Bovine Serum Albumin binding to CoCrMo nanoparticles and the influence on dissolution

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Abstract. CoCrMo alloys exhibit good mechanical properties, excellent biocompatibility and are widely utilised in orthopaedic joint replacements. Metal-on-metal hip implant degradation leads to the release of metal ions and nanoparticles, which persist through the implant's life and could be a possible cause of health complications. This study correlates preferential binding between proteins and metal alloy nanoparticles to the alloy's corrosion behaviour and the release of metal ions. TEM images show the formation of a protein corona in all particles immersed in albumin containing solutions. Only molybdenum release was significant in these tests, suggesting high dissolution of this element when CoCrMo alloy nanoparticles are produced as wear debris in the presence of serum albumin. The same trend was observed during extended exposure of molybdenum reference nanoparticles to albumin.

1. Introduction

Metal-on-Metal hip implants were intended to offer improved durability, releasing a lower volume of wear debris than traditional implant designs. However the implants may release particles and soluble wear debris that, once in the bloodstream, are surrounded by a diversity of chemical species and hostile environments. Several studies indicate that metal ion levels rise after surgery and could persist. Specifically, metal particles spread by lymphatic circulation can continue to release ions even after removal of the source of wear. A major concern is that these particles and/or ions may trigger an inflammatory response [1]. Most research has concentrated on corrosion/dissolution studies of bulk pieces of CoCrMo hip implants, exploring the influence of possible body environments, in either static or dynamic situations. However, the behaviour of the wear particles after release remains unclear.

It is well known that in a biological environment, the surfaces of biomaterials are modified by adsorption of biomolecules such as proteins, which mediates the interaction between cells and foreign materials. Metal nanoparticles could be fully coated with proteins, effectively becoming a complex biological unit that changes the way in which the body identifies the particles. Depending on the nature of the material, proteins can either enhance or inhibit metal dissolution: a corrosion protection effect is related to protein adsorption on the surface of a material, acting as barrier against corrosive body fluids; whereas complexation of a metal by the amino acids present in proteins could potentially increase corrosion in some materials. It is therefore interesting to investigate whether serum albumin, the most abundant protein found in the circulatory system, may bind to alloy wear debris particles and change the surface, bioavailability and chemical behaviour such as dissolution.

The purpose of this study is to investigate CoCrMo nanoparticle interactions with proteins, specifically isolating the effect of bovine albumin serum. Nanoparticles were generated in large


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amounts by ball-milling and were comparable in terms of size and composition to some of the nanoparticle wear debris produced by real hip implants [2]. To complement this study, Co_3O_4, Cr_2O_3 and Mo metal nanoparticles were used as reference materials as they may be associated with the surface of the CoCrMo hip implants.

2. Experimental techniques

CoCrMo high carbon gas atomized powders (ASTM75, ~ 60% Co, 27% Cr, 6% Mo) was purchased from Sandvik Osprey. The starting powder had a particle size of around 16 μm. Bovine Serum Albumin (BSA), heat shock, pH 7.0 was acquired from Fisher Scientific, and used during milling as a process control agent. The solution used was composed of 0.4 g of BSA diluted in 10 mL of deionized water. The powder was ball milled for 150 min in a high energy Spex 8000M mill using zirconia ceramic vials and balls. Before milling, the vial was filled with an inert gas (Argon) to avoid atmospheric contamination. A ball-to-powder weight ratio of 3:2 was used. After collection, samples were centrifuged in a Heraeus Megafuge for 10 min, at 25 °C and a centrifugal force of 8000 G. This procedure removes the excess BSA and coarse particles (>200nm), maintaining most of the fine particles in dispersion (20mg/mL). Co_3O_4, Cr_2O_3, Mo nanoparticles (which exhibit MoO_2 on the surface) were purchased from Sigma Aldrich and suspended in BSA (20mg/mL) for 24h, washed in distilled water and finally resuspended in ethanol. The washing procedure removed excess BSA due to its high affinity with ethanol.

In order to demonstrate the effect of milling fluid on the size and stability of CoCrMo nanoparticles when in the presence of serum proteins, the samples were analyzed by Transmission Electron Microscopy (TEM), Energy-Dispersive X-ray spectroscopy (EDX), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Zeta Potential (ZP) measurements.

3. Results and Discussion

Wear debris from hip implants was synthetically produced by milling CoCrMo ASTM75 gas atomized powder in Bovine Albumin Serum (BSA). Following milling, the size of the standard ASTM F75 powder decreased to ca. 0.005-1.8 μm (Figure 1). The reduction in particle size is mainly related to friction and impact during milling; however, the interaction with BSA could also play an important role in both the size reduction and the nanoparticle composition. The size fraction of 5-200 nm, commonly found in real wear debris in synovial fluid, was generated here by centrifugal sedimentation (10 min, 25 °C, centrifugal force of 8000 G) prior to testing and analysis.

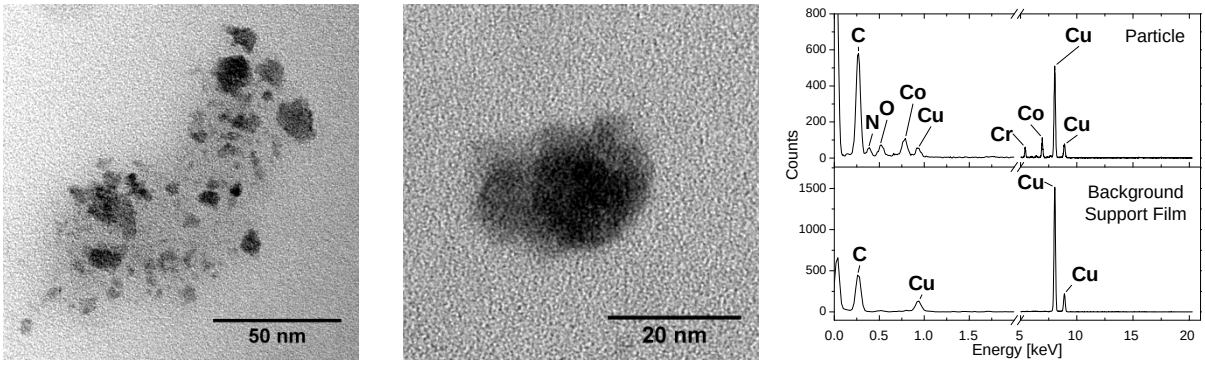


Figure 1. TEM analysis of synthetically produced CoCrMo nanoparticles on carbon support film; (A) a group of particles surrounded by a thick amorphous layer; (B) and (C) TEM/EDX of a representative particle, showing a composition consisting of cobalt and chromium with the presence of nitrogen and oxygen, note the deficiency in molybdenum signal (Mo $K\alpha$ is at 17.4 keV).

Initial TEM examination shows most of the CoCrMo milled particles were surrounded by a thick amorphous layer (note darker contrast surrounding the group of particles) that could potentially be a

protein corona (Figure 1). BSA is formed by a large sequence of amino acids, composed of carboxylic and amine groups, thus the presence of BSA could be distinguished from the TEM carbon support film by the detection of nitrogen. EDX spectra show that particles were mainly composed of cobalt and chromium with a deficiency in molybdenum (relative to the alloy composition), but also reveal the presence of nitrogen and oxygen. A comparison with the composition of the raw material before milling indicates a decrease in cobalt weight percentage in the milled material of only about 5%.

The reference materials showed an average size distribution of 100 nm, with standard deviation of 41nm. The particles where incubated as received for 24 h in BSA, washed and then analyzed by TEM. The results show the clear formation of a protein biofilm (generally less than 5 nm in thickness) on the surface of all the particles (see Figure 2).

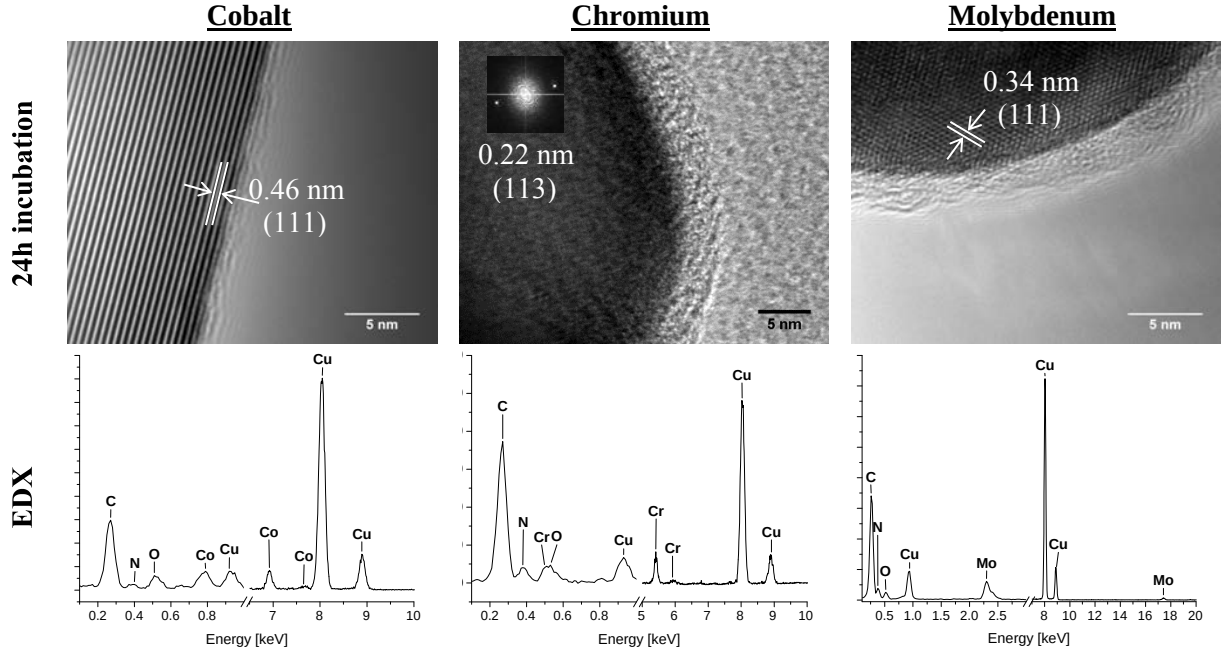


Figure 2. TEM +EDX of Co_3O_4 (JCPDS 42-1467), Cr_2O_3 (JCPDS 38-1479) and MoO_2 (JCPDS 32-0671) reference materials incubated for 24h showing a biolayer containing nitrogen surrounding the particles

Dissolution of the reference materials after one month immersion in a BSA containing solution was measured by ICP-MS (Figure 3) following centrifugation and ultrafiltration to remove any small nanoparticulates [2]. The levels of Co and Cr ions were found to be low, possibly as a result of biolayer formation inhibiting contact with the solution. Molybdenum however, showed high levels of dissolution in the presence of albumin protein in accordance with recent electrochemical corrosion studies of CoCrMo bulk alloy in BSA [4]. Molybdenum is added to the alloy to impart corrosion resistance and its depletion in the presence of BSA, even at pH 7, could leave the material open to greater corrosive attack.

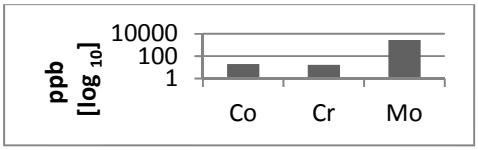


Figure 3. ICP-MS of the dissolution of Co_3O_4, Cr_2O_3 and MoO_2 reference materials incubated for a month in BSA containing stock

Electrostatic forces are expected to play a significant role in protein binding. Albumin, when ionized in water at pH > 4.9 (isoelectric point - IEP), is a negatively charged protein that often shows affinity for hydrophobic surfaces, however it does exhibit a distinct positive patch on one of the

triangular protein surfaces [5]. The zeta potential represents the surface charge in the presence of an aqueous solution when functional groups dissociate or ions adsorb onto surfaces from the solution. The IEP is the pH at which a species exhibits a net zeta potential (approximating to surface charge if specifically adsorbed ions are present) equal to zero. At the IEP of a protein, its structure is more hydrophobic, more compact and less stable due to the absence of inter-particle repulsive forces. Zeta potential titration results showed that the standard IEP of the metal oxide reference materials in water all changed to become closer to that of BSA presumably due to the surface coverage (Figure 4). Molybdenum oxide showed the highest affinity to BSA, and cobalt the lowest. The CoCrMo alloy particles milled in BSA also showed a similar IEP to BSA. Electrostatic forces mediate the protein corona formation, molybdenum (oxide) particles are negatively charged at all pH values > 2.5, whereas surface charge of chromium and cobalt oxides is positive below pH ~7 (Figure 4).

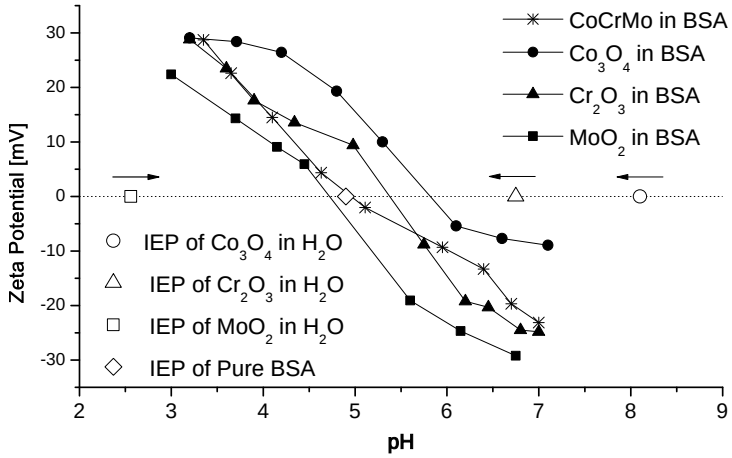


Figure 4. Zeta potential titration of reference materials incubated for 24h in BSA and CoCrMo milled in BSA at the same concentration. The solution pH was titrated from 7 to 3 using HNO_3.

4. Conclusions

TEM analysis suggests the formation of a protein corona on synthetic CoCrMo wear debris particles incubated with BSA and the particles were found to be deficient in Molybdenum by EDX. An interaction with proteins was also seen for elemental reference oxide materials where ICP-MS showed that the reference materials underwent dissolution of predominantly molybdenum ions under static incubation in the presence of BSA. Using the same amount of particles and protein concentration, Molybdenum nanoparticles showed the highest affinity to BSA, demonstrating that in the presence of serum proteins, the effective surface charge is very close to the IEP of BSA. Taken together these results indicate the importance of binding between albumin proteins and molybdenum, which may directly influence the strong dissolution of this element when CoCrMo alloy nanoparticles are produced as hip-implant wear debris in the presence of serum albumin.

Acknowledgements

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