INTRODUCTION:
In recent years metal-on metal hip resurfacing (MOMHR) has become a common alternative to conventional total hip arthroplasty (THA) in younger patients with osteoarthritis. More than 500,000 patients worldwide have received these prostheses, and currently around 40% of patients <50 years of age in UK receive an MOMHR rather than conventional THA. However, adverse events including failure of implant osseo-integration, periprosthetic fracture, femoral neck narrowing, and unexplained pain occur more frequently following MOMHR versus THA, resulting in a higher revision rate at 3 years post-surgery.

Metal (cobalt and chromium) species are released from MOMHR prostheses at higher levels than occur following THA. Physiological levels of cobalt and chromium are usually <0.25µg/L (0.005µM). Whole blood concentrations of cobalt and chromium after MOMHR are reported to be up to 4.6µM and 2.3µM, respectively. Hip joint synovial fluid levels are reported up to 30µM and 25µM, respectively.

In this study we examined the effect of cobalt (Co²⁺) and chromium (Cr³⁺, Cr⁶⁺) ions at clinically relevant concentrations post MOMHR implantation on number and bone resorptive activity of primary human osteoclasts derived from peripheral human mononuclear cells taken from volunteer donors. The effect of the ions on osteoclasts was tested under 2 conditions: the effect on newly forming osteoclasts; and that on mature osteoclasts.

METHODS:
Cell culture: Up to 100ml of human peripheral blood was taken from healthy volunteers following local ethics committee approval. The monocyte and lymphocyte fraction was isolated by Histopaque fractionation, seeded evenly onto pre-wetted dentine wafers and treated throughout a 21-day culture period with αMEM Glutamax (Gibco; Invitrogen) supplemented with 50 IU/ml of penicillin, 50µg/ml of streptomycin, 10% fetal calf serum, 25ng/ml of M-CSF, 30ng/ml of recombinant RANKL.

Metal ion preparations: Co²⁺, Cr³⁺, and Cr⁶⁺ ion solutions were made up over a concentration range from 0.01 to 200µM to represent the range of clinically reported concentrations in serum and synovial fluid of MOMHR patients. Solutions were made by serial dilution of cobalt (II) chloride hexahydrate, chromium (III) hexahydrate and chromium (VI) oxide in α-MEM. Metal ion treatments were incorporated into the cell feeding medium on day 2 and day 14 for newly forming and mature osteoclasts, respectively, and were changed 3 times a week thereafter. Cell formation and resorption activity assays: At the end of each experiment cell cultures were fixed in buffered formalin, TRAP stained, and counterstained with Gill’s Haematoxylin. The cultures were quantified for osteoclast number, total resorption and resorbing osteoclasts by point counting.

RESULTS:
Effect of Co²⁺: Co²⁺ had no effect on newly forming osteoclasts at concentrations from 0.01µM to 10µM, whilst 200µM reduced both osteoclast number, total resorption per disk, and dentin resorption per osteoclast (Figure 2, p<0.0001). Co²⁺ showed a dose-dependent effect on mature osteoclasts, with a reduction in osteoclast number at 10µM and 200µM (Figure 2, p<0.001), total resorption, and resorption per osteoclast at 200µM (p<0.0001 and p<0.001).

Effect of Cr³⁺: Cr³⁺ resulted in a trend to increased osteoclast number and resorption activity at sub-micromolar concentrations in newly forming osteoclasts, followed by a reduction in total resorption and resorption per osteoclast at 200µM (Figure 2, p<0.001 and p<0.0001 respectively). For mature osteoclasts, Cr³⁺ ions resulted in a dose-dependent reduction in the number of osteoclasts, total amount of resorption, and resorption per osteoclast (p<0.05, p<0.001 respectively).

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