Impact of Nanoparticles Released From Total Disc Replacement Prostheses: Cobalt Nanoparticles Cause Major Platelet Aggregation in Vitro While Chromium Nanoparticles Induce Platelet Lysis in Vitro

Poster ID: 1847

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Disclosures

No conflicts of interest to declare.
Introduction

• Total disc replacement (TDR) has been gaining popularity as an alternative procedure to spinal fusion surgery.
• It has proven to be clinically effective in the cervical (Findlay et al. 2018) and lumbar (Formica et al. 2019) spine in long-term follow-up studies.
• Corrosion due to electrochemical processes, shear stress and wear from joint articulation releases a significant amount of wear debris including nanoparticles (NPs) or ions, which can remain in local tissues or translocate into the bloodstream.
• However, potential toxic effects of NPs released from TDR implants on blood platelets have not yet been comprehensively investigated.

The aim of this study was to investigate the impact of cobalt (Co) and chromium (Cr) NPs on platelet function in vitro using quartz crystal microbalance with dissipation (QCM-D) methodology that measures NPs-induced platelet microaggregation.
The following NPs were chosen for this study: Co 28nm; CoO 50nm; Co$_2$O$_3$ 50nm; Co$_3$O$_4$ 30-50nm; Cr 35-45nm; Cr$_2$O$_3$ 60nm.

The ability of the tested NPs to induce platelet activation and aggregation was measured using:

- light transmission aggregometry,
- flow cytometry
- quartz crystal balance with dissipation (QCM-D) that measures platelet microaggregation under flow conditions
- confirmed by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and optical and immunofluorescence microscopy.

Blood was collected from healthy volunteers who had not taken any medications known to affect platelet function for at least 2 weeks prior to the study (e.g. NSAIDs). Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared and utilized in the experiments.
Results

Figure 1. Platelet aggregation monitored by light transmission aggregometry. Collagen was used as positive control.

Incubation of PRP with Co and Cr NPs resulted in a concentration-dependent increase in platelet aggregation with maximal effect at 50 μg/mL.
Figure 2. Representative flow cytometry recordings showing analysis of P-selectin on platelets in the presence and absence (resting) of chromium and cobalt nanoparticles (5 μg mL⁻¹). Collagen (5 μg mL⁻¹) was used as positive control. The bar graph represents quantitative analysis of P-selectin expression. An asterisk (*) marks statistically significant changes when compared to resting platelets.

A significant, but varying in intensity, increase in the number of P-selectin copies on platelet surface was observed when incubated with Co 28nm and Co₂O₃ 50nm NPs at a concentration of 5 μg/mL. The effect was not shown for Cr NPs.

Figure 3. Effects of chromium (5 μg mL⁻¹) and cobalt (5 μg mL⁻¹) nanoparticles on platelet-rich plasma using quartz crystal microbalance with dissipation. Data are expressed as mean ± standard deviation. An asterisk (*) marks statistically significant changes when compared to PRP alone.

At the lowest tested concentration (0.5 μg/mL), the largest increase in D occurred when platelets were incubated with Co 28nm. CoO 50nm and Co₂O₃ 50nm NPs caused significant increase in D. There was no significant change in D for Co₂O₄ 30-50nm, Cr 35-45nm and Cr₂O₃ 60nm when compared to PRP alone at the lowest tested concentration (0.5 μg/mL).
Figure 4. Representative micrographs of the surface of sensor quartz crystals as viewed by transmission electron microscopy showing start of platelet activation (A), major platelet activation (B) and increased accumulation of platelet aggregates (C) following perfusion of platelet-rich plasma in the presence of cobalt nanoparticles.

The images show that Co NPs lead to platelet activation including shape change, expression of pseudopodia and aggregation.
Figure 5. Representative micrographs of the surface of sensor quartz crystals as viewed by transmission electron microscopy following perfusion of platelet-rich plasma in the presence of chromium nanoparticles. Platelet encapsulation (A), swelling (B) and lysis (D) due to chromium nanoparticles is visualized. Figure 7E shows close-up of platelet lysis with empty arrows indicating NP encapsulation. Figure 7C shows combined image for the effects of chromium nanoparticles on platelets.
Discussion

• Aggregometry, flow cytometry and QCM-D all showed varied levels of platelet activation and aggregation due to both Co and Cr NPs.

• Interestingly, TEM showed two different mechanisms involved in interactions of Co and Cr NPs with platelets. While both NPs activated platelets, Co NPs resulted in classical features of platelet aggregation. In contrast, Cr NPs initially caused only low-degree platelet activation. This was promptly accompanied by encapsulation of platelets with Cr NPs that appear blocking aggregation. However, once platelets were encapsulated by Cr NPs they underwent lysis. Lysed platelet fragments clumped together due to Cr NPs and formed structures similar to platelet aggregates.

• We hypothesize that in vivo heterogenous structures of Cr NPs and fragmented platelets could cause further platelet activation and aggregation (thrombosis) as in vivo the number of platelets would exceed the number of NPs that could encapsulate them. Cobalt NPs caused typical platelet aggregation, therefore could potentially lead to thrombosis via standard platelet aggregation-associated mechanisms.
Summary points

1. Our study provides evidence that both Co and Cr NPs affect platelet function in vitro.

2. Currently, in the era of the development of nanotherapeutics and an increasing prevalence of total disc replacement surgeries, concerns over the toxicity of nanoparticles are warranted. We suggest that further in vivo studies should determine safe Co and Cr levels in humans and establish concentrations that potentially would warrant revision surgery to exchange Co-Cr implants with new or alternative bearings.