A Highly Sensitive Method for Isolation and Display Metallic Debris from Synovial Fluid

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Introduction

Analysis of metal wear particles is of growing importance in evaluating the performance of artificial joints. Wear particles can lead to inflammation, osteolysis and, eventually, implant failure. Although polyethylene particles may be extracted from fluids and tissues using base digestion, this approach is not suitable for recovering metallic particles, due to the corrosive effects of the base. Furthermore, since joint fluid has a very different composition, and far fewer particles than typical joint simulator lubricants, an ideal isolation method should produce complete digestion of these organic compounds, with minimal loss of particles and minimal agglomeration. This will facilitate improved understanding of the wear mechanisms that produced the particles, as well as their biological consequences. In the present study, a previously developed protocol for extracting particles from the bovine serum used in joint simulator testing (1) was extended for use with the synovial fluid from patients with prosthetic joints. These modifications were required because of the additional organic compounds (hyaluronic acid, etc.) contained in the synovial fluid. In an example application, titanium particles that had been generated by metal-metal wear after wear-through of a polyethylene tibial plateau were isolated and characterized.

Materials and Methods

1.2 mL of synovial fluid were available from a knee undergoing revision total knee arthroplasty. The fluid was rotated for 24 hrs to re-suspend any deposits and to homogenize the sample. It was then diluted to 3.0 mL using 0.02 µm filtered ultra-pure water and HEPES buffer. The diluted sample was digested with hyaluronidase for 8 hrs, benzonase for 8 hrs, and protease K for 48 hrs, and then was centrifuged (Beckmann Optima XL80) through a density gradient, such that the metallic particles were deposited onto a silicon wafer that had been coated with an organic adhesive. More specifically, a silicone coated polyallomer centrifuge tube was fitted with a resin plug, with the upper surface perpendicular to the direction of the centrifugal force, and a silicon wafer then was placed on the resin plug. The tube was 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 the digested joint fluid, and the tube was centrifuged for 4.5 hrs at 25 krpm in an SW60 rotor.

The silicon wafer with the adherent particles was removed from the tube, sputter coated with gold (PELCO SC7) and examined in a field-emission scanning electron microscope (FE-SEM, Zeiss Supra 40VP) at an acceleration voltage of 15kV. Images were taken from 1,000X to 37,000X, with fields of view ranging from 10,000 µm^2 to 7 µm^2. Energy dispersive X-ray spectroscopy (EDS – Thermo Noran 6) was used to identify the chemical composition of the particles. Using digital image analysis, the particles were outlined on each micrograph and several basic morphometric parameters (e.g., dmax) as well as the five morphometric descriptors specified by ASTM F1877-98 were computed.

Results

The metallic particles were well dispersed, with minimal contamination (Fig.1). As indicated by the EDS spectra (Figs.2 and 3) most of the particles were composed only of Ti oxide, with some also containing Al and V. Occasionally, particles of bone and stainless steel (both with density greater than 1.92 g/cc) were observed. Particle length (dmax, Fig.4) ranged from approximately 100 nanometers to 10 microns. The majority of the particles were round, but many (particularly the larger particles) were irregular and elongated in shape The texture of the particles tended to be rough, with only a few smooth or slightly roughened. The number, morphology and size of the metallic particles were consistent with their being generated by the metal-metal wear between the femoral condyle and the tibial baseplate.

Discussion

The new protocol enables the recovery of metallic particles from joint fluids, with minimal degradation. It can be applied to very small samples of fluid, such as the 1.2 mL in the present case. Because the protocol does not require filtration, there is greater recovery of the nano-sized particles, with far less tendency for agglomeration. This provides a more accurate evaluation of the size spectrum of the particles, and facilitates accurate analysis of the composition of individual particles that is not possible when they are agglomerated.

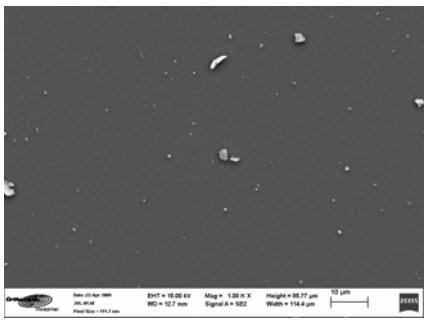

Figure 1 Low magnification micrograph of showing particles of different sizes

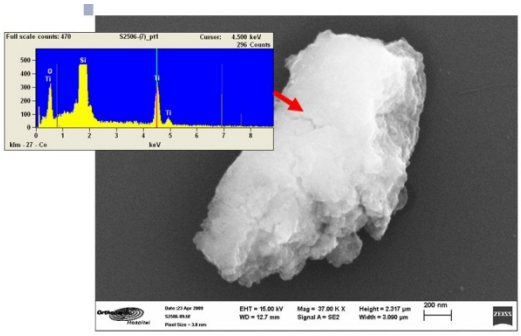

Figure 2 SEM image and chemical characterization of a typical Ti oxide particle

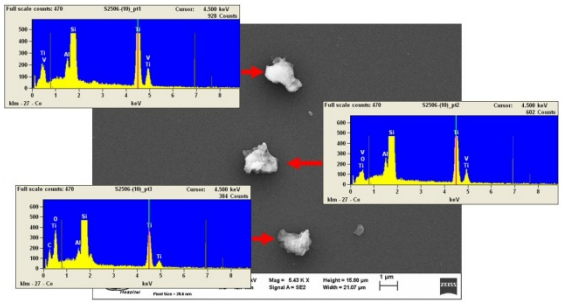

Figure 3 Chemical identification of three particles

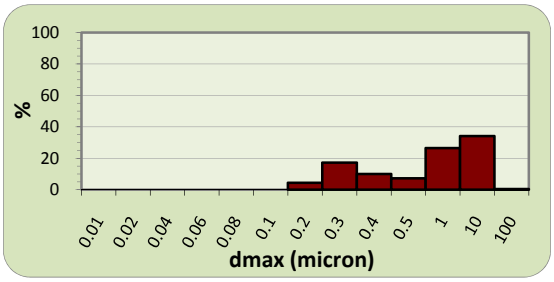

Figure 4 Size distribution of the Ti particles

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(1) F.Billi, P. Benya, E. Ebramzadeh, HA McKellop: *An Accurate and Extremely Sensitive Method to Isolate and Display Nanoparticulate Metallic Wear Debris for Morphometric Analysis.* 54th ORS,125 , 2008