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Metal-on-metal bearings in total hip arthroplasties: Influence of cobalt and chromium ions on bacterial growth and biofilm formation

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Abstract: Metal-on-metal (MOM) bearings involving cobalt–chromium (Co–Cr) alloys in total hip arthroplasties are becoming more and more popular due to their low wear. Consequences of corrosion products of Co–Cr alloys are for the most part unclear, and the influence of cobalt and chromium ions on biofilm formation has never been studied. Therefore, the aim of this study was to evaluate how Co–Cr ions affect bacterial growth, biofilm formation, and architecture. A collection of clinically isolated and commercially available bacterial strains were exposed to Co–Cr concentrations as found in serum and above as found in adjacent tissue. Planktonic growth of bacteria was inhibited by concentrations of 200,000/93,000 µg/L Co–Cr. Co–Cr concentrations up to 20/9.3 µg/L as reported to occur in serum revealed no consistent influence on bio-

film formation, but higher concentrations of 200,000/93,000 µg/L significantly reduced *Staphylococcus aureus* and CNS biofilm formation. As indicated by confocal laser scanning microscopy, no dead bacteria were encountered in the biofilms, and the metal ion concentrations used must be classified as growth-inhibiting and not bactericidal. Long-term clinical data on infection rates for Co–Cr MOM-bearings are not yet available, but the current results suggest that Co–Cr ions may yield these prostheses less prone to biofilm formation and subsequent infection. © 2008 Wiley Periodicals, Inc. *J Biomed Mater Res* 88A: 711–716, 2009

Key words: metal-on-metal bearing; infection; cobalt; chromium; biofilm formation

INTRODUCTION

Total hip replacement is a highly successful procedure with a regain of a relatively high quality of life and an almost instant pain relief. Success of the procedure resulted in younger cohorts of patients. Many of these younger patients want to return to a high level of activity and seek an implant that provides durability. Larger femoral heads were indicated,¹ but this tended to cause excessive wear in conventional prostheses (metal ball connected to large stem and a cup with polyethylene interface). Polyethylene wear and debris is a suspected cause of osteolysis around the implant,^{2,3} which led to the development of alternative bearings lacking a polyethylene/metal interface, like the recently reintroduced metal-on-metal (MOM) bearings. In contrast to metal-on-polyethylene

bearings, wear rates of MOM-bearings turned out to be impacted in a positive way by increasing the head size,^{4,5} yielding 20–100 times less debris than in traditional metal-on-polyethylene bearings.⁶ The remarkably low wear of MOM-bearings has led to a rapidly increasing popularity of MOM-articulation in the treatment of young and active patients.⁷

Although mid- and long-term clinical results of MOM-bearings appeared to have demonstrated excellent durability, recent studies show that there is at least one MOM-bearing system with periprosthetic osteolysis and aseptic loosening, which is possibly associated with hypersensitivity to metal debris.^{8,9} Additionally, MOM-articulations are not completely biologically inert, since they produce metal particles that can be found in, for example, blood and urine. These particles tend to corrode and serum levels of metal ions, mainly cobalt and chromium, become elevated.^{10–15} Cobalt and chromium are usually eliminated only slowly from the body by urine, and chromium is even retained in the body's tissues.^{10,16} These high cobalt and chromium serum

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TABLE I
The Percentage of Growth Stimulation/Reduction After 24 h of Metal Ion Exposure

	Co/Cr Ion Concentration ($\mu\text{g/L}$)			
	2/0.93	20/9.3	20,000/9300	200,000/93,000
<i>Staphylococcus aureus</i>				
<i>Staphylococcus aureus</i> 5296	4%	-4%	-8%	-12%
<i>Staphylococcus aureus</i> 7388	-3%	-7%	-10%*	-17%*
<i>Staphylococcus aureus</i> ATCC 12600	15%	9%	-15%	-35%*
<i>Staphylococcus aureus</i> ATCC 25923	-4%	0%	0%	-11%*
<i>Staphylococcus aureus</i> ATCC 51153	4%	4%	0%	0%
Mean (SD = 8%)	3%	0.4%	-7%	-15%*
CNS				
CNS 7391	4%	-6%	-20%	-45%*
CNS 5115	-9%	-7%	-11%	-20%
CNS 5295	7%	11%	0%	-4%
CNS 7319	-4%	0%	-8%	-8%
CNS 7349	-4%	4%	-32%*	-47%*
CNS 5147	-9%	-2%	-9%	-38%*
CNS ATCC 35984	10%	21%*	5%	-28%*
CNS ATCC 14990	-3%	0%	3%	-21%*
Mean (SD = 13%)	-1%	3%	-9%	-26%*

*Indicates a significant difference versus growth in the absence of metal exposure, i.e. 0% growth stimulation/reduction ($p < 0.05$).

Values are averages including standard deviations from three experiments with separately cultured bacteria, yielding an average mean standard deviation of 11%. Note that mean values in bold represent the mean including standard deviations over the collection of isolates involved. Growth reductions appear as negative numbers.

concentrations may have toxic effects, which include the increase of bone resorption, and theoretical risks of delayed-type hypersensitivity, organ toxicity, and altering of cell homeostasis.¹⁷⁻²² Furthermore, cobalt and chromium have been shown to be carcinogenic and mutagenic in human and animal models,²³⁻²⁶ which implies that systemic toxicity and cancer risk may be possible disadvantages of MOM-articulation.

Alongside these possible disadvantages, it is also conceivable that the risk of infection is influenced by metal ions. Infection still remains a significant complication following total hip replacement and as a conservative estimate, affects about 1-2% of all patients during the life time of an implant.²⁷ In case of infection, bacteria adapt a biofilm mode of growth on the surface of the prosthesis, which represents a basic survival mechanism of the organisms²⁸ to external (500-5000 times increased antibiotic resistance^{29,30}) and internal environmental factors (the host immune system). The increased antibiotic resistance of biofilms causes major difficulties in patient treatment. Removal and replacement of an infected implant is usually required to eliminate the infection with accompanying trauma and increased costs to the health service.³¹⁻³³ Copper and zinc are known for their bactericidal properties and impact on biofilm formation,³⁴ but no research efforts have been undertaken towards the specific influence of the cobalt-chromium ion combination on biofilm formation, despite extensive other studies into MOM-bearings.³⁵⁻³⁷

The aim of this *in vitro* study is to evaluate the influence of cobalt and chromium ions on bacterial

growth, biofilm formation, and architecture for a collection of clinically isolated and commercially available bacterial strains.

MATERIALS AND METHODS

Bacterial strains

Gram-positive organisms account for most bacteria found in infected hip arthroplasties. Coagulase negative staphylococcus (67%) was found to be the predominant organism, although *Staphylococcus aureus* (13%) is gaining importance.³⁸ Therefore, a total of 13 staphylococcal strains were used in this study (Table I), chosen to represent their frequency of occurrence in clinical infection. Eight strains were isolated with extensive biomaterial culturing³⁹ from explanted metal-on-polyethylene joint prostheses from individual patients with septic loosening and retrieved during revision surgery (Department of Orthopaedic Surgery at the University Medical Center Groningen, The Netherlands) and five additional strains were of ATCC origin.

Cobalt and chromium ions

Metal ion concentrations of 2/0.93; 20/9.3; 20,000/9300; 200,000/93000 $\mu\text{g/L}$ Co-Cr were applied throughout this study. The lowest Co concentration of 2 $\mu\text{g/L}$ was inline with previously found Co serum concentrations¹¹⁻¹⁵ and the proportion Co-Cr in this study was chosen similar to most MOM-bearings currently used in Europe ($\pm 61\%$ Co and 29% Cr). The second-lowest level of 20/9.3 $\mu\text{g/L}$ Co-Cr was chosen to represent higher serum levels, described in the literature. Higher concentrations of metal ions were

used, since the local concentration of metal ions in the synovial fluids is expected to be much higher than the serum concentrations.

Based on a previously described method,⁴⁰ 0.847 mg cobalt salt (CoCl₂·6H₂O, Sigma) and 0.475 mg chromium salt (CrCl₃·6H₂O, Merck) were dissolved in 10 mL tryptone soya broth (TSB) (Oxoid, Basingstoke, United Kingdom). These samples contained 200,000/93,000 µg/L Co–Cr and were diluted with broth to reach the concentrations and Co–Cr proportions needed for the experiments.

Planktonic growth evaluation

Three *S. aureus* strains and two CNS strains were randomly chosen for growth curve evaluation. These isolates were routinely cultured from frozen stock on blood agar plates at 37°C for 24 h. Precultures were inoculated with a single plate colony, grown in 10 mL TSB and incubated aerobically overnight at 37°C. From the resulting suspension, 1 mL was inoculated overnight with 45 mL TSB, 45 mL TSB with 20,000/9300 µg/L Co–Cr or 45 mL TSB with 200,000/93,000 µg/L Co–Cr. The absorbance at 600 nm (A₆₀₀) was determined using a spectrophotometer. All growth curve experiments were performed twice with separately cultured bacteria.

Biofilm formation in microtiter plates

All strains mentioned in Table I were routinely cultured from frozen stock on blood agar plates at 37°C for 24 h. Precultures were inoculated with a single colony from plate grown in 10 mL TSB and incubated overnight aerobically at 37°C. A bacterial suspension of 200 µL (2 µL pre-culture and 198 µL fresh TSB) supplemented with different concentrations of metal ions, was used to inoculate a well of a 96-well polystyrene flat-bottomed tissue culture plate (Falcon, Becton Dickinson, Oxnard, CA) for biofilm formation. After 24 h at 37°C, the growth media and planktonic cells in the 96-wells plates were removed from the biofilms by carefully replacing the volume of the wells twice with 200 µL 10 mM potassium phosphate, pH 7.0 by pipetting, while taking care that air-liquid interface passages over the biofilms were avoided. The wells were subsequently stained with 200 µL 1% crystal violet. After 30 min, excess stain was replaced with 200 µL demineralised water as described above, and the crystal violet was dissolved in 200 µL of ethanol–acetone (80:20 vol/vol). The absorbance at 575 nm (A₅₇₅) was determined using a microtiter plate reader (Fluostar Optima) to determine the amount of crystal violet, as a measure of biofilm growth. The influence of Co–Cr ions on biofilm formation was evaluated by measuring the percentage of growth stimulation/reduction according to

growth stimulation/reduction

$$= \frac{(A_{575} \text{ presence Co-Cr} - A_{575} \text{ absence Co-Cr})}{A_{575} \text{ absence Co-Cr}} \times 100$$

Thus inhibitory effects of the presence of Co–Cr ions appear as negative numbers in the outcome parameter. All

experiments included six replicate wells and were performed three times with separately cultured bacteria.

Biofilm architecture determination by confocal laser scanning microscopy

Two of the five strains used for growth curve evaluation, *S. aureus* 7388 and a CNS 5147, were used for visualizing biofilm architecture. These isolates were routinely cultured from frozen stock on blood agar plates at 37°C for 24 h. Precultures were inoculated with a single colony from plate grown in 10 mL TSB and incubated overnight aerobically at 37°C. From the above mentioned bacterial suspension, 25 µL was inoculated with respectively 3 mL TSB, 3 mL TSB with 2/0.93 µg/L Co–Cr or 3 mL TSB with 200,000/93,000 µg/L Co–Cr in a 6-wells polystyrene tissue culture plate (Costar). After 24 h of incubation at 37°C, biofilms were stained with calcofluor white (Bayer) to visualize extracellular polymeric substance (fluorescent blue), and with LIVE/DEAD BacLight viability kit (Molecular Probes Inc., Eugene, Oreg.), to visualize live (fluorescent green) and dead (fluorescent red) bacteria. After 15 min incubation in the dark, confocal images were collected using a Leica TCS-SP2 microscope with a 40× water objective. Images were obtained at 1–2 µm intervals down through the biofilm and the number of images, therefore, corresponded with the thickness of the biofilm. The confocal laser scanning microscopy (CLSM) experiments were performed twice with separately cultured bacteria.

Statistical analysis

Differences in optical densities of biofilms grown in the absence and presence of metal ions were analyzed for significance by the Student *t*-test for paired samples. A 95% (*p* < 0.05) confidence interval was applied for statistical significance.

RESULTS

Planktonic growth

Figure 1 summarizes the planktonic growth of *S. aureus* 7388 and a CNS 5147 in the absence and presence of different concentrations of Co–Cr ions. Clearly for these two strains as well as for the other three strains involved in planktonic growth experiments (data not shown), planktonic growth was not significantly influenced by Co–Cr as compared with the control when the ion concentrations were less than 20,000/9300 µg/L Co–Cr, but at the highest concentration of 200,000/93,000 µg/L Co–Cr all *S. aureus* and CNS strains showed significant growth reduction.

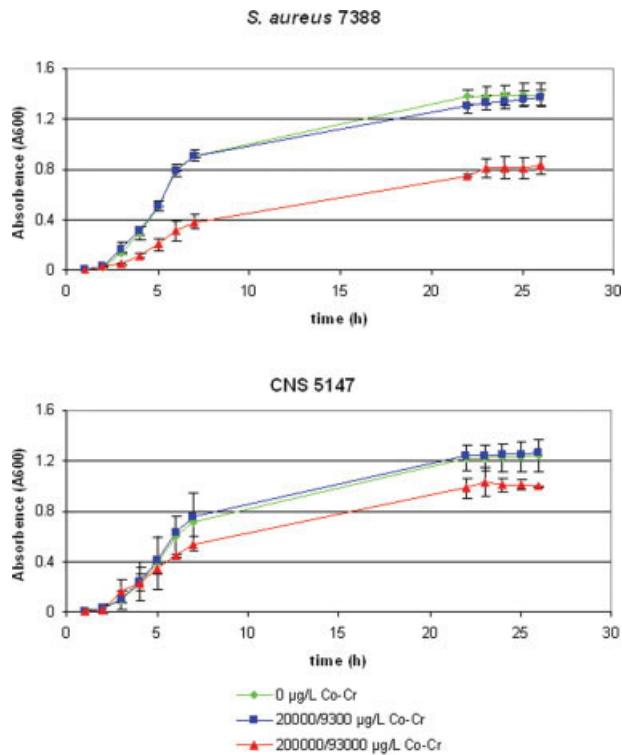


Figure 1. Growth curves of two clinically isolated strains: *S. aureus* 7388 and CNS 5147 in the absence and presence of metal ions. Error bars indicate the standard deviation of the mean calculated from two growth curve experiments with separately cultured bacteria. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Biofilm formation

Table I summarizes the effects of different concentrations of Co–Cr ions on biofilm formation of the *S. aureus* and CNS strains involved. Whereas most isolates show growth reductions that increase with increasing Co–Cr concentrations, some strains are clearly stimulated in their growth at low metal ions concentrations (*S. aureus* ATCC 12600 and CNS ATCC 35984). At the highest metal ion concentrations, however, all strains are reduced in their growth. When averaged over all isolates of a given species, it becomes clear that *S. aureus* and CNS are inhibited in their growth when Co–Cr concentrations are above 200,000/93,000 µg/L. At that concentration, CNS is slightly more affected than *S. aureus*.

Biofilm architecture

CLSM images of the *S. aureus* 7388 and CNS 5147 biofilms grown in the absence and presence of Co–Cr ions revealed a decrease in the number of live bacteria due to the presence of Co–Cr ions (see Fig. 2). Sectional analysis of each biofilm layer (about 1 µm in thickness) made it possible to demonstrate the three-dimensional structure of biofilms, and revealed that the biofilms formed in the absence of Co–Cr ions had a thickness of 42 µm (*S. aureus*) and

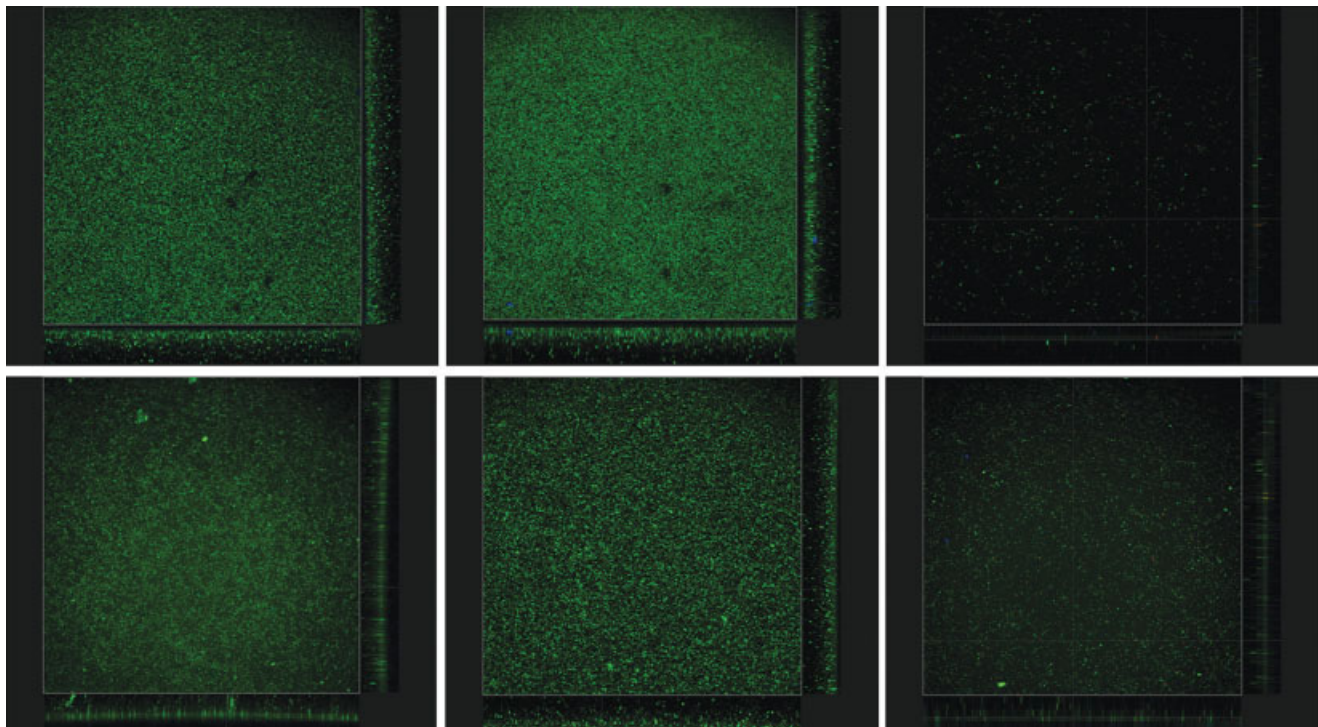


Figure 2. CLSM images (375 × 375 µm) of *S. aureus* 7388 (top) and CNS 5147 (bottom) biofilms, grown in the absence (left) and presence of 2/0.93 µg/L (middle) and 200,000/93,000 µg/L (right) Co–Cr ions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

35 μm (CNS), respectively. While in the presence of the highest concentration Co-Cr ions (200,000/93,000 $\mu\text{g/L}$) biofilm thickness and density is remarkably reduced to 15 μm and 8 μm , for *S. aureus* and CNS, respectively, confirming that CNS is slightly more affected. Neither dead bacteria nor slime were observed, regardless of the absence or presence of Co-Cr ions or the strain involved.

DISCUSSION

MOM-bearings for joint arthroplasty have regained popularity in the treatment of young patients because they offer wear rates low enough to prevent the bearing from wearing out in a lifetime, but little is known about the way infectious microorganisms behave toward the Co-Cr surfaces involved in MOM-bearings. Recently, Anwar et al. showed that wear debris from MOM-bearings accelerate the growth rate of planktonic bacteria.⁴¹ To our knowledge, this is the first study that focuses on the influence of cobalt and chromium ions on biofilm formation.

An intriguing novel finding from this study was that biofilm formation and planktonic growth were inhibited by a high dose of chromium and cobalt ions. The highest concentration of metal ions (200,000/93,000 $\mu\text{g/L}$ Co-Cr) used, reduced biofilm formation by 15% and 26% with respect to a control for two collections of *S. aureus* and CNS strains, respectively. The lower metal concentrations revealed no consistent influence on biofilm or on planktonic growth. CLSM images of the biofilm confirmed the results retrieved by light absorbance and showed that biofilm thickness and density were only affected by exposure to the highest concentration Co-Cr. The highest metal ion concentration caused a reduction in biofilm thickness of more than 50%.

The exact role of either cobalt or chromium in staphylococcal biofilm formation is still unclear and hypothetically involves competition with Fe for uptake in the cell. Iron is an important nutrient element required by the bacterial metabolism, and interference with its uptake could provide an effective mechanism to contain infection. This suggestion is confirmed by a study on the effect of cobalt on *Pseudomonas aeruginosa*,⁴² demonstrating inhibition of iron-dependent metabolic activities of the bacterium leading to growth retardation and cell death.

Cobalt chromium alloys had not been available before the 1950s and it was at that moment when the first designs of MOM-bearings were described. Initially, infection rates of early MOM-bearings developed in the 1960s, such as the McKee-Farrar arthroplasty were high and ranged from 0% to 6% with antibiotic prophylaxis and from 0.5% to 11%

without, but at that time in many centers no clean air enclosures were used.⁴² However, the durability of these designs was quite poor and at present the durability of the bearing surfaces has been improved by appropriate surface finishes and forging processes. Preliminary data over short follow-up times of these newly developed MOM-bearings show lower infection rates than of the early designs. Milosev et al. reported six revisions because of infection in a cohort of 640 total hip replacements after a 7.1-year follow up.⁴³ In addition, Korovessis et al. reported three infections in a consecutive series of 217 total hip replacements after 6.4 years.⁸ However, no large, long-term outcome studies are presently available.

Although the reductions in biofilm formation observed in the present study seem to be in line with the few clinical data on infection rates of MOM-bearings, it must be acknowledged that reliable information about the exact local concentrations of Co-Cr around prostheses is not available. However, in local antibiotic treatment it is recognized that local antibiotic concentrations can become up to 5000 times higher than serum levels,⁴⁴ which suggest that Co-Cr concentrations around a MOM-bearing may be as high as 100,000/46,500 $\mu\text{g/mL}$. In addition, serum levels of metal ions have demonstrated great variability from patient to patient.⁴⁵ Moreover, local concentrations of Co and Cr ions in the synovial fluids will probably exceed these serum levels significantly, particularly in poorly engineered implants or in case of increased wear rate because of malpositioning of the components, impingement, or loosening.

CONCLUSIONS

In conclusion, planktonic bacterial growth, biofilm growth and thickness were significantly reduced by Co-Cr concentrations of 200,000/93,000 $\mu\text{g/L}$, which are higher than observed in serum, but not unlikely around a prosthesis or in synovial fluid. This suggests that MOM-bearings may be less prone to biofilm formation and subsequent infection.

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