Introduction
Because of their potential for improved wear performance, there has been a revived interest in metal-metal bearings, made of cobalt-chromium-molybdenum alloys, as an alternative to the use of conventional metal-polyethylene bearings. However, metal ion toxicity remains a major concern for human implantation (1). Indeed, these ions are multiple sources of metallic corrosion products in the periprosthetic environment.

Previous studies demonstrated that both cobalt (Co\(^{2+}\)) and chromium (Cr\(^{3+}\)) ions induced mouse macrophage mortality in a dose-dependent manner (2). The results also suggested that Co\(^{2+}\) and Cr\(^{3+}\) ions induced apoptosis via a pathway involving caspase-3. The interest in apoptosis lies in the fact that it is under positive and negative regulation through evolutionary conserved biochemical pathways that are dependent on the death stimulus and cell context. Therefore, it offers specific targets for therapeutic intervention.

The caspase family of proteins plays a central role as initiators and executioners of apoptosis. The first aim of this study was to analyze the effects of Co\(^{2+}\) and Cr\(^{3+}\) ions on the activity of caspase-8 and caspase-3, respectively initiator and executioner of apoptosis, in macrophages.

Also, the Bcl-2 family of proteins plays a central role in the modulation of apoptosis. Some of them, such as Bcl-2, protect cells from apoptosis, while others, such as Bax, induce apoptosis. The second aim of the present study was to analyze the effect of Co\(^{2+}\) and Cr\(^{3+}\) ions on the expression of Bcl-2 and Bax to better understand the mechanisms leading to ion-induced apoptosis in macrophages.

Materials and Methods
U937 human macrophages were cultured in RPMI 1640 medium (Biomédia Canada, Drummondville, Québec) supplemented with 5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Macrophages were exposed for 0-24h to 0-10 ppm Co\(^{2+}\) (CoCl\(_2\), Fisher Scientific, Ville St-Laurent, Québec) and 0-500 ppm Cr\(^{3+}\) (CrCl\(_3\), Sigma Chemicals, Oakville, Ontario) at a concentration of 5 x 10\(^5\) cells/ ml of culture media. Macrophages alone served as negative control.

Caspase-3 and caspase-8 activities were measured by colorimetric assays based on the recognition of specific amino acid sequences (DEVD and IETD, respectively) (BioSource, Belgium). The presence of the active fragment of these caspases was measured by Western blot using specific antibodies against caspase-3 (Trevisien, Gaithersburg, MD) and caspase-8 (New England BioLabs, Mississauga, Ontario) antibodies.

In conclusion, our results suggest that the induction of human macrophage apoptosis by Co\(^{2+}\) and Cr\(^{3+}\) ions is complex and involves the modulation of both caspase and Bcl-2 families of proteins.

Results
Our results also show that Co\(^{2+}\) ions inhibited Bel-2 expression in a time-dependent manner with a significant effect after 16h and a maximal 52% inhibitory effect after 24h. Co\(^{2+}\) ions also inhibited Bel-2 expression in a dose-dependent manner. The inhibition was significant with 6 ppm Co\(^{2+}\) with a maximal inhibition of 52% with 10 ppm, Co\(^{2+}\) stimulated Bax expression in a time-dependent manner with significant stimulation after 8h and a maximal effect after 24h (1.55-fold control).

Cr\(^{3+}\) ions inhibited Bel-2 expression in a time-dependent manner with significant effect after 8h and a maximal inhibitory effect after 24h. The inhibition was faster than that observed with Co\(^{2+}\). This inhibition was also dose-dependent with a significant effect with 250 ppm Cr\(^{3+}\) and a maximal inhibition reaching 53% of control value after 24h with 500 ppm Cr\(^{3+}\). Cr\(^{3+}\) stimulated Bax expression in a time-dependent manner with significant stimulation after 8h and a maximal effect after 24h (2.24-fold control).

In parallel, Co\(^{2+}\) (10 ppm) and Cr\(^{3+}\) (500 ppm) ions induced a 2.5-fold increase in the expression of PARP fragment after 24h, confirming the induction of human macrophage apoptosis by these ions.

Discussion
Our results show a differential effect of Co\(^{2+}\) and Cr\(^{3+}\) on caspase-3 and caspase-8 activities in human macrophages. Caspase-8 is primarily activated by membrane-associated events (3). Our results suggest that Cr\(^{3+}\) interacts with cell membrane components to induce macrophage apoptosis. On the other hand, Co\(^{2+}\) does not interact with caspase-8 membrane-associated components and would therefore stimulate caspase-3 activity and apoptosis most likely through intracellularly located mechanisms.

Our results also suggest that the induction of human macrophage apoptosis by Co\(^{2+}\) and Cr\(^{3+}\) ions is occurring, at least in part, through the inhibition of Bel-2 and the induction of Bax expression. As observed in the present study in control cells, Bax expression per se does not harm cells under physiological conditions and can induced apoptosis after external stimulus (4). It appears that the ratio between Bel-2 and Bax is more important in the regulation of apoptosis than the level of each protein separately (5). In this regard, we observed significant increases of the Bax/Bel-2 ratios with both Co\(^{2+}\) (3.4 fold increase) and Cr\(^{3+}\) (7.0 fold increase).

In conclusion, our results suggest that the induction of human macrophage apoptosis by Co\(^{2+}\) and Cr\(^{3+}\) ions complex and involves the modulation of both caspase and Bel-2 families of proteins.

References