A Novel, Single-Step Method to Separate Multiple Types of Wear Debris from Tissues or Wear Simulator Lubricants

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Statement of Purpose: Wear debris in joint fluids or periprosthetic tissues may include particles of polyethylene (PE), metal alloys, PMMA cement and other materials. Previously, isolation techniques required a different process for each type of debris. In the present study, we developed a single-step separation protocol ("1-Step" method) for simultaneously separating and extracting all common types of wear debris from tissues or from serum lubricants of laboratory wear simulators.

Methods: The first part of the protocol was thorough digestion of lubricants or tissues by Proteinase K (1 mg/ml) in the presence of a detergent, 0.5% Sodium Lauroyl Sarcosine (SLS), and reducing agent, 5 mM Tris (2-carboxyethyl) phosphine (TCEP) at 50 ºC for 24 h. The digests were then adjusted to 50 mM TCEP and 20% SLS and boiled for 10 min. For tissues, a second digestion in 50 mM EDTA and 2% SLS was added to dissolve any bone particles, before adjustment to 20% SLS and boiling. It has been shown that enzymatic digestion does not alter the morphology or size of particles.[1]

Particle separation was then based on equilibrium buoyant density centrifugation. Step-gradients were prepared in siliconized polyallomer centrifuge tubes for the Beckman SW40 rotor. Steps were introduced to the tubes from the bottom (most dense) to the top by overlayering, which eliminated any opportunity for contamination of the steps by the sample. All steps below the sample contain 0.1 M Hepes, pH 7.5, and NaN_3. The steps were a) ρ=1.5 g/cc cesium formate, 1.5 ml b) ρ=1.4 g/cc cesium formate, 0.5% SLS, 1.0 ml c) ρ=1.3 g/cc cesium formate, 0.5% SLS, 1.0 ml d) ρ=1.2 g/cc cesium formate, 0.5% SLS, 1.0 ml e) Sample Digest, 4.0 ml f) water, 1.0 ml g) ρ=0.968 g/cc water/isopropanol, 1.5 ml and h) ρ=0.90 g/cc water/isopropanol, 1.0 ml. Centrifugation was 4 h at 25,000 rpm. Isolated particles were obtained by slicing the tubes from the top down. This prevented mixing between gradient domains (because they were separated by a knife blade) and it allowed easy, complete removal of particles without cross contamination. PE particles and lipid were obtained from the water and 0.968/0.90 g/cc interface regions of the gradient; cement particles from the 1.2/1.3 g/cc interface, and metal particles from the pellet beneath the 1.5 g/cc step. PE particles were separated from lipids through a second gradient centrifugation step. Metal and cement were harvested, diluted with water, briefly sonicated and transferred directly to 0.01 micron polycarbonate filter (Sterlitech) for collection and characterization by SEM and EDAX, before imaging and morphological analysis.

In the present study, the protocol was applied to **A)** bovine serum lubricant from a joint simulator wear test of a metal-on-metal prosthesis, **B)** bovine serum lubricant from a wear test of a CoCr ball paired with a PE acetabular cup, and **C)** 300 mg (wet weight) of capsular tissue adjacent to a cemented metal-on-metal hip resurfacing revised due to femoral stem loosening.

Results/Discussion: The 1-Step method separated each type of wear debris into a distinct band by buoyant density (Fig. 1), allowing recovery for analysis by sectioning of the tubes. In **Fig. A**, CoCr particles are pelleted in a donut shape caused by a dimple in the bottom of the tube. This MOM wear simulation contained only CoCr particles, and the lipids are concentrated in a thin band close to the top.

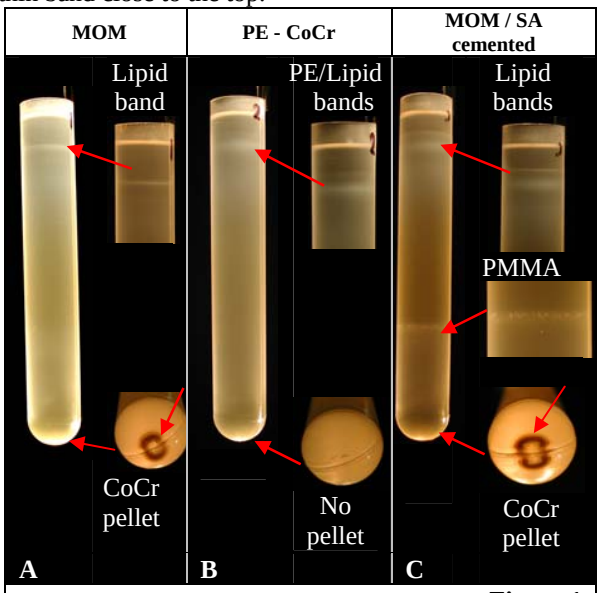

Figure 1

Fig. B shows clear bands of polyethylene and lipids, with no visible metal pellet, consistent with low wear of the CoCr ball in a metal-on-PE wear test.
Fig. C shows clear separation of bands of lipids, PMMA and metal debris (there was no PE component in this hip). SEM and EDAX analysis verified that the bands contained the expected materials.
In all 3 samples above, particles as small as 20nm were observed for every material.

Conclusions: The 1-Step method introduced in this study is the first to allow virtually all of the wear particles of different materials to be separated simultaneously from either peri-prosthetic tissues or *in vitro* wear simulator lubricants, using the same sample. Other advantages of the method include minimizing contamination and particle loss due to multiple steps and the ability to quantify and analyze the total amount of debris. In summary, the 1-Step method provides a powerful tool for isolation of wear debris from multiple sources, allowing new types of characterization and quantification that have not been possible with previous methods.

Reference: Catelas I. JBMR 2001; 55(3): 320-9

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