A Unified Isolation and Display Method for Either SEM or TEM Morphometric Analysis of Metallic Wear Debris

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Introduction

Reliable prediction of wear and biological reaction to wear debris depends on accurate evaluation of wear particle morphology and composition. Accurate analysis of wear particles is, however, limited by a multitude of problems. Specifically, inefficient and/or incomplete digestion of lubricant proteins can lead to agglomeration or loss of particles. Further, even in the presence of complete digestion, separation of the particles from lubricant digests by filtration or resin embedding can lead to particle loss and/or agglomeration. Additionally, nanoparticles can easily bind to proteins, be lost by adsorption to surfaces and/or adhesion to non-pelleting debris, or escape through filter pores.

Particles have been imaged either by SEM or TEM and, so far, the choice of one technique or the other has affected not only the display method, but often also the digestion method adopted. As a result, if SEM and TEM analysis were both found necessary, they had to be performed on different samples, producing results that might be easily compared or integrated.

We present an isolation and display method developed to analyze the same sample of wear particles with either SEM or TEM, enabling not only accurate and reproducible morphometric analysis but also complete integration of topographical and phase information.

Materials and Methods

Six lubricant samples from wear simulation tests of artificial metal-on-metal joint arthroplasties containing 20-95% serum were centrifuged in a SW32 rotor at 25 ºC and 30 krpm for 4 hr to concentrate the particles and remove serum. Pellets containing metal particles and protein precipitates were digested with continuous agitation in 5 ml of 8M urea, 0.1M Hepes, pH 7.5 containing calcium and Proteinase K (PK) (1mg/ml) at 37 ºC for 24h with periodic additions of PK and sonication. Calcium was then over titrated with EDTA and the samples were reduced with TCEP (50-100 mM). Samples were centrifuged through solutions of increasing density directly onto a mussel glue-coated TEM grid. Polyallomer centrifuge tubes were fitted with resin plugs with top surfaces perpendicular to the centrifugal force. Grids were placed and the tubes sequentially filled with CsTFA (ρ=1.92); wash solution (7M urea, 20 mM EDTA, 100 mM Hepes, pH 7.5, 10% w/v sucrose), and sample. Centrifugation was for 4.5 h at 25 krpm in an SW60 rotor. The grids were then collected, washed by continuous flow with ultrapure water and then analyzed using FE-SEM and STEM. EDS was performed for elemental analysis.

Morphometric analysis was performed by image analysis software at magnifications of ×1K, ×10K, and ×30K on SEM micrographs. The particles were outlined on each micrograph and basic morphometric parameters (e.g., dmax) as well as the five morphometric descriptors specified by ASTM F1877-98 were obtained. Bright field and dark field TEM analysis was also performed.

Statistical analysis was initially performed to evaluate the distribution of the particles on each wafer and to establish the minimum number of images that needed to be analyzed to assure that the distribution was representative of the serum lubricant sample.

Results

The use of the TEM grid allowed for SEM or TEM analysis, allowing a perfect integration of the information obtained with the two techniques.

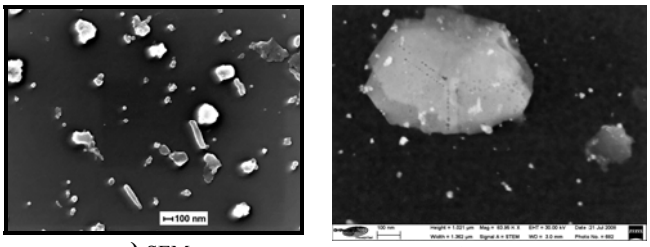
Figure 1a shows an SEM micrograph of a typical sample, and Figure 1b shows the STEM at a higher magnification, indicating phase distribution. TEM analysis allowed for distinction of crystalline and non-crystalline areas. Morphometric analysis (Figure 2) indicated that the largest percentage of the particles were round in shape and had the smallest size, approximately 40nm.

Digestion was complete and no residue was visible. Further, during centrifugation, the particles passed through a threshold boundary of 1.92 g/cc, eliminating non-metallic particles and other artifacts. EDS characterization was performed on a select number of particles confirming the presence of Cr and Co. Specifically, crystalline areas showed a relatively high Co peak with lower Cr and Mo peaks. EDS of noncrystalline areas showed relatively higher Cr and O peaks with no or low Co and Mo peaks.

Centrifugation to adhesive TEM grid provided a uniform distribution of particles that were readily distinguishable from the background.

The elimination of agglomerates, the distinct separation between the particles, and the excellent contrast between the particles and the background facilitated accurate image analysis and precise particle classification. Even at high magnification, the background caused negligible interference and allowed characterization of particles as small as 5nm.

With both low and high levels of wear debris, each sample could be concentrated or diluted to produce an optimum number of particles on each grid, allowing detailed characterization and reproducible classification, as confirmed by statistical analysis of different images of the same sample. As an example, the distribution of round, oval, rod and irregular shaped particles were remarkably similar between two runs of a typical sample, with differences in percentage of each shape below 4%, and the percentage of round particles within 1%. The observed power of the difference in distribution at α=0.05 was 90%.



a) SEM **b)** STEM

Figure 1

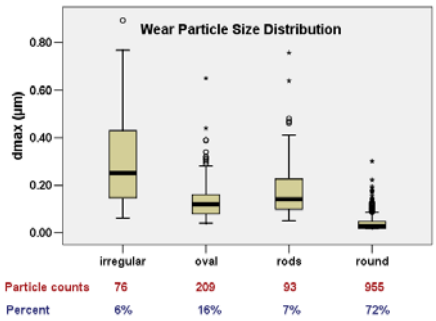


Figure 2

Discussion

The present method provided direct deposition of particles onto TEM grids without the use of filters, and without an embedding/cutting process, both of which are likely to interfere with particle distribution. The use of TEM grids allowed for analysis of the same sample using SEM and TEM.

As more automated and precise algorithms, and those aimed at 3D shape/volume and surface texture, become available, the present analysis platform will be ideally suited for automated morphometric analysis and study of wear particles as they relate to different wear mechanisms.

In summary, the present method overcomes several limitations of previous protocols, providing a powerful tool for accurate characterization of particulate debris from metal-on-metal joint replacements. By combining the information from SEM and TEM, particle characteristics may be more readily and reliably related to wear mechanisms that produced them.