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A novel method for isolation and recovery of ceramic nanoparticles and metal wear debris from serum lubricants at ultra-low wear rates

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ABSTRACT

Ceramics have been used to deliver significant improvements in the wear properties of orthopaedic bearing materials, which has made it challenging to isolate wear debris from simulator lubricants. Ceramics such as silicon nitride, as well as ceramic-like surface coatings on metal substrates have been explored as potential alternatives to conventional implant materials. Current isolation methods were designed for isolating conventional metal, UHMWPE and ceramic wear debris. In this paper, we describe a methodology for isolation and recovery of ceramic or ceramic-like coating particles and metal wear particles from serum lubricants under ultra-low and low wear performance. Enzymatic digestion was used to digest the serum proteins and sodium polytungstate was used as a novel density gradient medium to isolate particles from proteins and other contaminants by ultracentrifugation. This method demonstrated over 80% recovery of particles and did not alter the size or morphology of ceramic and metal particles during the isolation process.

Statement of Significance

Improvements in resistance to wear and mechanical damage of the articulating surfaces have a large influence on longevity and reliability of joint replacement devices. Modern ceramics have demonstrated ultra-low wear rates for hard-on-hard total hip replacements. Generation of very low concentrations of wear debris in simulator lubricants has made it challenging to isolate the particles for characterisation and further analysis. We have introduced a novel method to isolate ceramic and metal particles from serum-based lubricants using enzymatic digestion and novel sodium polytungstate gradients. This is the first study to demonstrate the recovery of ceramic and metal particles from serum lubricants at lowest detectable *in vitro* wear rates reported in literature.

1. Introduction

Modern ceramic-on-ceramic (CoC) bearings generate wear rates which are approximately twenty to eighty fold lower than the current generation highly crosslinked UHMWPE-on-metal bearings in total hip replacements [1,2]. The motivation for developing low wearing biomaterials and bearing combinations originates from clinical and experimental evidence which has revealed the role that wear debris plays in implant-associated osteolysis and adverse soft tissue reactions in patients implanted with devices [3–6]. Particle concentration and size are considered to be important factors affecting cytotoxicity, inflammatory cytokine release and bone resorption activity in macrophages. Wear particles in a critical size range of 0.1–1 μm are believed to be more biologically active in terms of osteolytic cytokine release [3]. The systemic distribution of wear debris in patients is also found to be dependent on particle size [7–9]. Furthermore, a number of studies have demonstrated the effect of particle morphology on phagocytic capacity and release of inflammatory cytokines in macrophages [10–12]. As a consequence, there is a large body of work investigating wear testing of orthopaedic bearing materials and, the subsequent characterisation of wear debris. Newborn calf serum (NCS) diluted with deionised water (protein concentration from 17 g/l to 30 g/l) is the lubricant used within mechanical simulators to simulate physiological wear mechanisms and wear rates with a variety of materials.

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Prior to characterisation, wear particles are isolated from the serum proteins together with other contaminants. A number of techniques using acid, base, or enzymatic digestion to break down the proteins, followed by isolation of the wear particles by chemical extraction, density gradients, or direct filtration have been developed. However, these methods were designed for isolation of debris from conventional UHMWPE, metals and alumina ceramic materials [13–19].

The latest generation of ceramic bearing materials such as silicon nitride (Si_3N_4) [20], as well as a number of surface engineered coatings have been explored as potential alternatives to UHMWPE and metal articulations [21–25].

Silicon nitride particles are found to slowly dissolve in aqueous fluids [26] and the wear debris released from silicon nitride articulating surfaces are predicted to slowly dissolve in biological fluids [24]. Current methods are not designed to isolate and recover wear debris in such situations.

As a very low wearing material, Si_3N_4-on-Si_3N_4 bearings have demonstrated wear rates comparable to alumina-on-alumina ceramic bearings [20]. Surface engineered coatings such as diamond-like carbon, chromium nitride, chromium carbonitride and silicon nitride coatings also demonstrated very low wear in comparison to their metal counterparts under standard conditions [21–24].

The amount of wear produced by modern CoC bearings is found to be as low as 0.01 mm^3/million cycles during hip simulator testing [1], whilst the volume of serum lubricant used in current wear simulators is usually several hundreds of millilitres. As a consequence, high sensitivity and high recovery have become critical for isolation of wear particles for these low wearing bearing combinations. For the same reason, it has been difficult to characterise wear debris and test the biocompatibility of very low wearing ceramic materials such as zirconia-toughened alumina (Biolox Delta, Ceramtec) and silicon nitride.

This has created the need for development of methodologies to isolate wear debris from simulator lubricants used in wear testing of ultra-low wearing materials and coatings. In addition, the ability to recover particles for further analysis such as biocompatibility testing would also be beneficial.

We have developed a novel method that is able to isolate any ceramic or metal wear particles denser than 1.6 g/cm^3 (density suitable for separation of serum proteins from metal, ceramic, or ceramic-like coating particles) from serum lubricants. The isolation process maintains the original size and shape of particles and demonstrates high recovery of particles for further analysis. The effectiveness of the method was tested by recovering and characterising Si_3N_4 nanoparticles and Cobalt-chromium alloy (CoCr) wear debris at very low wear rates in the order of 0.01 mm^3 per million cycles and low wear rates in the order of 0.1 mm^3 per million cycles.

2. Materials and methods

2.1. Materials

2.1.1. Particles

Si_3N_4 nanoparticles were chosen to test the isolation and recovery of ceramic particles. This was based on a number of reasons. Firstly, as mentioned previously, silicon nitride has shown potential as a monolithic bearing material and also as ceramic-like coatings for total hip replacements. Secondly, a large consortium of public and private sector European organisations (LifeLongJoints[1]) is currently developing very low wearing silicon nitride coatings for articulating surfaces and total hip replacements. The method developed in the present study will be utilised for the isolation of silicon nitride wear debris produced during the wear testing of these coatings. Lastly, the isolation of silicon nitride particles that slowly dissolve will further test the robustness of the isolation method. Commercially available Si_3N_4 nanoparticles (<50 nm nanopowder, Sigma-Aldrich) were used in the present study to test the isolation and recovery of ceramic particles from serum lubricants. These particles were stored in sealed containers to minimise surface oxidation in the presence of oxygen from air.

CoCr wear debris generated in a multidirectional pin-on-plate reciprocator were chosen to test the isolation and recovery of metal particles, as CoCr has been used consistently as an implant material and has recently been explored as a substrate for ceramic-like coatings [24].

2.1.2. Density gradient medium

Sodium polytungstate (SPT) was used as a novel density gradient medium due to its properties, such as its high solubility in water, the fact that it is nontoxic [27] and acts as a protein denaturant [28], coupled with a large density range of 1.1–3.0 g/cm^3 in water.

2.1.3. Particle characterisation instruments

A nanoparticle tracking analysis (NTA) based particle analyser (NanoSight LM10, Malvern Instruments UK) was used to measure particle size distribution and concentration (number of particles per ml) of Si_3N_4 particles dispersed in sterile water. NanoSight has been used to characterise polydisperse samples within the size range of 20 nm–1000 nm and is able to perform measurements on ultra-low concentrations of particles [29]. In the present study, NanoSight was used to characterise Si_3N_4 nanoparticles at ultra-low (0.023 $\mu g \cdot ml^{-1}$) and low concentrations (0.23 $\mu g \cdot ml^{-1}$).

A dynamic light scattering (DLS) based size analyser (Zetasizer Nano ZS, Malvern Instruments UK) was used to measure overall size range of Si_3N_4 aggregates, owing to its large particle size detection range of 0.3 nm–10 μm. This equipment required a minimum concentration of 0.1 $mg \cdot ml^{-1}$ of Si_3N_4 nanoparticles in sterile water for accurate results.

An Hitachi SU8230 high resolution cold-field emission scanning electron microscope (CFE-SEM) was used for high resolution imaging of Si_3N_4 nanoparticles and CoCr wear particles. Aztec Energy Energy-Dispersive X-ray (EDX) system integrated in the CFE-SEM, with a high resolution detector (80 mm^2 X-Max SDD, Oxford Instruments), was used for elemental analysis of the samples. Digital image analysis (Image-Pro Plus version 6.1, Media Cybernetics UK) was used to measure the particle size and shape descriptors.

2.2. Preparation of CoCr wear particles

Metal pins and plates were manufactured from medical grade cobalt-chromium alloy (ASTM F1537) with high carbon content (>0.2% wt) and their contact surfaces were polished to a smooth surface (Ra 0.01–0.02 μm). Subsequently, CoCr wear particles were generated in sterile water (Baxter, UK) in a six station multidirectional pin-on-plate tribometer as described previously [30]. The CoCr particle suspensions were collected after 330,000 cycles and frozen at −20 °C, before being used for particle characterisation or isolation experiments.

2.3. Characterisation of particles

Overall size range of the Si_3N_4 aggregates was measured using a Malvern Zetasizer (Section 2.1.3). The Si_3N_4 nanoparticles were suspended in sterile water (Baxter, UK) at a concentration of

[1] LifeLongJoints, Silicon nitride coatings for improved implant function. http://lifelongjoints.eu.