Macrophage Response to Nanometer-Size Chromium Oxide Particles

[1]VanOs R., [2]Beaulé P.E., [1,2]Catelas I.
[1]Mechanical Engineering, University of Ottawa; [2]Department of Surgery, University of Ottawa, ON, Canada

Statement of Purpose: Wear particle-induced periprosthetic osteolysis remains a major cause for aseptic loosening leading to implant failure. Previous studies have shown that the biological response to wear particles varies with particle composition, size, shape and concentration (1), with particles in the submicron-range being the most biologically active (2). Despite the low wear produced by metal-metal (MM) hip implants, there is a current concern about the long-term effects of exposure to wear products from such implants, which have been shown to produce a large percentage of 30-60 nm size chromium oxide particles (3). The biological effects of such particles remain largely unknown. Therefore, the purpose of this study was to analyze the effects of nanometer-size chromium oxide particles on macrophage response *in vitro*.

Methods: J774 mouse macrophages (ATCC) were exposed to commercially available round Cr_2O_3 particles at two different sizes (60 nm (Sigma) and 700 nm (Acros)) and with increasing concentrations (up to 1.5 million particles/macrophage for the 60 nm particles and up to 1000 particles/macrophage for the 700 nm particles). Particles at two different nanometer-sizes were analyzed to evaluate potential volume effects. Particles were first sterilized for 1-3 hours in 70% ethanol followed by 10 minutes in an ultrasonic bath to allow resuspension and 3 washes in phosphate buffered saline solution. Half a million macrophages were used for each condition, resuspended in 1 ml of medium containing the particles, except for the negative controls (no particles). Incubations were conducted in tubes for 18-24 hours at 37°C, 5% CO_2, and in a humidified environment, using a rotator for constant resuspension of the particles in the medium.

Cytotoxicity was evaluated by measuring the decrease in the overall viable cell number, cell mortality (apoptosis and necrosis), and TNF-α release after macrophage incubation with the particles. The overall viable cell number was measured using a hemacytometer. Particle phagocytosis was observed using standard light microscopy and by analyzing changes in the forward scatter (index of cell size) and side scatter (index of cell granularity) using flow cytometry as previously described (4). Cell apoptosis and necrosis were quantified by flow cytometry using the Annexin-V assay. Supernatants were collected and frozen at -20°C until TNF-α quantification by ELISA. Statistical analysis was performed using the ANOVA test (with 5% as the level of significance).

Results: Results showed that both 60 nm and 700 nm Cr_2O_3 particles caused a significant decrease in the overall viable cell number with increasing particle concentration (down to 43% compared to control with 1.5 million of 60 nm particles/macrophage ($p=0.0003$), and down to 40% with 1000 of 700 nm particles/macrophage ($p=0.002$)). When combining size and concentration into volume, results revealed a potential overall volume effect (Figure 1). Cell observation by light microscopy and changes in cell granularity depicted by flow cytometry revealed that both 60 nm and 700 nm Cr_2O_3 particles were phagocytosed by the macrophages in a dose-dependent manner. Early and late cell apoptosis remained at low levels for both particle sizes and at all concentrations analyzed, suggesting that chromium oxide particles did not induce apoptosis at these concentrations. However, cell necrosis significantly increased with increasing particle size and concentration (up to 8.4% over control with 1.5 million of 60 nm particles/macrophage ($p<0.0001$), and up to 9.4% over control with 1000 of 700 nm particles/macrophage ($p=0.0004$)). When combining size and concentration into volume, results revealed a potential overall volume effect (Figure 2). Finally, ELISA results did not show significant increase in TNF-α release over control at the particle concentrations analyzed.

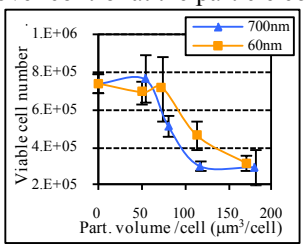

Fig. 1: Viable cell number after incubation with 60 nm and 700 nm Cr_2O_3 particles.

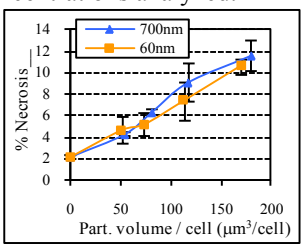

Fig. 2: Flow cytometry analysis of necrosis induced by 60 nm and 700 nm Cr_2O_3 particles.

Conclusions: Cytotoxic effects of nanometer-size Cr_2O_3 particles on macrophages were analyzed in vitro, by measuring the overall viable cell number, cell mortality (apoptosis and necrosis) and TNF-α release after cell exposure to particles. J774 mouse macrophages were chosen because of their morphological and behavior similarities with human macrophages at the implant site (5). Results demonstrated that, when present in large concentrations, Cr_2O_3 particles had a cytotoxic effect on macrophages, as depicted by a significant decrease in the overall viable cell number and a significant increase in necrosis with increasing particle concentrations. The 60 nm particles appeared to be less toxic than the 700 nm particles. When combining size and concentration, results suggested an effect of particle volume. Nevertheless, results also showed that the overall cytotoxic effects remained rather low with both nanometer sizes of Cr_2O_3, at the concentrations analyzed.

In conclusion, this study demonstrated that, unless present in large concentrations, nanometer-size Cr_2O_3 particles, i.e. a stable form of chromium oxide ceramic, had rather low cytotoxic effects on macrophages. However, other parameters (such as potential intracellular damage) remain to be analyzed.

References: (1) Shanbhag AS et al. J Biomed Mater Res. 1994;28(1):81-90. (2) Matthews JB et al. J Biomed Mater Res, 2000;52(2):296-307. (3) Catelas I et al. J Biomed Mater Res. 2003; 67(1):312-327. (4) Catelas I et al. J Biomed Mater Res. 1998;41:600-607. (5) Horowitz SM et al. J Bone Joint Surg Am. 1993;75A:802-813.