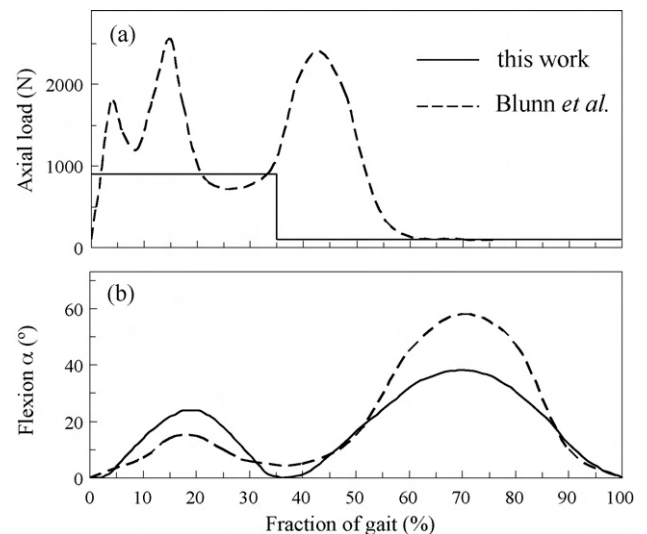


Fig. 2. (a) Axial load and (b) flexion angle as reported by Blunn et al. are shown for a realistic walking cycle (dashed curves) [15], in comparison with the coarse approximation utilized in this work (solid curves). The x-axis represents one complete walking cycle.

motor moves the femoral component with an actuating lever about the pivot point through the flexion angle α , as it is illustrated in Fig. 1(b). The actuator operates at a rate of 1 machine cycle per second. Maximum flexion angles of $\alpha_{\text{max}} = 24^\circ, 38^\circ, 51^\circ$ and 66° can be set. The femoral component is attached to the support frame via two elastic rubber spacers and the tibial tray is fixed to the floor of the lubricant container. Some rotational motion of the UHMWPE tibial component is possible in the horizontal plane as the component rests unattached in the tibial tray.

2.2. Walking cycle approximation

The UHMWPE component of the knee-prosthesis was actuated over a total of 1.5×10^6 machine cycles. Fig. 2 shows axial load and flexion angle, as a function of gait fraction, as they may be experienced during ‘walking’ [10]. In an attempt to coarsely approximate a ‘walking’ actuation of the prosthesis, a stance and a swing phase were simulated dynamically. For convenience, however, arbitrarily, one testing interval was chosen as 86,400 machine cycles. It may

be of interest that 86,400 machine cycles are roughly equivalent to 20 days of walking assuming 4320 steps per day. Every testing interval was divided into two distinct parts, as it is also shown in Fig. 2. The first part (35% of one testing interval) with an axial load of 900 N and maximum flexion angle $\alpha_{\max} = 24^\circ$ represented the stance phase. The remaining part (65% of the same testing interval) with a load of 100 N and $\alpha_{\max} = 38^\circ$ represented the swing phase.

During actuation the knee prosthesis was bathed in de-ionised water at room temperature. Water was chosen, since results can readily be compared with other published work [16–18]. The increased wear rate due to water lubrication, as compared to serum, was considered acceptable, since the focus of this work is on the spectrum of debris particles and their characterisation. The water lubricant and its debris content was removed regularly and replaced with a new sample of de-ionised water.

2.3. Mass loss of the UHMWPE component

Before the regular gravimetric measurements of the wear-induced mass loss were taken, loose debris was flushed with lubricant and the UHMWPE tibial component was dried for 3 h under a heat lamp. The uncertainty of the weighing was of the order of 1 mg. Fig. 3(a) shows the mass loss measured for the UHMWPE tibial component of the prosthesis as a function of the number of machine cycles.

In parallel with the gravimetric measurements, the surface area excavated by the femoral component at that point in time was measured on close-up photographs of the tibial component. The total excavated surface area, given in Fig. 3(b), includes both the lateral and medial wear areas. The excavated volume, or volume reduction, as it can be derived from the mass loss data in Fig. 3(a), is also shown in Fig. 3(b) as a function of the excavated surface area. Three distinct wear phases may be identified in Fig. 3. During Phase I, with an approximate wear rate of 150 mg/Mcycle, the contact area rapidly expands to wear in a UHMWPE surface area of about 21 cm². During Phases II and III the excavated area increases only marginally to ultimately 23 cm². However, beyond 0.75 Mcycle of actuation, which commences Phase III, the wear rate is reduced drastically to less than 20 mg/Mcycle.

Relatively high wear rates as observed in this work for Phases I and II have been reported before for immersion in a water bath. For example, wear rates of 45–75 mg/Mcycle have been reported

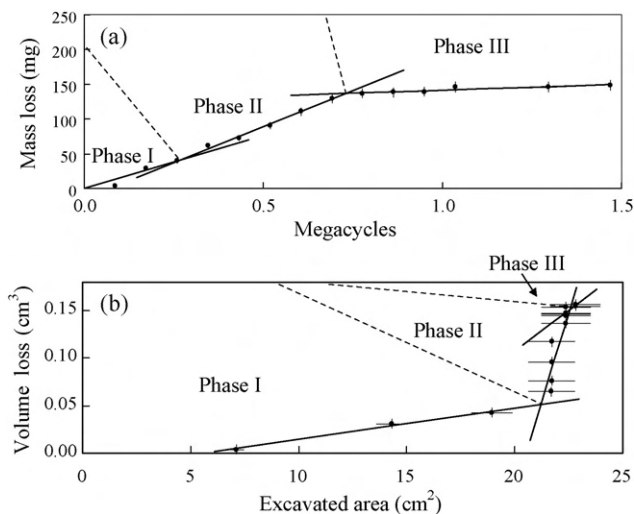


Fig. 3. (a) The mass loss of the UHMWPE tibial component plotted as a function of actuation cycles; and (b) the derived volume reduction shown as a function of the surface area excavated by the femoral component. The solid lines are linear fits to the data, whereas the dashed lines are to guide the eye.

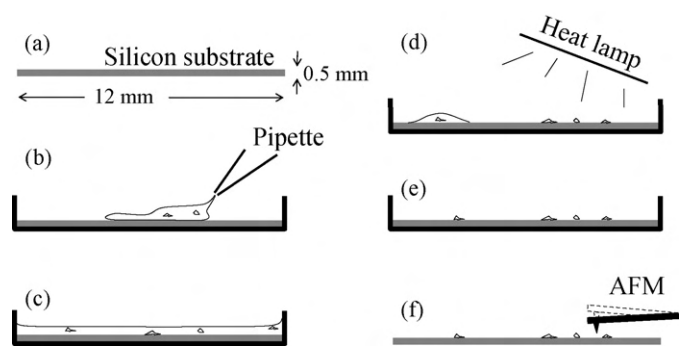


Fig. 4. Illustration how debris particles were extracted from the lubricant and prepared for atomic force microscopy. Details are given in the text.

for distilled water with a load of 1780 N and a water temperature of 37 °C [19,20]. The results for water may be compared with the considerably lower wear rates between 1.5 and 30 mg/Mcycle which have been reported for bovine serum at 37 °C, with maximum axial loads of 5500 N [21]. The difference between the two lubricants can be explained with the ineffectiveness of water to form a boundary lubricant film [11,13].

The drastic reduction of the wear rate observed in this work during Phase III of the joint actuation, when compared to Phases I and II, may be speculated to be due to the fact that the contact stress between the femoral and the tibial component is considerably lower during this Phase. This is due to the preceding increase in contact area during Phase I, a snug adaptation to this area during Phase II, and the formation of an agglomeration of particles or even the formation of a polymer film on the femoral component reducing its roughness [16].

2.4. Extraction of particle debris for AFM characterisation

In order to avoid filtration, debris particles have been extracted from the lubricant samples and prepared on mirror-finished silicon surfaces for AFM. Fig. 4 illustrates the procedure that was applied. P-type silicon wafers were readily available for this purpose and were found to be well-suited.

12 mm × 12 mm squares were cleaved from the wafer using a diamond pen (Fig. 4(a)) and cleaned by immersing in ethanol which was then expelled with nitrogen gas. The 0.5 mm thin silicon substrates were then placed at the bottom of matching square containers with inner dimensions of 12 mm × 12 mm (Fig. 4(b)).

For each sample the lubricant was agitated in an ultrasound bath for 30 min to ensure uniformity and reproducibility and a small amount of the lubricant was then taken up with a disposable pipette. This rejected any visible macroscopic debris. A calibrated and identical amount of lubricant was then dispensed onto the silicon substrate, ensuring complete surface coverage (Fig. 4(b) and (c)). Under a heat lamp the sample containers were completely dried over 24 h (Fig. 4(d)), so that the particle debris was resting on the substrate surface (Fig. 4(e)). Care was taken that the substrate was kept in a near-horizontal position, when it was removed from its container and placed under the AFM (Fig. 4(f)).

2.5. Atomic force microscopy of UHMWPE wear particles

The atomic force microscopy was performed with an NTEGRA instrument manufactured by NT-MDT. The instrument was operated in 'semi-contact mode', which is often also referred to as 'tapping mode'. A conical silicon tip (NT-MDT brand NSG01: height 10–15 μm, cone angle $\leq 22^\circ$) on a cantilever with a resonance frequency in the range 115–190 kHz and a force constant in the range 2.5–10 N/m was used. The AFM tip scans a small area on the flat

silicon substrate and directly probes the height variations due to debris particles resting on the substrate. The x , y and z response of the instrument was verified using a standard, confirming a lateral x , y resolution of 50 nm and a vertical z resolution of 10 nm.

Fig. 5 shows AFM data measured for wear particles on a mirror-finished silicon substrate. The particles were extracted from a lubricant sample of the prosthesis actuation studied in this work. Fig. 5(a) shows the data as a two-dimensional plot which illustrates the sensitivity and the measurement accuracy of the instrument in the horizontal x – y plane. Fig. 5(b) shows the same data as a three-dimensional projection which emphasizes the nanometre-scale precision of the instrument in the vertical z -direction (height).

3. Results and discussion

To give representative results, some 120 AFM measurements were carried out on particle debris from the prosthesis actuation described above. The projections in Fig. 5 are typical for the results obtained and show three distinct types of UHMWPE wear particles with sizes of the order or less than 1000 nm. The three particle types may be referred to as ‘pellets’, ‘tablets’ and ‘platelets’, as it is indicated in the figure. The three types may be distinguished with reference to their dimensions and, to a lesser degree, with reference to their geometry. The relative sizes and fundamental geometries which appear to be characteristic of the three particle types are illustrated schematically in Fig. 5(c).

‘Pellets’ are small particles approximating flat cuboid or cylindrical geometry with diameters of the order of 100–500 nm and much smaller thicknesses of the order of 10–50 nm. ‘Tablets’ have similar geometries, but are larger with thicknesses of the order of 100 nm. Finally, ‘platelets’ have the largest volume and often

approximate the geometry of flat cuboids or plates with sometimes fractured edges. The typical thickness of a ‘platelet’ is of the order of 20–200 nm, whereas the values for length and width are significantly larger and may be of the order of 1–2 μm .

The appearance of many ‘platelets’ is reminiscent of that of macroscopic ‘shavings’, which may indicate that ‘platelets’ are the result of material delamination on the UHMWPE tibial component induced by the actuation. Wear particles similar to the three types identified here have also been observed by other authors [9,10,14,22]. It is noteworthy that no particle has been observed resting on its edge on the silicon substrate. This may be due to the fact that for all three particle types illustrated in Fig. 5(c) the diameter tends to be an order of magnitude larger than the edge thickness and that the particles may float on the water just before they come to rest on the substrate surface.

Fig. 6 shows detailed AFM results for pellet- and platelet-type particles, which illustrate the strength of this technique. AFM is more sensitive in the z -direction. This is reflected in the figures through the use of a nm-scale for the vertical z -axis. It is clear from Fig. 6 that none of the particles are spherical, but that both particles types are considerably oblate, with the thickness tending to be an order of magnitude smaller than the lateral dimensions. Considering Fig. 6(a) and (c) only, in which the AFM data is plotted somewhat similar to what is obtained from SEM imaging, if the height information is ignored, the two particle-types shown may be mistakenly interpreted as having near-spherical geometry. Considering in addition the height information from AFM, which is not available from SEM imaging, the extreme-oblate nature of the wear particles is correctly identified.

In addition to individual particles, coagulations of particles have been observed, such as macroscopic clumps with up to millimetre dimensions. Another type of particle coagulation, a ‘fibril’, is shown

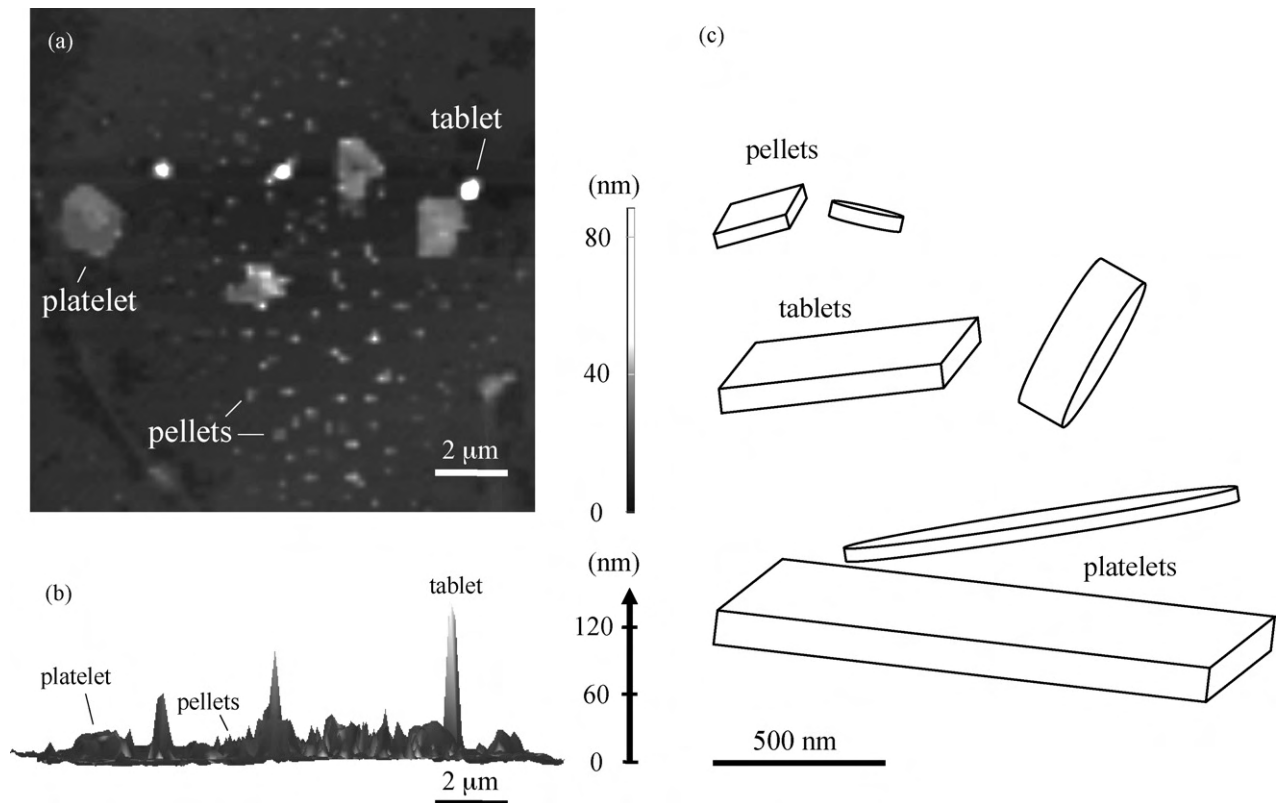


Fig. 5. (a) Two-dimensional projection of AFM data for wear particles extracted from the actuation lubricant. Three distinct particle types can be identified and are labelled in the figure. The grey-scale indicates the measured height. (b) Three-dimensional visualisation of the same data emphasizing the nm-scale precision of the instrument in the vertical direction. (c) Schematic illustration of the relative size and the fundamental geometries of the three major particle types observed.

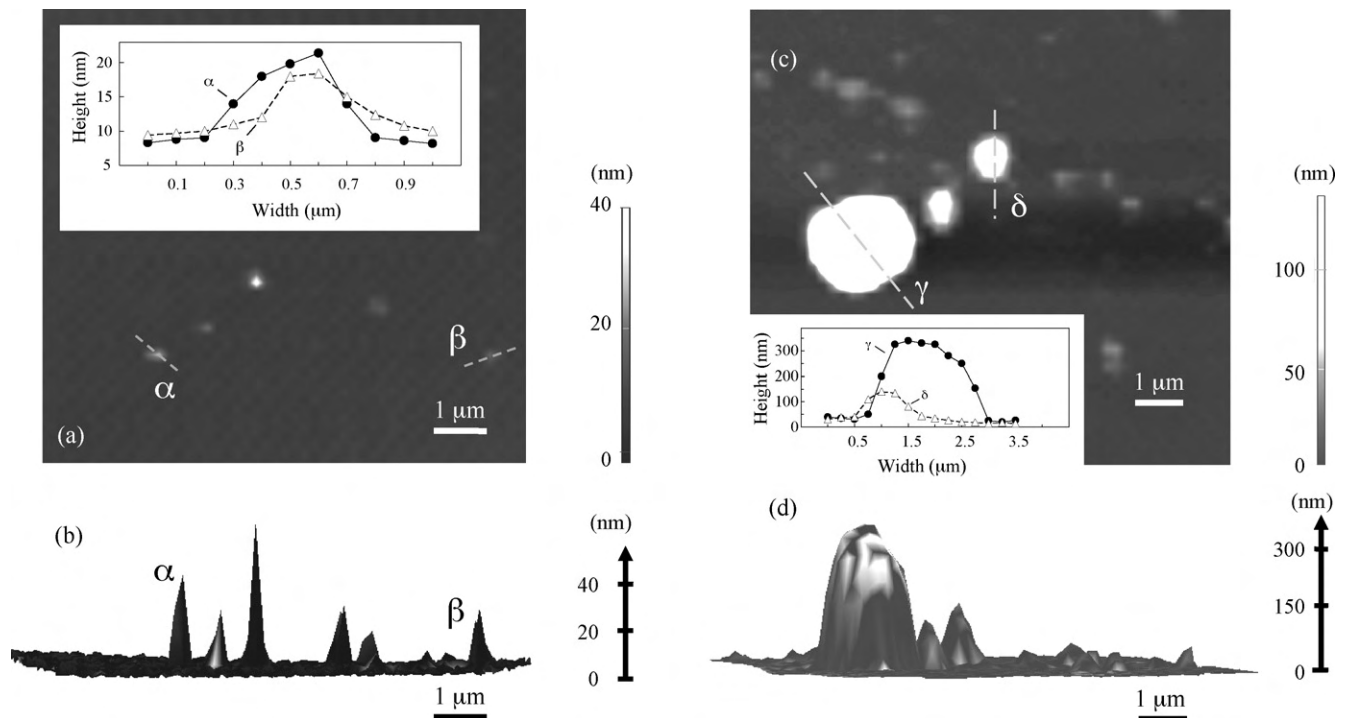


Fig. 6. (a, b) AFM measurements of pellet-type wear particles. The inset shows sections through the two particles α and β as measured with AFM. (c, d) AFM results for platelet-type particles. The inset shows sections through the two particles γ and δ as measured with AFM. The four sections are indicated in the figure with dashed lines. Note that, as with all AFM measurements, the z-scale is different from the x- and y-scales.

in Fig. 7. Several such fibrils have been observed, which may shed some light on the wear process. They consist of linear chains of typically ‘tablets’. The frequent and common appearance of such particle chains of varying length suggests that they may originate in the wear process with most of them breaking up into individual tablet-type particles.

Fig. 7 shows a fibril which may consist of more than 15 tablets. The distinct nature of the individual particles forming the chain is apparent from Fig. 7(b). The tablets are linked via their smallest surfaces, as it is illustrated in Fig. 7(c). This fact and the linearity of these coagulations may be evidence that the chains are not the result of a reordering process in the lubricant or during sample preparation. With a typical length of several microns and a width of about 1 μm , a fibril may be lifted off the UHMWPE tibial component by a micrometer-size asperity on the femoral component. It may be speculated that when the chain is agitated many fibrils break apart at their weak points, which are identifiable in Fig. 7(b), and are dispersed as ‘tablets’.

In order to assess the biological impact of a particular particle size or geometry, the abundance (quantity) of that particle type in the prosthesis lubricant in relation to the total particle number has to be determined.

This work has explored how the total particle abundance may be obtained from atomic force microscopy data. Samples were prepared as described above and illustrated in Fig. 4. Wear particles on 12 mm \times 12 mm silicon substrate were imaged with AFM over areas of 50 μm \times 50 μm . The images were taken at equidistant positions (–5.5 mm, –4.5 mm, . . . , –0.5 mm, 0.5 mm, . . . , 5.5 mm) on the substrate along the two main axes which intersect the substrate into four quarters.

For each image the AFM data was displayed as a two-dimensional projection in the x–y plane. Using appropriate software the projected area of each particle was evaluated. Assuming then near-circular geometry, which is a good approximation for most particle projections a diameter was calculated and assigned

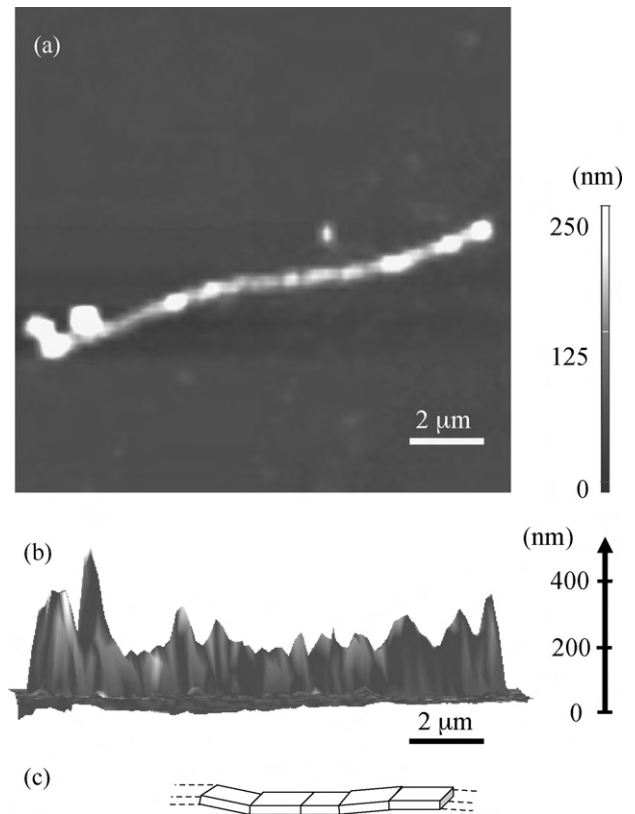


Fig. 7. AFM characterisation of a ‘fibril’. (a) Two-dimensional projection of the data. (b) Three-dimensional view of the data emphasizing the individual particles making up the fibril. Note that the vertical scale is much finer than the horizontal scale. (c) Schematic illustration of the fibril chain. Further details are given in the text.

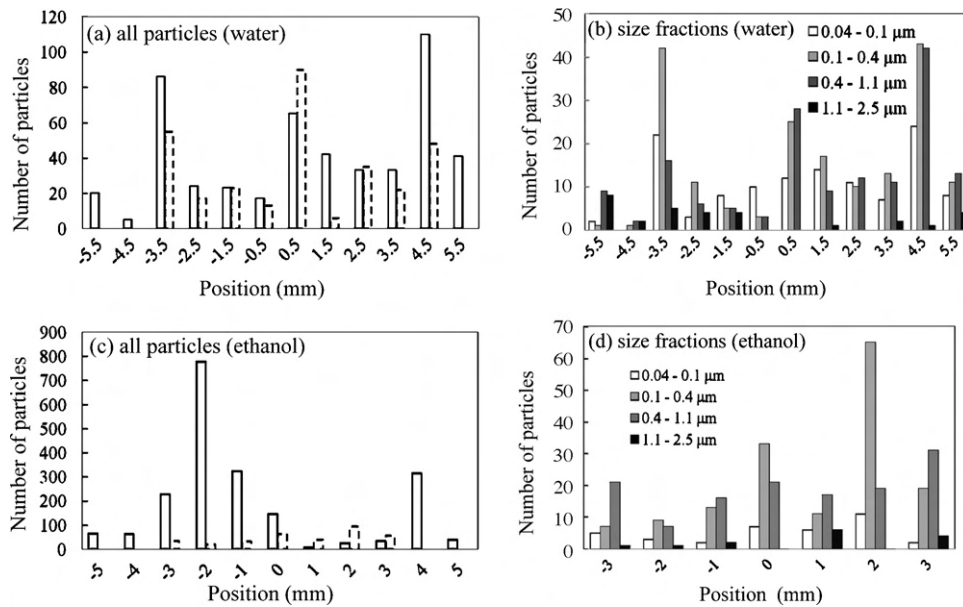


Fig. 8. Abundance distributions for UHMWPE wear particles as measured with AFM at different positions on a sample substrate: (a) the total particle count for two different AFM sample preparation techniques using a water medium; (b) the size fractions for sample preparation in a water medium, where 'size' refers to the largest diameter of the extreme-oblate particle shape; (c, d) the same as for (a) and (b), but for sample preparation in an ethanol medium. It is apparent that results across the substrate are inconsistent.

to each observed particle. Since the particles tend to be 'extreme-oblate' or 'plate-like' and of the type 'pellet', 'tablet' or 'platelet' and rest on one of their largest faces, as discussed above, the calculated diameter is characteristic of the large horizontal particle axes. The vertical thickness of the particles is very often in good approximation an order of magnitude smaller. The particles with calculated diameters of less than $2.5 \mu\text{m}$ were counted on each projection and a total count was established. The reliability of the software was verified manually for some of the projections.

Fig. 8(a) shows a characteristic result for one of the substrates as closed bars. It is apparent that the total particle count varies across the substrate and that this variation is statistically significant. Furthermore, the variation is not smooth, but irregular, making a reliable estimate of the total particle number impossible. The result for the other axis is similar.

In an attempt to avoid such irregularity, smaller substrates ($9 \text{ mm} \times 9 \text{ mm}$) were prepared with wear particles using otherwise

the same procedure as illustrated in Fig. 4. This was motivated by the suggestion that the effects of surface forces due to the edges of the container may be avoided (see Fig. 4(c)). However, the dashed bars in Fig. 8(a) show that this does not remove the irregularity of counts across the substrate.

Fig. 8(b) displays fractionated size distributions, where the observed particles on a $12 \text{ mm} \times 12 \text{ mm}$ substrate have been divided into four size fractions according to the calculated diameter discussed above. As in Fig. 8(a), the result varies irregularly across the substrate. No trends are apparent from the data, with apparent random results for each position.

Since it may be assumed that the particles are uniformly distributed in the lubricant container during the prosthesis actuation, the observed non-uniformity must be introduced during the preparation of the wear particles for AFM characterisation. A major factor in this may be the surface forces of the water lubricant and their actions during the drying process (Fig. 4(d)). In an attempt to

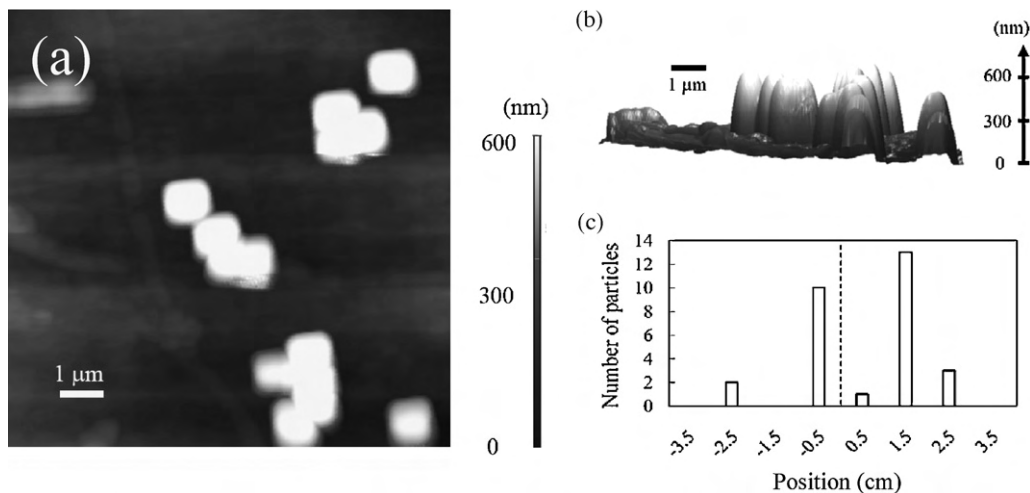


Fig. 9. Results from an experiment assessing the uniformity of abundance of identical polyethylene particles across filter paper; (a) two-dimensional projection of AFM data for position 1.5 cm on a piece of filter paper; (b) the corresponding three-dimensional projection; (c) abundance of the polyethylene particles at 8 different positions across the filter paper.

observe such an effect, the wear particles were prepared on a silicon substrate as before, however, after drying the particles were washed-up again using ethanol and the container was agitated. The ethanol was then allowed to dry and AFM was performed. Fig. 8(c) and (d) shows an example of the results for this approach. Again, both the total particle count and the abundance distributions vary irregularly across the substrate.

It may be concluded that with the procedures explored the assumed uniform distribution of the wear particles in the lubricant is not transferred onto the AFM substrate. Thus quantitative information about the size-dependent particle distribution in the lubricant may not be obtainable with AFM. However, similar selection effects may occur during filtration or during sample preparation for SEM. In order to test this assertion, a commercial sample of standard, regular size polyethylene particles was washed up with de-ionised water and filtered through 0.2 μm . Results are shown in Fig. 9.

It has been found that particle counts across the filter are inconsistent (Fig. 9). The experiment performed in this work is not representative of published studies which have determined the size and quantity of UHMWPE wear particle. However, this result gives support to the view that uniformity across a microscopy substrate or filter medium needs to be established in detail, and quantitatively, before reliable abundance distributions may be extracted for UHMWPE wear particles.

4. Conclusions

This work has explored atomic force microscopy as a new characterisation technique for UHMWPE wear particles and as an alternative to filtration and SEM. AFM offers the possibility to determine the size, shape and abundance of such wear particles through direct measurement. Results show that typical wear particles can be identified, surveyed, and measured in all three spatial dimensions with a precision on the nanometre scale. Importantly, the geometry of individual particles can be unambiguously established and measured. The precise geometrical information may shed light on some of the wear processes at the submicron level as for example the delamination of UHMWPE material or the origin, and possible break-up, of particle chains.

It has been shown that wear debris can be transferred from the lubricant onto a substrate suitable for AFM. However, with the methods explored here, in which the lubricant is evaporated, the particles do not precipitate uniformly onto substrates suitable for AFM. Consequently, information about the total particle abundance and information about fractionated abundance distributions in the joint lubricant is lost when lubricant samples are prepared for AFM. Such non-uniform precipitation of polyethylene particles from a liquid is likely driven by the complex actions of viscous and surface forces on the particles.

Importantly, it has been shown for samples of identical polyethylene particles suspended in water that conventional filtration results in similar non-uniformities of the particle count over the filter medium. This raises doubts about the validity of published abundance distributions for UHMWPE wear particles, for which uniformity over the substrate or filter medium was not specifically established.

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