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Metal wear particles: What we know, what we do not know, and why

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Abstract

The importance of wear particle characterization for orthopaedic implants has long been established in the hip and knee arthroplasty literature. With the increasing use of motion preservation implants in the spine, the characterization of wear debris, particularly metallic nature, is gaining importance. An accurate morphological analysis of wear particles provides for both a complete characterization of the biocompatibility of the implant material and its wear products, and an in-depth understanding of the wear mechanisms, ion release, and associated corrosive activity related to the wear particles. In this paper, we present an overview of the most commonly-used published protocols for the isolation and characterization of metal wear particles, and highlight the limitations and uncertainties inherent to metal particle analysis.

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Keywords: Wear particles; Morphology; Characterization; Metal-on-metal; Digestion protocol

Background

In the past decade, there has been growing acceptance of the use of motion-preservation implants in the spine. Prior experience with orthopaedic devices, such as hip and knee implants, has shown that particulate wear debris can be associated with postoperative complications such as peri-implant osteolysis.^{1–7} In the last 20 years, new materials have been developed for use in total hip and knee arthroplasty that effectively reduce the total amount of wear generated in situ. These materials include cross-linked polyethylenes, as well as combinations of metal-on-metal and ceramic-on-ceramic materials for articulating surfaces.

The introduction of modern metal-on-metal bearing surfaces for hip replacement components has resulted in renewed interest in the analysis of metal particles due to concerns for the potential long-term effects of metal wear products in vivo. Despite their increasing popularity, it is widely recognized that modern metal-on-metal bearings for total hips can generate extremely small sized metal particles.^{8–10} For a given period of usage, the total volume of metal wear debris from a metal-on-metal articulation may be much smaller compared to the wear volume of polyethy-

lene from a polyethylene-on-metal articulation.⁹ However, due to the smaller metal particle size, it is estimated that the number of metal particles can be up to 100 times greater than the number of polyethylene particles.^{8,10,11} The small size of metal particles vastly increases the total surface area of the metal exposed to the aggressively corrosive body environment, increasing the propensity to release metal ions. Moreover, concerns have been raised regarding the toxicity, corrosion, and ion release associated with metal wear debris; although the long-term implications related to carcinogenicity remain unknown to date.^{12–16}

As motion preservation gains traction as a treatment paradigm for spinal disorders, the need for accurate characterization of the wear particles generated at articulating surfaces in the spine is growing in importance. An accurate morphological analysis of wear particles can provide an understanding of the specific mechanisms of wear, and also allows for a more complete characterization of the biocompatibility of the implant materials employed. Previous experience in particle characterization for large joint orthopaedic implants has shown that these techniques are complex and on the leading edge of scientific development. However, we do stand to gain significantly from this body of knowledge as it relates to particle characterization for spinal implants. In this paper, we present an overview of the most commonly used published protocols to isolate, display,

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and morphologically and chemically characterize metal wear particles, and highlight the advantages and limitations in each.

The necessity and importance of particle characterization

In the literature, the most widely used particle characteristic is particle size which is evaluated in different ways, normally estimating the Feret's diameter (the longest distance between any 2 points along the particle boundary) or the equivalent circle diameter (the diameter of the circle that would have the equivalent area as the particle). Shape, if evaluated, is normally assessed qualitatively or semi-qualitatively. Most of the previous particle analysis work in orthopaedic biomaterials has focused on polyethylene wear debris. It has been suggested that small (less than 1 μm) polyethylene particles can be phagocytosed more easily than larger particles and that elongated particles may induce a stronger cellular reaction than rounded particles.^{17,18} More recently, the importance of metallic wear particle analysis has gained interest in the community. As a result, it is now recognized that particles interact differently with the biological environment depending on a multitude of particle characteristics including size, shape, surface topography, and chemistry.^{6,19–24}

The tribological properties of materials strongly affect the characteristics of wear particles produced during implant use. Conversely, particle characteristics can often provide information regarding not only the magnitude of wear but also the types of underlying wear mechanisms in action. For example, under normal conditions of use, the continuous generation and removal of the protective layer of a metal surface may produce extremely small particles in round or oval shapes. This generally indicates mild abrasive wear. In contrast, more severe service conditions will produce greater number of irregularly shaped and larger particles whose chemistry is closer to that of the bulk material, indicating severe abrasion. It has been recently hypothesized that microstructural changes at and below the surface are responsible for the generation of nanometer-sized wear debris with metal-on-metal implants.²⁵

There has also been increasing interest in the evaluation of biological response to particles, and, in particular, to metal wear particles. It is important to note, therefore, that evaluation of host bioreactivity to wear debris requires first and foremost a full morphological and chemical characterization of the debris. Clearly, a well-established and validated method to characterize wear debris is necessary to facilitate rigorous bioreactivity testing, identify the underlying wear mechanisms, and understand the material degradation phenomena.

Laboratory wear simulations provide an accurate and reliable prediction of an implant's wear performance. In addition to providing the amount of wear, it is important for laboratory wear tests to also analyze the morphology, dis-

tribution, and chemical composition of the wear particles generated. Furthermore, the validation of wear simulation studies requires an accurate and direct comparison of the particles from the wear simulator to particles extracted from peri-implant tissues. Therefore, the isolation protocol should ideally be designed so that it can be used for the isolation of particles from both laboratory wear simulation studies and from explanted tissues. Unfortunately, metal particles obtained from tissues have already gone through some extent of degradation in vivo, complicating such comparisons.

In addition to wear simulations, laboratory studies of bioreactivity also have the potential to provide vital information in predicting clinical performance of new materials. For such studies ideally, the same method used to isolate and characterize particles from wear simulations should also be used to isolate and characterize particles for prediction of bioreactivity. This would ensure that the particles provided for cellular bioreactivity testing have the same morphology, size distribution, and chemical composition as those produced during the wear process. This fundamental observation leads to an important consequence: the isolation process must be designed so that particles can be fully characterized and virtually free of any contaminant or residue.

A synopsis of the information that can typically be obtained from analysis of wear particles is illustrated in Fig. 1.

In recent years, numerous novel implants have been introduced for the spine. Typically, FDA requirements for approval of a new implant include, among others, results of fatigue and wear testing.²⁶ Increased recognition of the importance of wear particle analysis has resulted in regulatory organizations requiring particle analysis as a routine component of implant fatigue and wear testing, general procedures of which are described in various ASTM and ISO documents.^{27,28} So far, these documents provide general guidelines but do not outline the details of the methodology required to conduct reliable and reproducible particle isolation and characterization. Specifically, an FDA guidance document²⁶ specifies, "You should . . . extract the wear debris from the test solution using a filter with a pore size that allows collection of sub-micron particles," and that ". . . you should characterize the wear debris." As discussed below, these requirements are not well-defined and overlook the complexities of the process of particle analysis. Substantial challenges remain towards the goal of establishing a single, accurate and repeatable method for the analysis of metal particles generated by implant wear for universal application in the orthopaedic and spine communities.

Past accomplishments

Many different techniques and approaches have been used over the years in order to establish a reliable and repeatable methodology for the isolation and characterization of metal wear particles from tissues or from lubricants used for wear simulations.^{8,9,29–47} A thorough review of

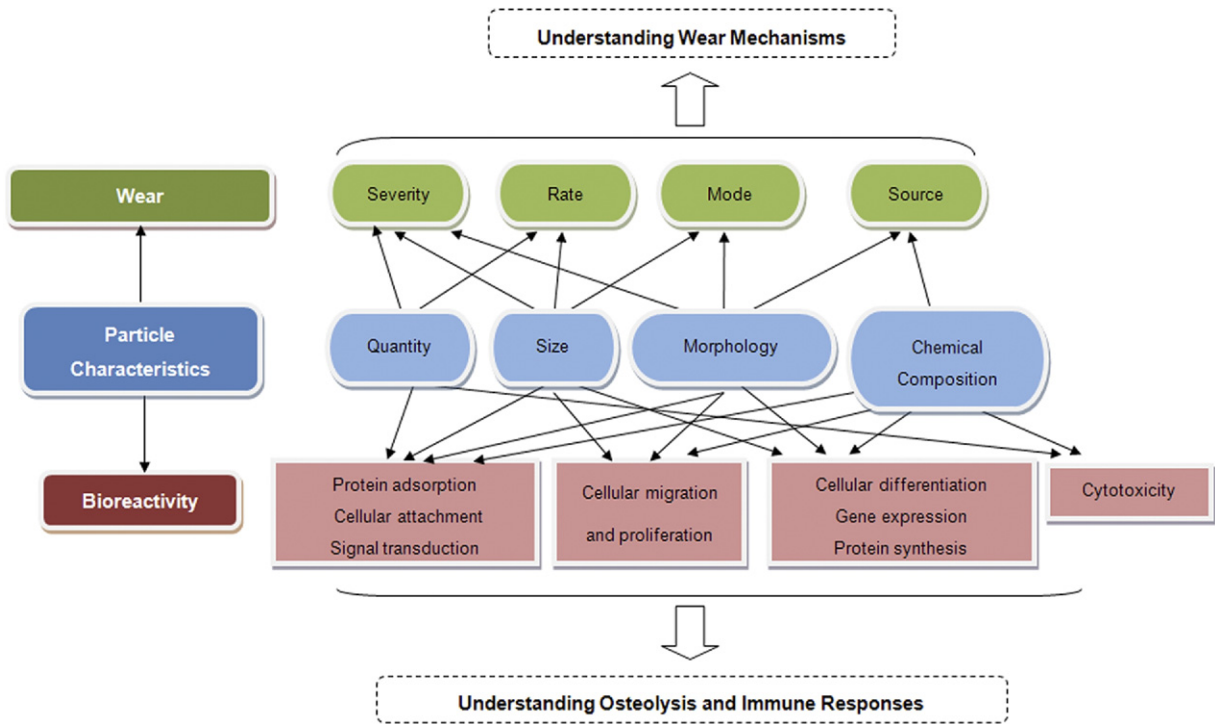


Fig. 1. Interrelations among wear particle characteristics, wear mechanisms and biological activity.

these methods is beyond the scope of this article. However, in this section, we briefly summarize the most significant past accomplishments of key investigators towards particle isolation and analysis.

Any particle analysis protocol essentially consists of 4 parts: digestion and isolation, display and image acquisition, morphological and chemical characterization, and statistical analysis.

Digestion and isolation

The first step in the analysis of particles is their isolation from serum lubricant solutions, tissues, or synovial fluid. Each of these is characterized by the presence of different proteins, lipids, and other constituents such as hyaluronic acid. Particles can bind to these macromolecules or be trapped along with other residues. In order to free the particles from any substance that eventually might interfere with their characterization, researchers have used different approaches.

Selection of each particular approach also depends on the

type of particles and type of media in which the particles are dispersed. Particles from the wear of metal alloys are of 2 types: ceramic particles, which are oxides or carbides and thus fairly inert, and metal alloy particles coming from the bulk materials, thus having all the characteristics of the metal constituents including susceptibility to corrosion and elution processes. For example, it has been established that the use of an aggressive alkaline solution, such as sodium hydroxide which is typically used for the isolation of polyethylene particles,³⁰ has a detrimental effect on metal particles, affecting both their shape and chemical composition.^{37,48} Various (and widely different) protocols involving the use of enzymes and detergents have been developed with the goal of digesting the proteinaceous content of the solution, while at the same time preserving the characteristics of the particles as closely as possible.^{8,9,37,46,47}

For the digestion and isolation of metal particles, various methods have been published over the years, but 4 are most commonly recognized (Table 1). Chronologically,

Table 1
Protocols for metal particle isolation/characterization

Authors	Digestion	Display	Imaging	Size analysis	Shape analysis	Size/Shape distribution	Evaluation of sample distribution
Schmiedberg	Papain + pK (NaOH wash)	Drop Evaporation	SEM	Y	N	N	N
Doorn	Papain + pK	Nebulization	TEM	Y	N	N	N
Catelas	Papain + pK	Resin Embedding	TEM	Y	Y	Y	Y
Brown	Papain + p-K + yeast lytic enzyme + Zymolyase	Sequential Filtration	SEM	Y	N	Y	N

Table 2
Steps involved in enzymatic protocols for metal particle isolation

STEPS	Schmiedberg ⁴⁰	Doorn ¹⁵	Catelas ¹⁰	Brown ⁴
Washing	6	7	9	12
Centrifugation	7	4	6	2
Dilution	2	4	3	8
Digestion	2	2	2	12
Boiling	-	4	3	5
heating	-	-	-	1
Transfer	3	-	-	
Supernatant removal	7	4	6	

Schmiedberg et al⁴⁶ applied a double enzymatic protocol using papain and proteinase-K followed by a final wash with NaOH. Doorn et al⁸ applied an enzymatic protocol to isolate particles from tissue and used a combination of reagents to first defat and denature the samples. Enzymatic digestion with papain and then proteinase-K in Tris-HCl was adopted for the digestion step. Catelas et al³⁷ applied an enzymatic protocol similar to the one developed by Schmiedberg et al. Sodium dodecyl sulfate (SDS) was used as a detergent. Digestion was performed using papain and proteinase-K in Tris-HCl at 65°C and 55°C, respectively. More recently, Brown et al⁴⁷ applied a multi-step protocol involving the use of 4 different enzymes (papain, proteinase-K, yeast lytic enzyme, and Zymolyase) and various other reagents including SDS, MOPS, TCEP, Tris-HCl, HEPES buffer, Sodium Phosphate buffer, β -mercaptoethanol and Elmaclean 65 solution (Table 2).

Display and image acquisition

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are the 2 main tools used for imaging particles. Both instruments, if equipped with energy-dispersive x-ray spectroscopy (EDS), can give information on the chemical composition of the particles, discriminating those of interest from contaminants and residues. Furthermore, SEM and TEM can collect other information that can be used to better understand the wear mechanism that created the particles. It should be noted that SEM and TEM can provide complementary data. SEM can be used to obtain information about the surface topography of the particles, and TEM can be used to understand the crystallinity and thus the phase composition of the constituent alloy. Both instruments provide images containing size and shape information.

SEM and TEM function in fundamentally different ways, and, therefore, have different limitations with regard to particle analysis. The TEM beam, in fact, passes through the sample, and the image obtained is the projection of the object in the path of the beam. Depending on the orientation of the particle to the beam, the error associated with its dimensions and any following estimation of its shape and size may not be trivial. Therefore, great caution is required in analyzing TEM images for size and shape estimations. On the other hand, until recently, TEM provided much

greater magnifications than SEM, allowing visualization and chemical characterization of extremely small particles.

The greatest consideration when performing TEM analysis for imaging isolated wear particles is sample preparation, which is more difficult for TEM than it is for SEM and strongly dependent on operator skills. The quality of particle sample preparation determines in large part whether or not the micrograph will be clear and representative of the entire particle distribution. Vastly different methods have been adopted during the years to prepare particles for characterization either by SEM or TEM. Filtering for SEM preparation has been the most commonly used technique, although resin-embedding, nebulization, and drop evaporation have been also employed. Similarly, Schmiedberg et al⁴⁶ evaporated 5 μ L of solution onto a carbon planchet for SEM analysis. In contrast, Doorn et al⁸ used a nebulizer to display the particles on a copper grid for TEM analysis. Catelas et al³⁷ developed an original procedure to embed particles in resin and then obtain slices of about 90-nm thickness for TEM analysis. Brown et al⁴⁷ used vacuum filtration to collect particles onto filters for SEM analysis.

Morphological and chemical characterization

All investigators who have conducted particle analysis have provided an estimation of particle size. Some have also included size distribution as a part of their analysis. In contrast to determining size distributions, analysis of particle shapes typically requires subjective assessments by the observer. For example, in determining size distribution, Schmiedberg et al⁴⁶ defined length and width as the longest axis through the particle and the shorter line segment that spans the length at a 90° angle. However, the shape information was not quantified; rather, the authors observed that “fragments appeared to be predominantly spherical in shape, but some ovoid fragments were also observed.” Doorn et al⁸ reported the median size of particles regardless of their shape; however, those authors reported most of the particles to be round in shape.

Unlike previous authors, Catelas et al³⁷ reported quantification of shape as well as size distributions. For size estimation, the length, defined as the maximum dimension, and the width, defined as the maximum orthogonal dimension, were used. For shape and morphology estimation, the ratio of length to width was used to categorize particles into round, oval, and needle-shaped.

More recently, Brown et al⁴⁷ evaluated a maximum diameter to calculate the size distribution of particles and generically categorized them as round or irregular shape particles.

Statistical analysis

Statistical analysis is used to estimate characteristics of the general population using sample observations. It follows that microscopic images (micrographs) of each sample should be representative of the overall sample. It may be necessary to obtain several different micrographs of the

same sample to determine the morphological distribution of the whole sample.

There is currently no consensus among researchers on the best procedure to follow to evaluate the size distribution of particles in a sample. Schmiedberg et al.⁴⁶ evaluated a total of 20 particles and, assuming a normal distribution, reported mean and standard deviation of length and width. By comparison, Doorn et al.⁸ reported the widest diameter of the particle on a much larger population. A different number of particles were characterized for each sample (from 1 to 580) and no indication about the accuracy of the distribution was given. Catelas et al.³⁷ reported the mean maximum dimension (length), the mean maximum orthogonal dimension (width), and shape of an average of 300 particles evaluated on different micrographs. In a more recent article, up to 1300 particles from up to 10 micrographs were analyzed.⁴⁹ It must be acknowledged that Doorn et al analyzed particles from tissues, explaining probably why the authors had a different number of particles for each sample, as wear greatly varies depending on the patients.

Discussion

The particle analysis methods discussed above have contributed significantly towards our understanding of the nature of particulate wear debris. However, a consensus still does not exist as to which method should become the standard for routine use. The absence of a universally accepted method for particle analysis and the use of different techniques to isolate and characterize particles make it difficult to compare results from different studies. Despite the lack of consensus, attempts have been made to establish standards for a universally accepted method.

As discussed below, accurate analysis of wear particles is limited by multiple problems. The lubricants used in laboratory wear simulations are typically serum solutions containing high concentrations of protein. Digestion of lubricant protein is necessary to retrieve the debris efficiently. However, inefficient and/or incomplete digestion of lubricant proteins can lead to agglomeration or loss of particles (Fig. 2). In particular, nanoparticles can easily bind to proteins or be lost by adsorption to surfaces and/or adhesion to nonpelleting debris. That is, particles, especially in the nanometer size, can bind to centrifuge tubes surfaces or to lipids and proteins or fragments of them. Furthermore, even in the presence of complete digestion, separation of the particles from lubricant digests can lead to particle loss and/or particle agglomeration; thus the choice of the technique to retrieve particles from the digest lubricant can affect the final distribution. Moreover, information on particle morphology, particle size, and particle size distribution is as critical a component as designing and conducting the digestion and isolation methods that are used to generate the particle images. Because of these difficulties, the errors associated with particle characterization can be very high.

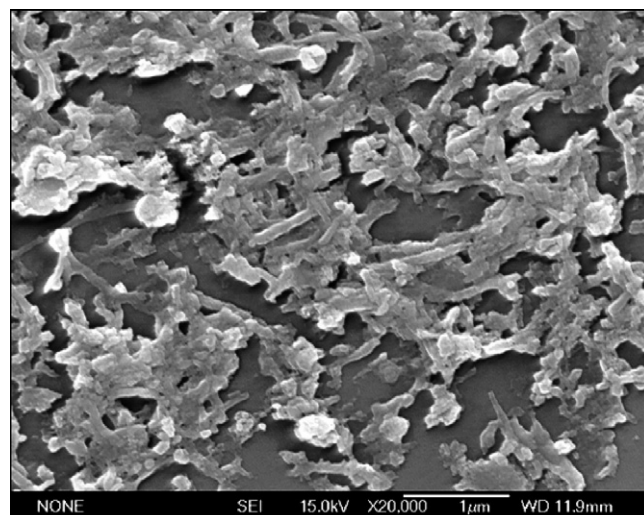


Fig. 2. Metal wear particle and organic residue after double enzymatic digestion.

Digestion and isolation

As indicated above, the process of digestion involves several steps to obtain particles that are as clean as possible by the removal of any proteinaceous content and residue. Initially, researchers used fewer steps in their digestion protocol; but over time, in the interest of obtaining purer and better-separated particles, more steps were added to the digestion protocols. All of the particle isolation protocols discussed here have involved a large number of steps, as shown in Table 2, and all of them involved several centrifugation steps to purify and collect particles. However, there is an optimum balance between loss of particles and purification. In fact, every purification step introduced into a protocol can potentially lead to loss of particles or be aggressive enough to distort their physical and chemical characteristics.

A discussion of specific side effects of each reagent in the published digestion protocols is beyond the scope of this review. However, it is worth mentioning that if centrifugation is involved in isolating nanoparticles, any substance that can affect their buoyant density can be a source of loss of particles.

In consideration of these issues, any enzymatic digestion protocol needs to be optimized and fine-tuned so that the number of steps and enzymes can be reduced to a minimum. Similarly, reagents need to be selected so that their interaction with particles is minimal.

In the final analysis, the reasoning behind digestion and isolation protocols to obtain purified particles is 2-fold: the need to obtain particles that can be easily identified and characterized, and the need to access contaminant-free particles that can subsequently be fed to cells in bioreactivity tests. However, the images obtained using protocols established so far frequently depict apparent residues and agglomerates, indicating that these needs have only been partially addressed.

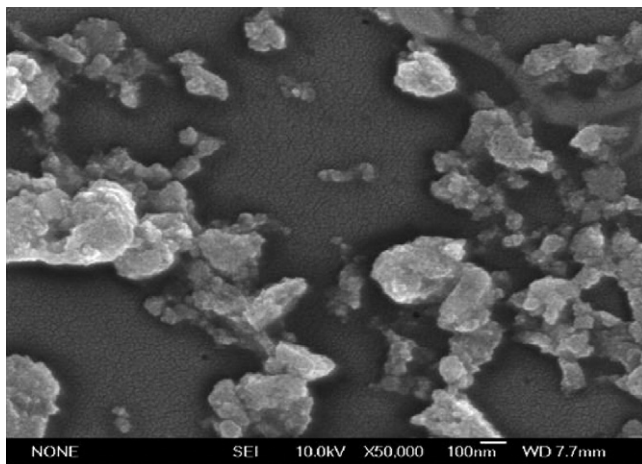


Fig. 3. Metal wear particles on a polycarbonate filter.

Display and image acquisition

The ultimate goal of an efficient display technique should be clear and sharp images of well separated particles that allow for the use of an image analysis software to automatically or semi-automatically measure and calculate the features associated with each single particle. Thus the contrast between the background and the observed particle should be as high as possible so that the error associated with image analysis is minimized. Moreover, the background should be chosen for absence of features that could interfere with the recognition of particles. Ideally, the background should be as smooth and as uniform as possible. If the particles are not well separated, their chemical composition cannot be accurately identified through EDS. Consequently, it may not be possible to discriminate particles of interest from contaminants.

Most researchers have used filtration in order to separate particles.^{9,47} Filtering is the easiest way of collecting particles on a support that can then be used for SEM analysis. However, filter membranes normally present a distribution of pores that induce agglomeration by a funnel-effect. This effect occurs because the washing solution (and thus the particles) is forced to follow a path that is not straight, concentrating the wear particles around the pores (see Fig. 3). Furthermore, some of the pores can easily get clogged by particles bigger than the pore size or by residue, thus reducing the overall filtering area and increasing the possibility of further agglomeration. Lastly, the use of membranes of specific pore size introduces an artifactual threshold in the size of recovered particles, since most of the particles smaller than the pore size are lost. The number of particles lost through pores may be significant; consequently, the resulting size distribution might not represent the actual distribution of particles in the sample. Furthermore, displaying on filter membranes may be unacceptable in terms of contrast between the background and the particles, or because the pores/features on the filter can incorrectly be counted as particles or may be difficult to distinguish.

Although they are quite different, both nebulization and drop evaporation may very likely lead to agglomeration of particles. This is due to natural formation of droplets that may hold several particles together, eventually forming agglomerates once the liquid has evaporated. If the evaporation is carried out at high temperature, the agglomerates can be extremely compacted, destroying the resolution of individual particles. Furthermore, during nebulization, it is probable that particles of different sizes are unevenly nebulized and distributed, remain trapped, or fall away from the target.

Embedding in resin for TEM analysis has gained popularity in the particle analysis community. Unfortunately, embedding, if not done accurately, can also be a source of particle agglomeration. Specifically, the particles are pelleted at a bottom of a centrifuge tube and thus initially forced together into potential artifactual agglomerates. The resin is then poured into the tube and allowed to cure on top of the pellet, leaving no possibility for particle separation. Furthermore, some of the particles can remain attached at the bottom of the tube or be lost due to partial embedding. Slices of 90–100 nm are then cut once the resin is cured. As acknowledged by some authors, slices may contain particles whose orientation could lead to erroneous evaluation of the projected dimension obtained through TEM. Consequently, there may be an artificial skewing of particle size, because larger particles may not be sectioned and the resin will simply tear around them, leaving only the smaller particles in the successful sections. Finally, particles may be stacked too close on top of each other within the slice to be discriminated.

Size and morphological distribution

Analysis of wear particle size and morphological distributions has often been limited to the evaluation of few parameters; eg, length and width, precluding a detailed characterization and discrimination. This is typically compounded by the fact that image analysis is normally performed in the presence of significant amounts of agglomerates and, in general, using poorly contrasted images. Consequently, the operator is forced to select the particles manually and then extract the parameters of interest either manually or via dedicated software, making the entire process tedious and heavily dependent on the operator's skills and judgment.

For estimation of size distribution, a few simple descriptors are often sufficient and established in the literature. On the other hand, description of morphology has to be done using a much more elaborate analysis. Morphological analysis should give an accurate description of the shape and texture of the observed object. Typically, this cannot be extrapolated by applying simple algorithms to dimensional measurements. The difficulty of proper morphological analysis is often underestimated by researchers in the orthopaedic field.

A more accurate description of particle size and shape should involve the use of multiple parameters and morpho-

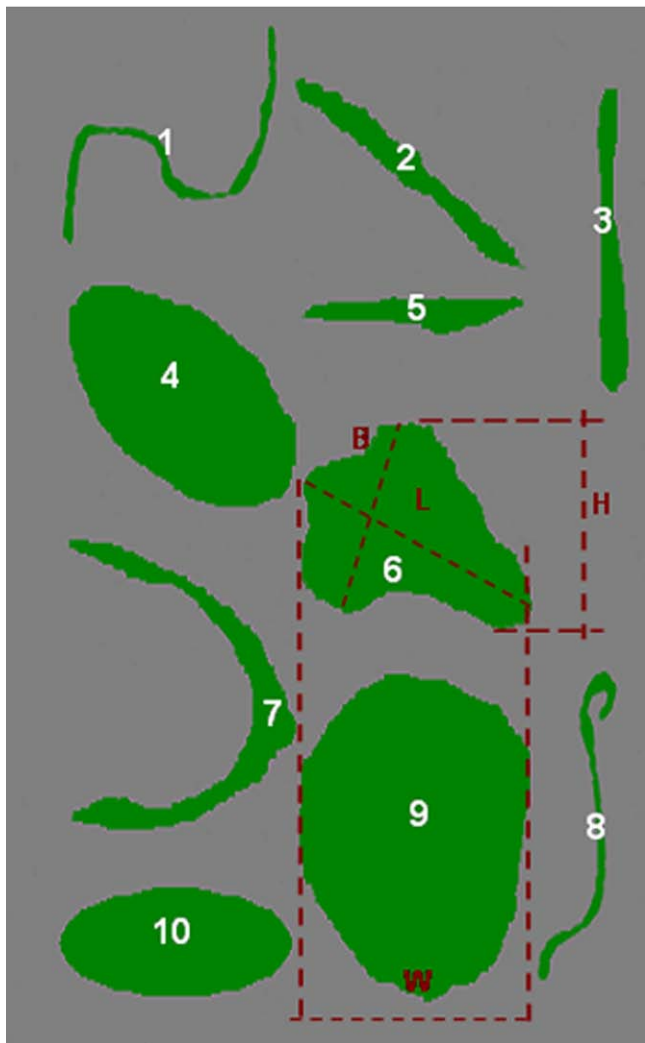


Fig. 4. Example of particles outlined from SEM micrographs for morphological characterization.

logical descriptors calculated via image analysis software. ASTM F1877-98 standard specifies different parameters to evaluate the size of each particle and to further define morphological descriptors.²⁸ Commonly measured characteristics are width (W), height (H), d_{max} or length, d_{min} or breadth, fiber length (FL), fiber breadth (FB), perimeter (P), and area (A). These data can be used to calculate the 5 morphological parameters specified in the standard, ie, equivalent circle diameter (ECD), aspect ratio (AR), elongation (E), roundness (R), and form factor (FF). Figure 4 shows an example of the particle length, breadth, height and width for different types of particles. These shapes are outlined from SEM particle images and grouped together for demonstration purposes. Table 3 shows the shape and morphological descriptors obtained for each of the objects in Figure 4.

A simple example demonstrates how each of these parameters alone is hardly sufficient to fully characterize a given particle. In Figure 4, particles 6 and 4 have the same height and length but are very different in shape. Most of the

particles shown have the same d_{max} but very different shapes. A combination of parameters might be useful for a more precise separation. Orientation of the particles in 3-dimensional space also has an influence on the evaluation of the size when only 1 parameter is chosen to evaluate size distribution. The use of artificial intelligence software could help in automatically discriminating particles of different shapes. These difficulties indicate that the complications associated with morphological analysis are far from resolved.

It is not clear whether, and if so how, differences in shape and morphology as described above may affect bioreactivity. However, in the absence of characterization, it is virtually impossible to detect such clinical importance.

Statistics

Appropriate statistical analysis is crucial in describing and comparing distributions of particle size and shape descriptors. Different micrographs of the same sample should be compared to ensure that they provide the same distribution estimates; otherwise, the previous steps in the method should be questioned. This necessary step has not been reported in many of the commonly cited publications on metal particle analysis. Another critical point is to analyze and average a large enough number of particles from different micrographs, after having classified them by shape. Likewise, comparison of the results of one study to the next among different investigators or using different methods can only be made using proper statistical estimates of the distributions.

It is well established (and it stands to reason) that the distribution of particles is far from normal; however, comparison of particle distributions is often conducted using analyses intended only for normal distributions. Specifically, the most common error in statistical representation of particle distributions is the use of means, standard deviations, and other parametric analysis such as *t* tests and analysis of variance, all of which are valid only if distributions are normal in the general population. Fortunately, appropriate statistical methods are available for describing and comparing any general type of distribution (eg, P and Q plots). However, the literature on metal particle analysis has not taken advantage of these methods so far.

Table 3
Morphological characterization of particles in Fig. 4

Object #	SHAPE	ECD	AR	E	R	FF
1	Fibril	0.31	1.96	48.0	0.06	0.06
2	Rod	0.37	8.33	16.6	0.09	0.17
3	Rod	0.35	5.80	11.4	0.11	0.23
4	Oval	0.84	1.97	1.7	0.48	0.73
5	Rod	0.32	7.30	13.4	0.09	0.20
6	Oval	0.79	1.54	3.0	0.46	0.59
7	Fibril	0.51	1.23	29.5	0.16	0.10
8	Fibril	0.28	3.61	33.6	0.06	0.09
9	Round	1.10	1.02	1.0	0.89	0.83
10	Oval	0.64	2.90	2.7	0.34	0.62

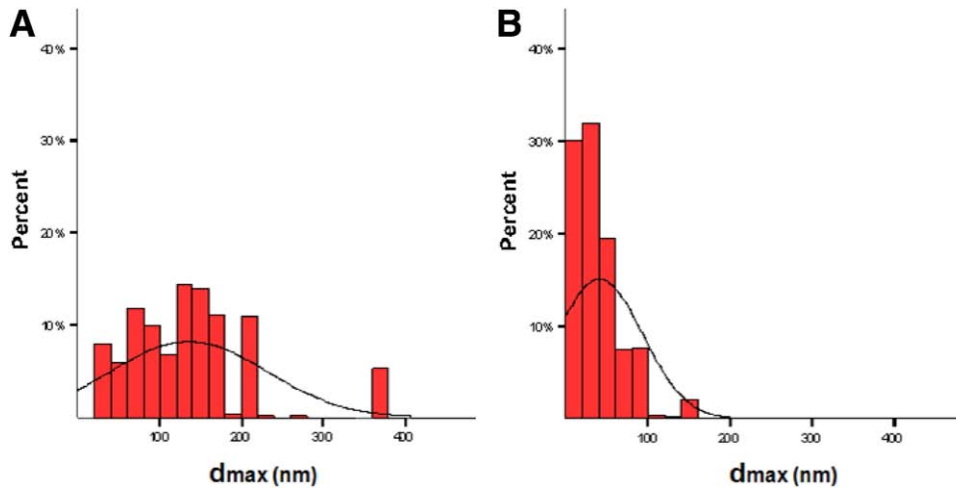


Fig. 5. Effect of number of particles on apparent size distribution: (A) 30% of the particles visible on the micrograph are taken into account; (B) 75% of the particles visible on the micrograph are taken into account.

The next most common source of error in statistical representation of particles of an image is disproportionately counting a greater fraction of large or small particles, giving rise to bias towards that fraction compared to what is actually present in the whole sample. Figure 5 shows the d_{max} calculated for the same image, but counting a different number of particles. In the first image, only 30% of the particles are taken into consideration, while in the second one, 75% of the particles are included in the distribution. Clearly, including a greater fraction of the particles in this sample results in a substantially more accurate estimate of the peak d_{max} .

Another potential source of error in estimation of size distribution is the image magnification. For example, an image at a given magnification can give a good estimate of the bigger particles, but may not be sufficiently detailed to allow a good estimation of the smaller particles. Several images taken at different magnification should thus be considered. In this case, the contribution of each image to the final distribution must be accurately taken into account.

It is important to accurately evaluate the distribution of particles across different images. The minimum number of particles to be analyzed to ensure statistical accuracy in estimating the general distribution of size and morphology is often overlooked by researchers. More often than not, the sample simply does not contain enough particles, perhaps due to processing time and expense.

Comparison of distribution of the particles on each single micrograph is a strong indicator of the uniform dispersion of particles and the accuracy of the measurement. However, so far, the state of dispersion of isolated particles is a characteristic that has not been taken into account. The relative number of primary (single) particles in comparison to agglomerates could produce useful parameters that could help the researcher to better understand the results obtained with a specific protocol.

Conclusion

Metal particle analysis consists of multiple, complex processes that span several disciplines in science and technology. Each of the steps (digestion and isolation, display and image acquisition, morphological and chemical characterization, statistical analysis) requires taking advantage of the state of the art in technology and knowledge in the respective field. In part, due to the technical difficulties and the many challenges involved in each step, investigators have established significantly different techniques to address each of the problems. Consequently, each of these different techniques may produce entirely different results for a given sample. Therefore, the results obtained using different techniques cannot be compared without taking into consideration the differences among these complex, multi-step processes. The lack of agreement in particle isolation and analysis methodology also poses tremendous challenges in studies of the host biological response to particulate wear debris. While there is a need to better characterize and control the particle size, shape, and distribution for cell culture or animal studies, there is no consensus in the methods needed to accomplish this.

Today, the machinery is in place to isolate, visualize and chemically characterize the metallic wear particles from implants with articulating surfaces. In spite of the various standards and guidance documents, there is currently no consensus in the community on the methods used to generate these data. Equally important is that there is also no consensus on the methodology for the analysis and interpretation of the results. Therefore, data on wear particle characterization must be critically assessed by the reader in view of the limitations of each specific set of techniques employed. In general, these data do provide valuable information on the potential characteristics of the wear particles. However, caution is still recommended in extrapolating particle analysis results to a predictive statement of clinical

host response to a particular implant design. Further research must be conducted in order to develop and validate refined techniques within these interrelated areas of wear and biology to arrive at a reasonable set of specifications and requirements for the design and material selection of implants for motion preservation implants for the spine and, more generally, for arthroplasty devices.

References

- Jacobs JJ, Campbell PA, Y TK. How has the biologic reaction to wear particles changed with newer bearing surfaces? *J Am Acad Orthop Surg* 2008;16 Suppl 1:S49–55.
- Carr AM, DeSteiger R. Osteolysis in patients with a metal-on-metal hip arthroplasty. *ANZ J Surg* 2008;78:144–7.
- Goodman SB. Wear particles, periprosthetic osteolysis and the immune system. *Biomaterials* 2007;28:5044–8.
- Groot D, Gosens T, Leeuwen NC, Rhee MV, Teepeen HJ. Wear-induced osteolysis and synovial swelling in a patient with a metal-polyethylene wrist prosthesis. *J Hand Surg Am* 2006;31:1615–8.
- Ingham E, Fisher J. The role of macrophages in osteolysis of total joint replacement. *Biomaterials* 2005;26:1271–86.
- MacQuarrie RA, Fang Chen Y, Coles C, Anderson GI. Wear-particle-induced osteoclast osteolysis: the role of particulates and mechanical strain. *J Biomed Mater Res B Appl Biomater* 2004;69:104–12.
- Hallab NJ, Cunningham BW, Jacobs JJ. Spinal implant debris-induced osteolysis. *Spine* 2003;28:S125–38.
- Doom PF, Campbell PA, Worrall J, Benya PD, McKellop HA, Amstutz HC. Metal wear particle characterization from metal on metal total hip replacements: transmission electron microscopy study of periprosthetic tissues and isolated particles. *J Biomed Mater Res* 1998;42:103–11.
- Tipper JL, Firkins PJ, Ingham E, Fisher J, Stone MH, Farrar R. Quantitative analysis of the wear and wear debris from low and high carbon content cobalt chrome alloys used in metal on metal total hip replacements. *J Mater Sci Mater Med* 1999;10:353–62.
- Firkins PJ, Tipper JL, Saadatizadeh MR, et al. Quantitative analysis of wear and wear debris from metal-on-metal hip prostheses tested in a physiological hip joint simulator. *Biomed Mater Eng* 2001;11:143–57.
- Williams P, Clarke I. The role of wear debris and biological response in the assessment of total joint performance. *J Bone Joint Surg Br* 2004;86-B(Suppl IV):437.
- Lewis AC, Ladon D, Heard PJ, Peto L, Learmonth I. The role of the surface chemistry of CoCr alloy particles in the phagocytosis and DNA damage of fibroblast cells. *J Biomed Mater Res A* 2007;82:363–72.
- Cobb AG, Schmalzried TP. The clinical significance of metal ion release from cobalt-chromium metal-on-metal hip joint arthroplasty. *Proc Inst Mech Eng [H]* 2006;220:385–98.
- Brown C, Fisher J, Ingham E. Biological effects of clinically relevant wear particles from metal-on-metal hip prostheses. *Proc Inst Mech Eng [H]* 2006;220:355–69.
- Rakshit DS, Ly K, Sengupta TK, et al. Wear debris inhibition of anti-osteoclastogenic signaling by interleukin-6 and interferon-gamma. Mechanistic insights and implications for periprosthetic osteolysis. *J Bone Joint Surg Am* 2006;88:788–99.
- Willert HG, Buchhorn GH, Fayyazi A, et al. Metal-on-metal bearings and hypersensitivity in patients with artificial hip joints. A clinical and histomorphological study. *J Bone Joint Surg Am* 2005;87:28–36.
- Ren W, Yang S-Y, Fang H-W, Hsu S, Wooley PH. Distinct gene expression of receptor activator of nuclear factor-[kappa]B and rank ligand in the inflammatory response to variant morphologies of UHMWPE particles. *Biomaterials* 2003;24:4819–26.
- Ingham E, Fisher J. The role of macrophages in osteolysis of total joint replacement. *Biomaterials* 2005;26:1271–86.
- Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. Macrophage/particle interactions: effect of size, composition and surface area. *J Biomed Mater Res* 1994;28:81–90.
- Campbell P, McKellop H, Alim R, et al. Metal-on-metal hip replacements: Wear performance and cellular response to wear particles. In: JA D, RL K, RM P (eds). Cobalt base alloys for biomedical applications. ASTM: 1999. Volume STP 1365, p. 193–209.
- Chang BS, Brown PR, Sieber A, Valdevit A, Tateno K, Kostuik JP. Evaluation of the biological response of wear debris. *Spine J* 2004;4(6 Suppl):239S–44S.
- Endo M, Tipper JL, Barton DC, Stone MH, Ingham E, Fisher J. Comparison of wear, wear debris and functional biological activity of moderately crosslinked and non-crosslinked polyethylenes in hip prostheses. *Proc Inst Mech Eng [H]* 2002;216:111–22.
- Ingham E, Fisher J. Biological reactions to wear debris in total joint replacement. *Proc Inst Mech Eng [H]* 2000;214:21–37.
- Sommer B, Felix R, Sprecher C, Leunig M, Ganz R, Hofstetter W. Wear particles and surface topographies are modulators of osteoclastogenesis in vitro. *J Biomed Mater Res A* 2005;72:67–76.
- Buscher R, Tager G, Dudzinski W, Gleising B, Wimmer MA, Fischer A. Subsurface microstructure of metal-on-metal hip joints and its relationship to wear particle generation. *J Biomed Mater Res B Appl Biomater* 2005;72:206–14.
- FDA. Guidance for industry and FDA staff: Preparation and review of investigational device exemption applications (IDEs) for total artificial discs. In: Services USDoHaH, administration FaD, health CfDaR (eds). Volume 1637, 2008.
- ISO_17853-2003. Wear of implant materials - Polymer and metal wear particles - Isolation, characterization and quantification.
- ASTM_F1877-98. Standard practice for characterization of particles.
- Schmalzried TP, Jasty M, Rosenberg A, Harris WH. Polyethylene wear debris and tissue reactions in knee as compared to hip replacement prostheses. *J Appl Biomater* 1994;5:185–90.
- Campbell P, Ma S, Yeom B, McKellop H, Schmalzried TP, Amstutz HC. Isolation of predominantly submicron-sized UHMWPE wear particles from periprosthetic tissues. *J Biomed Mater Res* 1995;29:127–31.
- Maloney WJ, Smith RL, Schmalzried TP, Chiba J, Huene D, Rubash H. Isolation and characterization of wear particles generated in patients who have had failure of a hip arthroplasty without cement. *J Bone Joint Surg Am* 1995;77:1301–10.
- McKellop HA, Campbell P, Park SH, et al. The origin of submicron polyethylene wear debris in total hip arthroplasty. *Clin Orthop Relat Res* 1995;311:3–20.
- Doorn PF, Campbell PA, Amstutz HC. Metal versus polyethylene wear particles in total hip replacements. A review. *Clin Orthop Relat Res* 1996;329(Suppl):S206–16.
- Hirakawa K, Bauer TW, Stulberg BN, Wilde AH, Secic M. Characterization and comparison of wear debris from failed total hip implants of different types. *J Bone Joint Surg Am* 1996;78:1235–43.
- Schmalzried TP, Campbell P, Schmitt AK, Brown IC, Amstutz HC. Shapes and dimensional characteristics of polyethylene wear particles generated in vivo by total knee replacements compared to total hip replacements. *J Biomed Mater Res* 1997;38:203–10.
- Wirth MA, Agrawal CM, Mabrey JD, et al. Isolation and characterization of polyethylene wear debris associated with osteolysis following total shoulder arthroplasty. *J Bone Joint Surg Am* 1999;81:29–37.
- Catelas I, Bobyn JD, Medley JB, et al. Effects of digestion protocols on the isolation and characterization of metal-metal wear particles. I. Analysis of particle size and shape. *J Biomed Mater Res* 2001 Jun 5;55:320–9.
- Visentin M, Stea S, Squarzoni S, et al. Isolation and characterization of wear debris generated in patients wearing polyethylene Hylamer inserts, gamma irradiated in air. *J Biomater Appl* 2005;20:103–21.
- Tipper JL, Galvin AL, Williams S, et al. Isolation and characterization of UHMWPE wear particles down to ten nanometers in size from in

- vitro hip and knee joint simulators. *J Biomed Mater Res A* 2006;78:473–80.
40. Wagner CN, Shabaik AH. Preparation and characterization of wear debris of orthopedic materials for biocompatibility studies. *J Biomed Mater Res* 1976;10:653–70.
 41. Blumenthal NC, Cosma V, Jaffe W, Stuchin S. A new technique for quantitation of metal particulates and metal reaction products in tissues near implants. *J Appl Biomater* 1994;5:191–3.
 42. Jacobs JJ, Shanbhag A, Glant TT, Black J, Galante JO. Wear debris in total joint replacements. *J Am Acad Orthop Surg* 1994;2:212–20.
 43. Margevicius KJ, Bauer TW, McMahon JT, Brown SA, Merritt K. Isolation and characterization of debris in membranes around total joint prostheses. *J Bone Joint Surg Am* 1994;76:1664–75.
 44. Salvati EA, Evans BG, Betts F, Doty SB. Particulate debris in cemented total hip replacement. *Instr Course Lect* 1994;43:277–88.
 45. Savio JA III, Overcamp LM, Black J. Size and shape of biomaterial wear debris. *Clin Mater* 1994;15:101–47.
 46. Schmiedberg SK, Chang DH, Frondoza CG, Valdevit AD, Kostuik JP. Isolation and characterization of metallic wear debris from a dynamic intervertebral disc prosthesis. *J Biomed Mater Res* 1994; 28:1277–88.
 47. Brown C, Williams S, Tipper JL, Fisher J, Ingham E. Characterisation of wear particles produced by metal on metal and ceramic on metal hip prostheses under standard and microseparation simulation. *J Mater Sci Mater Med*. 2007;18:819–27.
 48. Catelas I, Bobyn JD, Medley JJ, Zukor DJ, Petit A, Huk OL. Effects of digestion protocols on the isolation and characterization of metal-metal wear particles. II. Analysis of ion release and particle composition. *J Biomed Mater Res* 2001;55:330–7.
 49. Catelas I, Bobyn JD, Medley JB, Krygier JJ, Zukor DJ, Huk OL. Size, shape, and composition of wear particles from metal-metal hip simulator testing: effects of alloy and number of loading cycles. *J Biomed Mater Res A* 2003;67:312–27.