Particulate and Ion Forms of Cobalt-Chromium Alloys Result in Different Biological Responses on Preosteoblasts

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Cobalt-Chromium alloys are widely used in total joint arthroplasty due to its good abrasive resistance and mechanical behavior. However, recent studies have suggested that elevated ion forms of Co^{2+} and Cr^{3+} are present in failed metal-on-metal joint replacement recipients. Aseptic loosening has become the most common major long-term complication following total joint arthroplasty, particulate wear debris generated at the bearing-surface are believed to play a critical role in pathogenesis of aseptic loosening.We hypothesize that CoCr debris particles and its ions may challenge osteoblastic cells and play an important role in regulating the differentiation/activation of osteoclasts. In addition, the metal ion forms may behavior differently than the particulate form during the aseptic loosening process. The current study tested our hypothesis by investigating the biological responses of preosteoblast to CoCr-alloy particles and metal ions Co^{2+} and Cr^{3+}.

Methods:

Preosteoblast culture and induction. Mouse-originated preosteoblastic cells (MC3T3-E1) were purchased from ATCC. Cells were cultured in Alpha Minimum Essential Medium with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 100U/ml penicillin and 100 mg/ml streptomycin. For osteoblast differentiation, 10 mM β-glycerol phosphate, 100 uM L-ascorbic acid, and 100 nM dexamethasone were added to the above medium.

Assessment of cells proliferation. Aliquots (200 uL) of 2.5x10^4 MC3T3-E1 cells were dispensed into each well of the 96-well plates in the presence of the CoCr particles (at 0.3 or 5 mg/mL), or metal ions Co^{2+}, Cr^{3+} (respectively or in combination) at 62um and 500um. Cells in osteoblast-induction medium without particles or ions were cultured as a control group. All groups were cultured for 3 days at 37C and 5% CO2 atmosphere before 3-(4,5)-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed to determine the mitochondrial activity. The plate was read at 590 nm using a microplate spectrophotometer, and cell responses were expressed as a proliferation index, which was calculated by normalization with the baseline proliferation (metal-free controls).

Assessment of cells cytotoxicity. Aliquots (200 uL) of 5x10^3 MC3T3-E1 cells were dispensed into each well of the 96-well plates in the presence of the CoCr particles at 15, 30, 62.5, 125, 250 or 500 μg/mL, or metal ions Co^{2+}, Cr^{3+} (respectively or in combination) at 31, 62,125, 250, 500, and 1000 μm. Cells cultured in osteoblast-induction medium without particles or ions were provided as a control group. Cells were cultured for 6 days. CytoTox 96® Cytotoxicity Assay was used to measure dead/live cell ratio. LDH activity in conditioned supernatant and lysed cells was also assayed respectively by colorimetric reaction with Substrate Mix. The optical density (OD) values were recorded spectrophotometrically at 490 nm and calculated for cell viability.

ALP activity assay. Aliquots (600 μL) of 6x10^4 MC3T3-E1 were dispensed into each well of the 48-well plates in the presence of the CoCr particles at 30 or 125 μg/mL, metal ions Co^{2+}, Cr^{3+} (respectively or in combination) at 62 or 250 μM. Cells were cultured for 3 days before lysis in CelLytic M (Sigma-Aldrich, St. Louis, MO) and centrifuged for 15 min at 12,000 rpm. The supernatant was saved for protein assay. Cell lysates were assayed using the ALP Kit (Sigma-Aldrich). Based on the colorimetric reaction with hydrolysis of p-nitrophenyl phosphate, ALP activity was determined by OD values read at 405 nm on a microplate spectrophotometer. Enzyme activity was normalized against protein concentration of each sample.

Real-time PCR. To gain insights of the molecular basis of the biomaterial particle and ion effects on the preosteoblastic cells, real-time polymerase chain reaction (RT-PCR) was performed to screen the gene expression of Lrp5, NFAT, OSX, Runx2, RANKL, OPG, as well as TNF, IL-6, IL-8R and MCP.

Results:

As differentiation and proliferation proceeded, the preosteoblasts exhibited osteoblasts-like spindle or polygonal morphology in the osteoblast-induction medium, and the phenotype of osteoblastic cells was confirmed with elevated ALP activities. High concentrated CoCr-particles (500μg/ml) and Co^{2+} ions (500μM) significantly inhibited the proliferation of MC3T3 cells ($p<0.01$), while no significant influence was noticed at low-dosed particle (30μg/ml) and ion (62μM) concentrations. Similarly, significantly higher cytotoxic effects were observed on cells challenged with high concentrations of particle and ions. Furthermore, ALP expression was significantly diminished following CoCr-particle and Co^{2+} ions interactions at both high and low concentrations ($p<0.01$). However, Cr^{3+} ions alone appeared to have no influence on the cellular ALP activity in comparison with control group.

PCR data suggested that CoCr-particles and Co^{2+}, Cr^{3+} significantly stimulated MCP-1 expression at both high and low concentrations (CoCr-particles and Co^{2+}, $p<0.01$; Cr^{3+}, $p<0.05$). However, it appears that the metal particles exhibited much stronger influence than the ion forms in promoting MCP-1 gene expressions ($p<0.01$), and the activation appeared in dose-depended pattern.

TNF expression was also significantly stimulated by CoCr-particles (up to 10 folds), and Co^{2+} (up to 6 folds) ions in dose-dependent manner (0.3mg/ml CoCr-particles and 62uM Co^{2+} $p<0.05$, others $p<0.01$). Cr^{3+} ions again had no significant influence on TNF expression compared to the control group. Among groups, the metal particles exhibited stronger promoting influence than most ion forms ($p<0.01$), and the activation appeared dose-depended. Combination of the two ions showed synergistic effects than the individual ion stimulation of TNF expression ($p<0.01$). Similarly, IL-6 expression was significantly stimulated by CoCr-particles and individual ions, and the activation appeared in dose-depended pattern except in Cr^{3+} group, Again, the particles exhibited much stronger influence in promoting IL-6 gene expressions compared to the ion forms ($p<0.01$).

NFAT expression was significantly stimulated by CoCr-particles and Co^{2+} ions ($p<0.01$), but not by Cr^{3+}, and the activation appeared in dose-depended pattern. Cells interacted with combination of Co^{2+} and Cr^{3+} ions exhibited significantly higher expression of NFAT than the individual type of ions. RANKL expression was significantly stimulated by various concentrated CoCr-particles and high dose of Co^{2+} ions ($p<0.01$), and Cr^{3+} again had no significant influence. It appears that the Co^{2+} ions exhibited much stronger influence in promoting RANKL gene expressions compared to the metal particles ($p<0.01$). OPG expression was significantly reduced by all the groups except the low dose Cr^{3+} group, and the inhibition effect appeared in dose-dependent manner. Among groups, it appears that the Co^{2+} ions had more significant influence than particle groups in reducing OPG expressions ($p<0.01$).

Lrp-5 expression was significantly stimulated by the particles and ions. However, cells with high dose of Co^{2+} interaction resulted in significant inhibition of Lrp-5, compared to other groups including controls.

OSX expression was significantly reduced by all the groups ($p<0.01$), and the deactivation appeared in dose-depended pattern. Among groups, high concentrated Co^{2+} ions (500μM) showed much stronger inhibition influence than other groups ($p<0.01$).

Runx2 expression was significantly inhibited by CoCr-particles and high dosed Co^{2+} ions ($p<0.01$). However, low concentration of Co^{2+} ions appeared to have a stimulation effect to pre-osteoblasts. Cr^{3+} ions again did not show significant influence on Runx2 expression compared to the control group.

Discussion:

The study provides evidence that both CoCr alloy particles and the metal ions interfere with the pre-osteoblastic cells on their proliferation, maturation, and functions. Previous studies have suggested that mature osteoblastic cells were impaired in their ability to synthesize collagen and mineralize matrix in response to metallic particles. This study further suggested that the CoCr particles and its individual ions (Co^{2+} Cr^{3+}) interacted with the preosteoblasts and influenced their differentiation to osteoblasts. It appears that the cells behavior differently to ion forms of the metal with the particulate form. Particulate CoCr alloy exhibited much stronger influence in promoting expressions of chemotaxis and inflammatory mediators such as MCP-1, TNF, NFAT and IL-6. For the regulation of RANKL/OPG radio, metal ions showed stronger effects in up-regulating RANKL and inhibiting OPG gene expressions. As to osteogenesis, Co^{2+} ions significantly diminished gene expressions of OSX, Runx2 and Lrp-5, compared to the particulate alloy and Cr^{3+} groups. It also appears that Cr^{3+} ions alone in the tested concentrations did not influence the gene expressions of TNF, NFAT, Runx2 and RANKL.