A New Method for Simultaneous Separation of Metallic and UHMWPE Wear 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. Since joint fluid has a very different composition and far fewer particles compared to simulator lubricant, an ideal isolation method should produce complete digestion of these organic compounds (hyaluronic acid, nucleic acid), with minimal loss of particles and minimal agglomeration. This facilitates 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 was extended for use with the synovial fluid from patients with prosthetic joints. Titanium and UHMWPE particles that had been generated by metal-metal wear after wear-through of a polyethylene tibial plateau were isolated and characterized.

Materials and Methods:
A 1.2 mL sample of synovial fluid, a surgical discard from a revision total knee arthroplasty, was homogenized by rotation for 24 hrs. It was then diluted to 3.0 mL using 0.02 µm filtered, deionized water and HEPES buffer. The diluted sample was digested with hyaluronidase for 8 hrs, benzonase for 8 hrs, and protease K for 48 hrs. For metal particles, a siliconized 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, coated with organic adhesive, was placed on the plug. The tube was sequentially filled with layers of CsTFA (p=2.0); wash solution (7M urea, 20 mM EDTA, 100 mM Heps, pH 7.5), and the digested joint fluid, and the tube was centrifuged for 4.5 hrs at 84,000 G in an SW60 rotor (Beckmann Optima L80 XP). Conversely, UHMWPE was centrifuged (Beckmann Optima L80 XP) through three separate density gradients (1 SLS & 2 IPA) over three days for purification. The purified UHMWPE sample, now in IPA, was diluted with water and then centrifuged onto a silicon wafer coated with a monolayer of organic glue using an ingenious tube insert to hold the wafer at the top of the tube allowing the particles to rise up onto the wafer due to their lower density. The tube was centrifuged for 4 hrs at 84,000 G in an SW60 rotor. The silicon wafers with the adherent particles were removed from the tubes, sputter coated with iridium (SBT IBS/e) and imaged 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 µm² to 7 µm². Energy dispersive X-ray spectroscopy (EDS – Thermo Noran System 6) was used to identify the chemical composition of the particles. The particles were digitally outlined on each micrograph and several basic morphometric parameters including the five morphometric descriptors specified by ASTM F1877-05 were computed. UHMWPE particles were identified en masse via FTIR (Thermo Nicolet iS10 with Centaurâ€ sign microscope).

Results:
The metallic particles were well dispersed, with minimal contamination. As indicated by the EDS spectra, most of the particles were composed only of Ti oxide, with some also containing Al and V. Occasionally, particles of bone and stainless steel were observed. Particle length (dmax) ranged from ~ 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 UHMWPE particles were well separated and evenly dispersed on the wafer, with minimal agglomeration and contamination. UHMWPE Particle length (dmax) ranged from ~10 nanometers to 10 microns. Round, oval, irregular (knotted) and fibril shaped particle were observed.

Discussion:
The new protocol enables the recovery of both metallic and UHMWPE 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.

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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