Orthopaedic Grade Cobalt Chromium Alloy Particle Corrosion and Biological Evaluation

Danielle de Villiers1, Agata Nyga2, Terry Tetley2, Akramul Hoque1, Alister Hart3, Julia C. Shelton1.

1Queen Mary, University of London, London, United Kingdom, 2Imperial College, London, United Kingdom, 3University College London, London, United Kingdom.


Introduction: Early failure of metal-on-metal bearings has highlighted the need for appropriate in vitro evaluation, both mechanical and biological [1]. Corrosion of cobalt and chromium from the bearing surface and wear particles has been acknowledged; ionic products from these processes can disseminate around the body with elevated cobalt and chromium levels reported in the blood, urine and joint of patients who have received metal-on-metal implants [2]. Wear particles around the implanted joint have been shown to be predominantly chromium based [3-5] as have those produced in hip simulator studies [6, 7], yet several in vitro studies have suggested that cobalt may be the primary cause of the biological responses observed clinically [8-10]. It is unclear whether this biological response generated in the body is due to the cell’s exposure to particles or ions or a combination. The use of wear particles obtained through a method of particle generation, storage and later biological testing may expose cells to ions as opposed to particles. The corrosion behaviour of orthopaedic grade cobalt chromium alloy has not been fully reported. This study aims to investigate the influence of storage on particle corrosion and determine the difference in biological response between particles and ions.

Methods: Two CoCrMo cylinders, composition defined according to ASTM F75-12, were manufactured and placed parallel to each other on a modified lathe (Harisson M300, UK) with one rotating cylinder and one static cylinder creating a line contact. Particles were generated over 24 hours at a speed of 125 rpm and a counterbalance load of 24 N using 25 % new-born calf serum diluted with pure deionised water. The fluid was collected after 24 hours and spun for 1 hour at 4000 rpm (PK 131, ALC, USA) to remove particles larger than 1 µm. Ions and nanometre sized particles were subsequently separated by further centrifugation (70Ti Rotor, Beckman Coulter, USA) at 165,000 g for 1 hour at 20°C. For biological evaluation, this separation was performed immediately and the ionic supernatant removed. Fluid from further tests was stored in 5 mL samples at 20, 4 and -20°C for between 1 to 4 weeks before separation. The concentration of total (ions plus particles) cobalt and chromium as well as the ionic concentrations (from the supernatant) were determined using graphite furnace atomic absorption spectrometry (Varian, UK), providing the mass of particles generated. Particles which were not used for biological testing were characterised following the protocol developed by Billi et al [11] and compared to particles produced from 48 mm CoCrMo heads and cups tested in an orbital hip simulator under ISO 14242-3:2009 [12] conditions.

To assess the effect of these nanoparticles and ions on U937 macrophages viability a colourimetric assay, MTS, was performed using CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, Southampton, UK). MTS measures the activity of various dehydrogenase enzymes in viable cells. CoCr particles were dispersed in serum-free RPMI at stock concentration of ~ 60µg/mL of Co mass. U937 were exposed to CoCr particles at concentrations of 3 to 30 µg/ml of Co mass and ions for 6 and 24 hours. After incubation media was aspirated and 100 µL of serum-free RPMI media containing 10% of
MTS reagent (0.5 mg/mL in PBS) was added to cells. Plates were incubated from 4 hours at 37°C. The absorbance was read at 490 nm using Infinite F50 plate reader (TECAN). The cell viability was calculated as a percentage of non-treated cell viability. Inflammatory response to CoCr particles and ions was assessed by measuring the release of inflammatory cytokines: IL-1β and TNF-α using Enzyme Linked Immunosorbent Assay (ELISA) (R&D Systems, Minneapolis, USA).

**Results:** Nanometre sized CoCr particles were generated from the lathe cylinder method which were comparable to those produced from the head and cups tested in an orbital hip simulator (Table 1). Immediately following generation, approximately 60% of the total cobalt was ionic while only 6% of the total chromium was ionic. Rapid further corrosion of cobalt was observed to occur within a week of storage regardless of temperature (Fig 1S, increasing to 80% ions and after 4 weeks 90% of the cobalt present was ionic. Chromium corrosion occurred at a slower rate and this rate was temperature dependent. Storing samples at 20°C this corrosion occurred most rapidly, increasing to 24% ions after 4 weeks of storage compared to 10% ions when stored at -20°C.

Cell viability was observed to decrease at concentrations of 30 µg/mL of particles and also caused a concentration-dependent increase in TNF-α release (Fig 2). The ions tested alone had no significant effect on cell viability even at concentrations of 400 µM.

**Discussion:** The body is exposed to cobalt and chromium in the form of wear particles and ions yet these particles can dissociate leaving only the ionic form when investigated through retrievals [3]. The current study has shown that both cobalt and chromium particles have the potential to corrode but that this is far more rapid in cobalt. During 24 hours of particle generation, particles may have already changed to leave only 40% of the total cobalt remaining as particles, which may decrease further to as little as 10% in particulate form if stored for 4 weeks. This may significantly influence results obtained from biological investigations. Wear particles, as opposed to ions, have been shown in the current study to influence cell viability and therefore it is necessary to preserve particles by separating them from the medium prior to use. Although once resuspended for biological evaluation, these particles will likely continue to ionise, this would occur clinically thereby better representing a condition in which the cells would experience in vivo.

**Significance:** Nanometre size metal particles can rapidly ionise with cobalt predominantly produced in ionic form after 24 hours. CoCr particles appear to influence cell viability more than the ions produced and therefore immediate separation of particles and ions are required to achieve conditions close to that experienced clinically.
Table 1: Summary of particle characteristics produced by different methodologies

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Hip Simulator (ISO Standard)</th>
<th>Lathe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode particle size, nm</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Particle distribution</td>
<td><img src="image1" alt="Graph A" /></td>
<td><img src="image2" alt="Graph A" /></td>
</tr>
<tr>
<td>Particle morphology</td>
<td><img src="image3" alt="Image A" /></td>
<td><img src="image4" alt="Image A" /></td>
</tr>
</tbody>
</table>

Effect of CoCr (Lathe) nanoparticles on U937 viability after 24 hour exposure

- **n=4**
  - ![Graph B](image5)

Effect of CoCr (Lathe) ions on U937 viability after 24 hour exposure

- **n=10**
  - ![Graph C](image6)

TNF-α release from U937 after 24 hrs exposure to CoCr (Lathe) nanoparticles

- ![Graph D](image7)

Figure 2: Cell viability in presence of a) particles and b) ions and c) TNF-α release from U937 exposure to nanoparticles after 24 hours.