A novel technique for the detailed size characterization of wear debris

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The accurate and detailed characterization of artificial joint wear debris is important in determining both the wear rate of prostheses and understanding the role that the debris plays in the development and progression of aseptic loosening. The novel application of low angle laser light scattering (LALLS) to the particle size characterization of ultra high molecular weight polyethylene (UHMWPE) wear debris is described. The results demonstrate that both *ex vivo* and *in vitro* origin wear debris samples, at concentrations typical of those produced via an alkali-digestion retrieval route, can be reproducibly analyzed via LALLS. Because the LALLS route enables particle size analysis of the entire debris sample to be acquired non-destructively and whilst in suspension, artefacts associated with filtering, drying and agglomeration of debris are avoided, in contrast to currently used techniques such as filtration and scanning electron microscopy (SEM) observation.

1. Introduction

The role played by artificial joint wear debris in the promotion of osteolysis is currently the subject of extensive research. It is generally accepted that a foreign body reaction occurs due to the presence of wear particles. However there is uncertainty as to the exact mechanism of osteolysis activation. Many studies published to date have examined the *in vitro* interaction of debris-like particles with macrophages. These studies have shown a number of particle parameters including size [1], material [2], morphology [3] and surface charge [4] to be capable of eliciting an adverse cellular response. It is therefore important that investigations to quantify the *in vivo* and *in vitro* response to wear debris should be underpinned by accurate and comprehensive debris particle characterization.

Much characterization of wear debris reported to date has utilized two dimensional information gained from either optical or scanning electron microscopy (SEM). Typical processing protocols include alkali digestion of either debris-loaded *ex vivo* tissue, or bovine serum for *in vitro* origin debris, followed by filtering to isolate debris particles from the digest suspension [5–7]. The debris-loaded filter membrane must then be dried and conduction sputter coated prior to SEM examination. This route enables imaging of individual particles and can thus provide useful morphological information of the debris. However, although adequate for sizing the debris in terms of absolute size range and modal size, this technique falls short of being able to provide the detailed information required to completely characterize the debris distribution.

The use of SEM observation to infer the size distribution and the mass distribution of the debris is unsatisfactory in a number of regards. Although random sampling procedures can minimize observer bias, the use of only a small number of sample micrographs taken from across the filter membrane introduces the possibility of misrepresentative sampling. The necessarily small number of particles characterized can introduce both systematic and observer bias which can be compounded by sampling errors. Image analysis techniques may improve upon the sampling statistics by increasing the number of particles characterized, but the accuracy of the imaging software is dependent upon resolution of discrete particles. Research within the authors' laboratory found that aggregation of particles subsequent to filter drying was a common occurrence and caused clumping of particles. Furthermore, because this phenomenon is not independent of particle size, a systematic bias towards the particle size least likely to agglomerate may be introduced.

The aim of this study was to establish a method for the detailed, accurate and reproducible characterization of wear debris. The authors believe that the outlined method, which utilizes low angle laser light scattering (LALLS), offers significant advantages over current techniques and will be of much use for the evaluation of joint prostheses of both *in vitro* and *ex vivo* origin. The rationale for investigating LALLS for the characterization of wear debris is that the entire debris sample is analyzed non-destructively, whilst in suspension and thus artefacts associated with filtering, drying and agglomeration of debris are avoided. Further, it is anticipated that

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the ability to completely size the debris will have application in assessing the distribution of wear debris sizes in tissue samples from osteolytic and fibrous tissue, or periprosthetic and lymphatic tissue.

2. Experimental procedure

The novel aspect of this technique is the use of a LALLS particle analyzer (Mastersizer S, Malvern Instruments, UK) to characterize wear debris. The LALLS technique is capable of quantifying particles lying within the size range 0.05 to 1000 μm, which encompasses the reported range of *ex vivo* and *in vitro* wear debris particle sizes. Within the instrument, particles are suspended within a carrying fluid and recirculated through an optical window placed in the path of a He-Ne laser beam. A passing particle causes diffraction of the laser beam, quantification of which enables calculation of the equivalent spherical volume of the particle using Mei theory. The LALLS method relies upon the fact that the diffraction angle is inversely proportional to particle size. Requirements for satisfactory operation of LALLS include the preparation of a representative suspension of the sample of particles, the use of an optically transparent carrying media and a sufficient concentration of particles to produce an acceptable signal to noise ratio. Subject to these conditions, the technique has been shown to be accurate, repeatable and reliable and has found wide application within the pharmaceutical, petrochemical and food industries.

In order to assess the suitability of LALLS for the assessment of artificial joint wear debris a preliminary investigation was undertaken to characterize PMMA powder ($\rho = 1.17\,\mathrm{gcm}^{-3}$) with a broad particle size distribution, encompassing that reported for *ex vivo* UHMWPE wear debris. The PMMA particle size range, incorporating sub-micron to approximately 200 μm particles, was validated by SEM microscopy. To determine the optical suitability of digested bovine serum as a carrying media, two samples were assessed by LALLS, PMMA powder dispersed in distilled water and PMMA powder dispersed in alkali-digested bovine serum [6]. The second objective of this study was to investigate LALLS for the characterization of *ex vivo* wear debris. In particular, it was important to ascertain if site-specific wear debris could be isolated from digested *ex vivo* tissue samples at a sufficiently high concentration for analysis by LALLS. Five tissue samples taken from three patients at revision hip joint surgery by Mr I. M. Pinder, Freeman Hospital, Newcastle were selected for investigation. Joint type and retrieval site information can be seen in Table I. The debris retrieval protocol of Campbell *et al.* [5, 7] was used because it produced wear debris particles of a high purity and at high concentrations within a colorless medium. To complement this work, samples of alkali digested and filtration retrieved *ex vivo* wear debris were also examined by SEM.

3. Results

Results of the LALLS characterization of PMMA powder are plotted in Fig. 1. This plot shows the PMMA particle size distributions by number and by volume for both distilled water and digested bovine serum carrying media. Table II summarizes the statistical parameters that are useful in describing the number and volume distributions. In Table II, D(n,0.1) represents the size interval that includes the first 10% of the number distribution, D(n,0.5) the size interval that includes the first 50% of the number distribution, (i.e. the median particle size) and D(n,0.9) the size interval that includes the first 90% of the number distribution. Similarly, D(v,0.1), D(v,0.5) and D(v,0.9) represent the 10%, 50% and 90% size intervals of the volume distribution of particles. D[4,3] represents the equivalent volume mean, also known as the De Brouker mean [8].

The LALLS results for the five *ex vivo* tissue samples are reproduced in Table II. The particle size distributions by number and by volume for two *ex vivo* tissue samples, C and D, both retrieved from the acetabulum region, are plotted in Fig. 2. A typical SEM image of filtered and agglomerated *ex vivo* origin UHMWPE wear debris can be seen in Fig. 3.

4. Discussion

The preliminary study of PMMA powder dispersed within different carrying media showed a good level of agreement between the size range determined by the particle analyzer and that observed via SEM. Inspection of Fig. 1 and reference to Table II confirms the compatibility of digested bovine serum as an optically suitable carrying media for the LALLS technique. The distributions obtained from the two carrying media show close correlation, particularly for the particle size distributions by volume. This is emphasized by the similarity of the De Brouker means, found to be within 1.5% of each other. The distributions by number display marginally poorer correlation. This may be a consequence of the disparity between the refractive indices of digested serum and distilled water, the effect of which is more pronounced for the smaller particle sizes. The number and volume distributions differ markedly for both samples, reflecting the significant contribution that relatively few large particles make to the volume distribution. The number distribution is comparatively insensitive to low numbers of large particles. By way of illustration, consider the particles that lie within the size range 0.58–0.67 μm in sample B, the particle analyzer reports that 15.27% of the particles measured were of this

TABLE I *Ex vivo* tissue sample retrieval site information

Sample	A	B	C	D	E
Patient	1	1	1	2	3
Retrieval site	Greater trochanter	Neck of femur	Acetabulum	Acetabulum	Acetabulum
Prosthesis type	PCA	PCA	PCA	PCA	Charnley

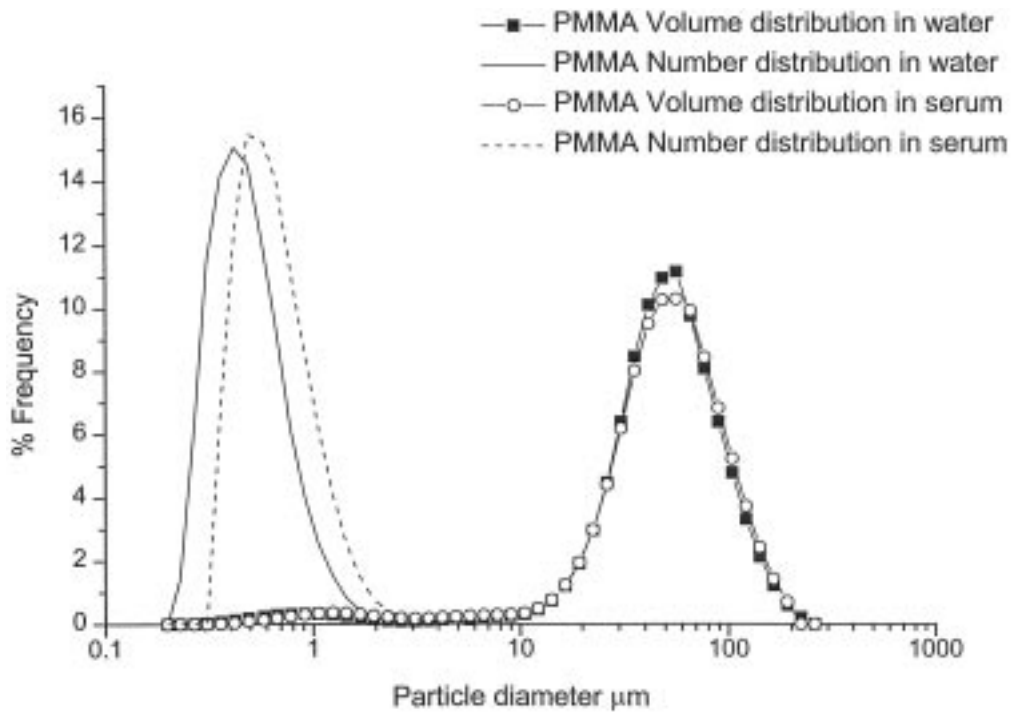

Figure 1 LALLS particle size distribution plots by both number and volume for PMMA powder dispersed within distilled water and digested bovine serum carrying media.

size but also that they accounted for only 0.13% of the total volume of particles present. *In vitro* joint simulators are an important tool in prosthesis development and commonly operate with bovine serum as a synovial fluid substitute. These results show that LALLS offers a viable method of particle size characterizing such wear debris.

The experimental addition of PMMA particles to the circulating fluid of the LALLS analyzer enabled measurements to be acquired at optimal particle concentrations. However, the concentration of retrieved *ex vivo* debris particles is a function of both the amount of debris located within the original tissue sample and the debris retrieval protocol. Furthermore, the LALLS analyzer required a minimum circulatory fluid volume of 25 cm^3 for operation. The particle size analyzes of the five typical *ex vivo* debris samples showed a range of debris yields, all of which were of sufficient concentration for satisfactory application of the LALLS technique. Fig. 2 shows the number and volume distributions of two separate samples from similar prostheses taken from two patients. A high degree of similarity between these two distributions by number can be seen. This is reflected in the similarity of the number median diameters of Table II. Further it can be seen that, with the exception of sample A, all the *ex vivo* debris samples have number median diameters of approximately 0.7 μm. This is apparently independent of prostheses type and tissue sample harvesting site. This agrees with the debris retrieval work of Campbell *et al.* [9] who observed no difference in size distribution by number between differing joint types.

In contrast to the similarity between the size distributions by number, a profound difference in the De Brouker mean, the equivalent volume mean diameter and the size distributions by volume was recorded for the *ex vivo* tissue samples. This variation was found to occur between joint types, patients and even within sampling sites on a single patient, as shown in Table II. Although the small sample size employed in this preliminary proving study dictates that no firm conclusion be drawn from this data, it does suggest that if a difference in debris production or particle mobility should exist, then the LALLS technique would be capable of quantifying it.

The necessity to look beyond the number distribution of wear debris particle sizes was proposed by Fisher *et al.* [10]. Tipper *et al.* [6] reported a wide variation in the

TABLE II LALLS characterization of PMMA powder dispersed within distilled water and digested bovine serum carrying media

	Distribution parameter	Particle diameter of PMMA powder dispersed in distilled water (μm)	Particle diameter of PMMA powder dispersed in digested serum (μm)
Number distribution	D(n,0.1)	0.32	0.44
	D(n,0.5)	0.50	0.67
	D(n,0.9)	0.90	1.23
Volume distribution	D(v,0.1)	24.28	22.96
	D(v,0.5)	55.43	55.42
	D(v,0.9)	115.59	114.14
	D[4,3]	63.75	62.81
Particle concentration (vol. %)		0.0287	0.0233

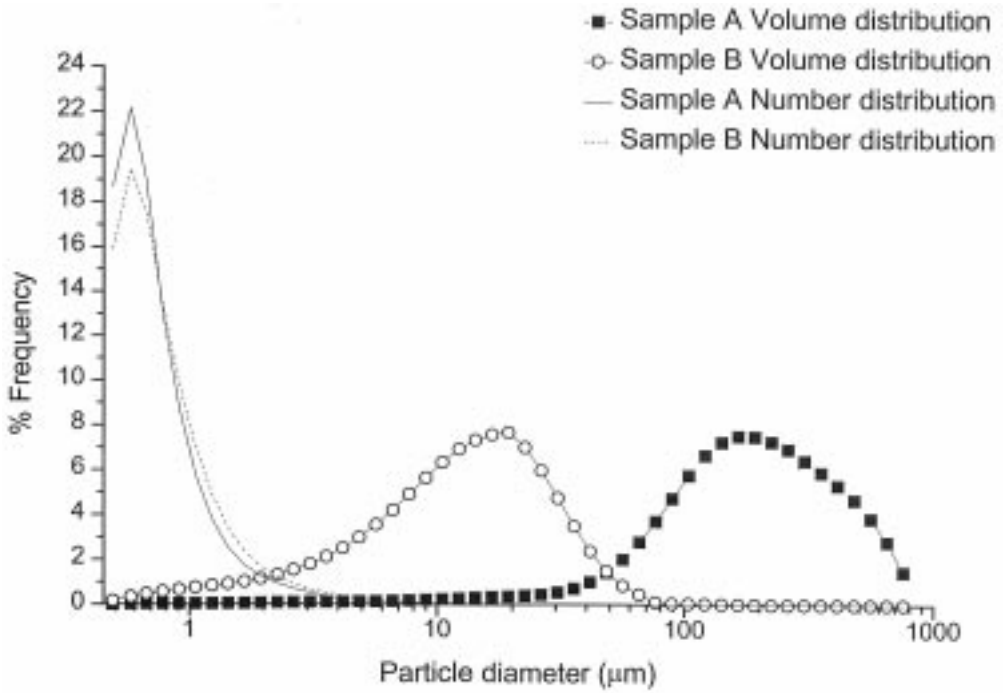

Figure 2 LALLS particle characterization of *ex vivo* wear debris retrieved from the acetabular tissue of two patients during revision surgery. The graphs show the debris size distributions by number and by volume.

mass, and by inference volume, distributions of *ex vivo* wear debris. Using filter membranes of pore diameters 0.1 and 10 μm, they reported that particles larger than 10 μm in size constituted between 3 and 82% by mass of the retrieved debris. Using the LALLS technique the variation to which these figures attest could be explored to a much greater depth than has previously been possible. In contrast to filtration retrieval, LALLS enables multiple size banding of the debris and provides particle sizing from the entire debris sample. For example, for a typical *ex vivo* debris sample of concentration 0.009 vol. % and a median particle size of 0.78 μm, a single analysis of the 25 cm^3 sample volume represents the particle sizing of approximately 10^{12} wear debris particles. Because the LALLS technique is non-destructive, the particle characterization data can be further augmented by direct SEM observation.

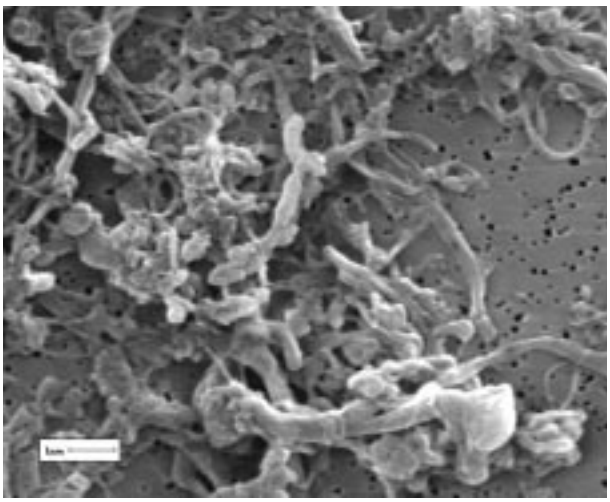

Figure 3 SEM micrograph of *ex vivo* wear debris particles on a 0.1 μm pore filter membrane. Debris was retrieved from *ex vivo* tissue using the Campbell *et al.* Protocol [7].

5. Conclusion

It is apparent from our investigations that LALLS represents a significant advance in the assessment of wear debris, offering a number of advantages over currently used debris characterization techniques such as filtration and SEM observation. Because the LALLS route enables particle size analysis of the entire debris sample to be acquired, whilst in suspension, artefacts associated with filtering, drying and agglomeration of debris are avoided. We have shown the technique to be

TABLE III LALLS characterization of *ex vivo* wear debris

	Distribution parameter	*Ex vivo* wear debris sample particle diameter (μm)				
		A	B	C	D	E
Number distribution	D(n,0.1)	2.84	0.55	0.54	0.54	0.54
	D(n,0.5)	3.54	0.74	0.76	0.72	0.72
	D(n,0.9)	9.67	1.19	1.57	1.31	1.36
Volume distribution	D(v,0.1)	11.27	0.95	3.15	67.32	6.19
	D(v,0.5)	34.83	8.87	14.01	198.84	42.69
	D(v,0.9)	68.62	21.88	34.27	520.77	156.59
	D[4,3]	37.90	10.12	17.09	249.69	64.54
Particle concentration (vol. %)		0.0076	0.0026	0.0087	0.1471	0.0565

equally applicable to the characterization of both *ex vivo* and *in vitro* origin wear debris and will therefore find application in the clinical assessment of osteolysis and in the validation of joint simulators and prostheses. The ability to completely size the debris will have application in assessing the size distribution of wear debris in site-specific tissue samples. Furthermore, because the technique is non-destructive, LALLS should prove well suited to the characterization of particulates prior to cellular response studies.

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