The effect of metal ions in solution on bacterial growth compared with wear particles from hip replacements

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The biological significance of cobalt-chromium wear particles from metal-on-metal hip replacements may be different to the effects of the constituent metal ions in solution. Bacteria may be able to discriminate between particulate and ionic forms of these metals because of a transmembrane nickel/cobalt-permease. It is not known whether wear particles are bacteriocidal.

We compared the doubling time of coagulase negative staphylococcus, *Staphylococcus aureus* and methicillin resistant *S. aureus* when cultured in either wear particles from a metal-on-metal hip simulator, wear particles from a metal-on-polyethylene hip simulator, metal ions in solution or a control.

Doubling time halved in metal-on-metal (p = 0.003) and metal-on-polyethylene (p = 0.131) particulate debris compared with the control.

Bacterial nickel/cobalt-transporters allow metal ions but not wear particles to cross bacterial membranes. This may be useful for testing the biological characteristics of different wear debris. This experiment also shows that metal-on-metal hip wear debris is not bacteriocidal.

In 2006, orthopaedic surgeons in the United Kingdom used metal-on-metal bearings for 45% of hip replacements in men less than 54 years old.¹ The current generation of hip resurfacing arthroplasties were only introduced in 1997.² The bearing surfaces from a total hip replacement (THR) produce particulate wear debris. These are most commonly metal-on-polyethylene, which generates 100 mm³ of 500 nm particles per year.³,⁴ In contrast metal-on-metal bearings generate 1 mm³ of 40 nm cobalt-chromium particles per year.³ Particulate metal debris may also originate from the modular junctions, such as at the trunnion and the interface between screw-heads and acetabular components, where micromovement occurs. The non-bearing surfaces corrode, producing nickel, cobalt and chromium ions in solution despite the use of relatively inert alloys of cobalt-chromium (American Society for Testing and Materials (ASTM) F75) or stainless steel (316L). The wear particles from metal-on-metal hip replacements may result in toxicity as seen by DNA damage in lymphocytes,⁵,⁶ a reduction in peripheral CD8⁺ T cell counts,⁷ and a vaguely-litic, lymphocytic inflammatory response.⁸ The metal-on-polyethylene wear debris does not have these effects.

Gram-negative and gram-positive bacteria possess transmembrane nickel/cobalt-permeases which enable uptake of these metal ions.⁹ Cobalt solutions of 30 parts per billion (ppb) have bacteriocidal effects against *Helicobacter pylori*,¹⁰ possibly caused by the uptake of cobalt instead of nickel, which is an essential element for bacteria. The nickel/cobalt-permease of *Staphylococcus aureus* is very similar to that found in *H. pylori* and so it is reasonable to assume that cobalt may also be toxic to *S. aureus*.

Despite strict antiseptic operative techniques, prophylaxis with systemic antibiotics and the use of laminar flow theatres, the infection rates in total joint replacement surgery still range between 1% and 3%.¹¹⁻¹³ The incidence of deep infection is between 0.5% and 1% with most deep infections presenting within a year of surgery.¹⁴ Revision surgery has an even greater risk.¹⁵ The organisms most commonly implicated are coagulase negative staphylococcus, *S. aureus* or multi-resistant relatives of these organisms.¹⁶ The infection rate following metal-on-metal resurfacing of the hip is 1.3%,² which is comparable to rates following standard hip replacement.

There are no reports of the effect of metal-on-metal or metal-on-polyethylene wear debris.
on bacterial growth. We wished to determine whether wear debris from metal-on-metal hip replacement is toxic to bacteria.

Materials and Methods
We used standard microbiological methods to determine whether the addition of metal-on-metal or metal-on-polyethylene wear debris to bacterial culture media affected the proliferation of bacteria commonly found in infected hip replacements. We compared this with the response to nickel and cobalt ions in the lubricant solution without wear debris as controls. All chemicals were from Sigma-Aldrich (Poole, Dorset, United Kingdom). All solutions were made up with sterile purified water and stored at 4°C unless otherwise stated.

Wear debris from THR (metal-on-polyethylene) and resurfacing arthroplasty with cobalt-chromium (ASTM F75) (metal-on-metal) were obtained from hip simulators by courtesy of Corin PLC (Corin Group PLC, Cirencester, United Kingdom). The samples were produced using a bovine serum lubricant, gamma irradiated for sterilisation and then refrigerated at 4°C. In each case, a 1 litre polyethylene container was used to store the lubricant from a standard hip simulator run between 1.2 and 1.5 million cycles. It is routine practice to change the lubricant in the simulator after every 300 000 cycles and it is accepted that the wear rate has reached a steady state after 1 million cycles. The metal-on-polyethylene prostheses had cobalt-chromium (ASTM F75) heads and stainless steel (316L) stems. Samples of unused gamma irradiated refrigerated bovine serum were used as controls. The amount of cobalt and chromium wear debris in each sample was calculated by dividing the mass reduction from the prosthesis into the predicted proportions for the cobalt-chromium alloy and this was converted into a concentration according to the volume of lubricant.

Bacterial strains. The following strains were used: National Collection of Type Cultures fully sensitive S. aureus, fully sensitive coagulase negative staphylococcus and a wild strain of methicillin resistant S. aureus (MRSA) from the microbiology laboratory. Each strain was removed from the maintenance plates with a plastic ring and suspended in conventional culture media (BioMérieux Corporation, Marcy l’Etoile, France).

Preparation of the culture media. Cultures were prepared in 25 ml plastic universal containers. Two ‘test’ media and three ‘controls’ were used. Culture media contained 4 ml of bovine serum and 0.5 ml of sterile water. The control medium contained either nickel or cobalt ions as positive controls or wear debris from the hip simulator (either metal-on-metal or metal-on-polyethylene).
Nickel chloride and cobalt chloride solutions were diluted to produce final ion concentrations in each culture medium of 10 parts per million (ppm). Then 0.5 ml ppm including 0.5 ml of each bacterial suspension were used to inoculate each culture medium to a 0.5 McFarland equivalent (in comparison with a 0.5 McFarland standard (Bio Mérieux Corporation)). Each culture broth was incubated at 37°C under aerobic conditions for 48 hours.

Determination of the growth of each bacterial strain in each culture medium. Aliquots of 0.5 ml were taken at 0 hours, 24 hours and 48 hours, tenfold serial dilutions were made in purified sterile water and 20 µl of each dilution were plated on to Columbia blood agar in triplicate. The plates were incubated for 24 hours and the surviving colonies were counted from suitable dilutions. The dilution factors were then reversed and the viable counts were expressed as colony forming units/millilitre (CFU/ml). A mean of triplicate readings at each dilution was taken to calculate the final CFU/ml.

Doubling times, the interval required for all cells in a generation to divide, were calculated assuming growth through binary fission according to the equation:

\[ G = \frac{t}{3.3 \log(b/B)} \]

where \( G \) is the doubling time, \( t \) is the recorded interval in minutes, \( b \) is the final CFU/ml and \( B \) is the original CFU/ml.

‘Two-way’ (replicated) analysis of variance (ANOVA) tests were used to compare the growth of each bacterium in each different culture medium over three time periods using MINITAB (Minitab Ltd., Coventry, United Kingdom).

Results

The rates of growth were similar for all bacteria. The culture media with either type of particulate debris, metal-on-metal or metal-on-polyethylene, all differed from the controls but were similar to each other. In the presence of particulate debris, all bacteria exhibited increased growth rates as shown by a 50% reduction in generation time compared with the controls (Fig. 1, Table I). This accelerated growth was statistically significant for metal-on-metal debris (\( p = 0.003 \)) but not for metal-on-polyethylene debris (\( p = 0.131 \), Table I).

The metal ion solutions of cobalt and nickel dramatically altered the rate of bacterial growth compared with both the particulate debris and control media. In the culture media containing nickel ions all colonies of organisms were eliminated by 24 hours, which was statistically significant when compared with the controls (\( p < 0.001 \)). In the control media containing cobalt ions, generation times were prolonged indicating a retardation of growth, but this result was not statistically significant (\( p = 0.465 \), Fig. 1, Table I). Cobalt chloride medium was tested as 10 ppm with each organism. The metal-on-metal wear debris and metal-on-polyethylene wear debris culture media were tested three times with each organism. Nickel chloride at 10 ppm and the control culture media were tested four times with each organism demonstrating the reproducibility of each result (Fig. 2). The levels of CFU/ml at the onset of the experiments were consistent in all the culture media, indicating that the initial inoculating loads were comparable in all samples. The mean initial inoculum for coagulase negative staphylococcus was \( 1.07 \times 10^5 \) (95% confidence interval (CI) \( 0.61 \times 10^5 \) to \( 1.52 \times 10^5 \)), for \( S. \) aureus \( 1.69 \times 10^6 \) (95% CI \( 1.39 \times 10^6 \) to \( 1.99 \times 10^6 \)) and for MRSA \( 2.32 \times 10^6 \) (95% CI \( 1.45 \times 10^6 \) to \( 3.19 \times 10^6 \)). No contaminants were noted on any plates at any time confirming the effectiveness of sterilisation with gamma irradiation.

The metal-on-metal debris had an estimated cobalt concentration of 6 ppm. The metal-on-polyethylene debris had an estimated nickel concentration of 4 ppm.

Discussion

This in vitro, microbiological investigation showed that metal particle debris from hip replacements is not toxic to bacteria. We had expected to find the opposite given that there is in vivo evidence showing that these particles are toxic to human cells. This study also showed that the response of bacteria to cobalt or nickel is dependent on its physical form, with metal ions having different effects compared with metal nanoparticles. It is, of course, widely understood in organic chemistry that the order and type of atoms in a molecular structure will dictate the chemical and therefore biological properties of the molecule.

Metal-on-metal bearings yield nanoparticles, typically of 40 nm in diameter, containing mostly cobalt and chromium which are in equilibrium with circulating protein-bound cobalt and chromium. In solution, as metal ions, both these metals have a relatively high affinity for transferrin and will mainly access cells via transferrin receptors, as has been well described for transferrin-bound iron. In contrast, nanoparticulate cobalt and chromium will be taken up by a variety of cells through constitutive pinocytosis and other endocytic mechanisms which have evolved for viral surveil-

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**Table I.** Generation times for bacteria in each culture medium. Nickel eliminated all bacteria resulting in a negative value with a true generation time which was infinite (\( p < 0.001 \)). The increased rate of growth with metal-on-metal (M/M) wear was statistically significant (\( p = 0.003 \)) using analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Generation times (mins)</th>
<th>Control</th>
<th>M/P</th>
<th>M/M</th>
<th>Nickel</th>
<th>Cobalt</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>516.9</td>
<td>356.8</td>
<td>219.3</td>
<td>-193</td>
<td>793.6</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>553.9</td>
<td>278.4</td>
<td>236.5</td>
<td>-140</td>
<td>611.3</td>
<td>631.2</td>
</tr>
<tr>
<td>MRSA</td>
<td>435</td>
<td>258.3</td>
<td>304.2</td>
<td>-138</td>
<td></td>
<td>611.3</td>
</tr>
<tr>
<td>p-value (ANOVA)</td>
<td>-</td>
<td>0.131</td>
<td>0.003</td>
<td>&lt; 0.001</td>
<td>0.465</td>
<td></td>
</tr>
</tbody>
</table>

* CNS, coagulase negative staphylococcus; MRSA, methicillin resistant S. aureus
† M/P, metal-on-polyethylene
The effects of nickel and cobalt ions range from being essential elements needed for bacterial survival at low concentrations, to being lethal to bacteria at high concentrations. Concentrations of metal following metal-on-metal hip replacement have been measured in a range of body fluids, but most have not specified whether the metal is in solution as ions or insoluble as particles. The highest concentration we have recorded is 10 ppm, found in the synovial fluid of a patient with a failed metal-on-metal THR. Davies et al found cobalt concentrations at 137 ppb in synovial fluid. Concentrations of nickel during revision of stainless steel on polyethylene THRs can be found in bone marrow as high as 2760 ppb compared with up to 810 ppb at primary THR.

Graphs showing the growth of coagulase negative staphylococcus, S. aureus and MRSA over 48 hours in control and test culture media. Nickel is bacteriocidal to all bacteria. Cobalt retards the growth of all bacteria. The growth rates are increased with metal-on-metal and metal-on-polyethylene wear debris. a) Control group, b) nickel chloride solution 10 ppm, c) cobalt chloride solution 10 ppm, d) metal-on-metal wear debris from hip simulator and e) metal-on-polyethylene wear debris from hip simulator (CFU/ml; colony forming units/millimetre).
We have shown differences in the rates of bacterial growth with cobalt in particulate form compared with cobalt ions in solution. Solutions of cobalt ions of 10 ppm had a generation time four times longer than that of metal-on-metal wear debris. We have also shown that particulate metal wear debris from a metal-on-metal hip resurfacing simulator containing 6 ppm of cobalt is not toxic to those bacteria commonly relevant to hip surgery, including coagulase negative staphylococcus, S. aureus or MRSA, after 48 hours.

Our controls behaved as expected. All organisms proliferated in bovine serum without wear debris, with generation times of approximately 500 minutes all were eliminated when exposed to a nickel solution of 10 ppm. Nickel and cobalt ions were antibacterial, and metal-on-metal and metal-on-polyethylene wear debris promoted bacterial growth.

Nickel is a toxic heavy metal known to have antibacterial effects, as is cobalt to a lesser extent. In this experiment, we used a concentration of a solution of 10 ppm of nickel, considerably lower than other commonly-used antibacterial solutions, such as Betadine (Purdue Pharma, Stamford, Connecticut) with 10 000 ppm iodine, and hydrogen peroxide with 30 000 ppm. Consideration might be given to the use of nickel containing solutions or nickel containing prostheses which may leach out nickel to reduce the incidence of the infected metal implants. Toxicity studies should be carried out to find out whether low concentrations of nickel or cobalt solutions might be useful for washing prostheses intra-operatively.

Particulate debris, of any composition, promotes growth by providing a scaffold, but metal wear debris at 40 nm in diameter is too small to act as a scaffold unless the particles are aggregated.

We conclude that the response of bacteria to nickel, cobalt and chromium is dependent on the physiochemical form of the metal. Metals in solution as ions may be useful as antibacterials whereas when in nanoparticulate form, such as wear debris from metal-on-metal bearings, they are not toxic to bacteria.

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References