Nanotoxicology of metal wear particles in total joint arthroplasty: a review of current concepts

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ABSTRACT: Metal-on-metal (M-M) joint replacement has raised concerns about the long-term effects of metal wear debris and corrosion products. This review summarizes the current concepts in biological reactivity to metal wear particles, ions, and corrosion products. Attention is focused on Co-Cr-Mo alloy since it is the most diffused and discussed material in arthroplasty. (Journal of Applied Biomaterials & Biomechanics 2010; 8: 1-6)

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Clinical performance of metal-on-metal implants

Metal-on-metal (M-M) arthroplasty has been very successful and attractive due to very low wear. In hip joint reconstruction, the possibility to produce larger diameter implants compared, for example, to metal-on-polyethylene, has greatly improved stability. Therefore, M-M total hip arthroplasty and hip resurfacing have been increasingly used to treat younger, more active patients. However, the subsequent longer period of exposure to metal bearing degradation products (wear particles, corrosion products, and metal ions) has caused concern about the long-term biological effects, as supported by the number of patients that undergo revision surgery due to adverse tissue response (1).

Tissue reactions to Co-Cr prostheses include reaction to metal particulate and metal sensitivity. Reaction to particles has been associated to high wear and characterized predominantly by macrophage inflammatory response (Fig. 1). Metal sensitivity is characterized by a predominantly lymphocytic response but not necessarily high wear. Usually, the periprosthetic tissue shows rare metal wear particles (Fig. 2). This response termed “aseptic lymphocytic vasculitis associated lesion” (ALVAL) (2, 3) is thought to be a reaction to metal ions with development of metal-protein complexes (haptens) that stimulate an immune reaction. Recent reports have reported painful effusion or a cystic/solid mass at the bearing site, which have been termed “pseudotumors” (4-7). Willert et al first reported a form of Type 4 delayed hypersensitivity (DTH) response that was more prevalent in patients with modern generation M-M hips (8). Histologically a sensitivity reaction is characterized by diffuse and perivascular infiltrates (Fig. 3) of T and B lymphocytes, but the stimulus for their presence and the type of immune reaction they represent have not been clearly identified (3). Plasma cells, high endothelial venules, massive fibrin exudation, accumulation of macrophages with droplike inclusions, and infiltrates of eosinophils and necrosis have also been reported (3, 9, 10). Similar types of reactions have been reported in association with corrosion products from co-balt chromium non-articulating surfaces, for example, from modular head-morse taper neck (11, 12). Campbell et al related persistent pain, most commonly in the groin, and development of a large effusion to metal sensitivity (13). ALVAL and other forms of painful periprosthetic tissue reactions could reflect variations over a spectrum of metal sensitivity, or a dose-related immune response to wear particles or ions.

Both metal reactivity and metal sensitivity can cause periprosthetic bone resorption (osteolysis) and can be associated with septic or aseptic loosening. Levels of metal ion in serum or whole blood greater than 10 ppb can be an indication of increasing wear damage that could prelude an increased risk of an adverse tissue reactions (14).

Despite these evidences, the mechanisms through which metal nanoparticles and/or metal ions cause adverse physiological effects in peri-implant cells remains unclear, but is increasingly more evident that their physicochemical features play a central role in governing their cellular uptake and subsequent intracellular fate.

Unique reaction to M-M implants is due to the simultaneous presence of metal wear nanoparticulate, corrosion products, and metal ions. Additionally, wear nanoparticles can themselves undergo a corrosion process contributing to the total level of dissolved ions. Researchers have focused their attention mainly on Co and Cr, since Mo and Ni and other minor elements, although part of the alloy, do not seem to be present in sufficient amounts to elicit any
immunological response. Hereinafter, a review of the current concepts in metal nanoparticles and Co and Cr ions immunology is presented.

IMMUNOLOGICAL REACTION TO NANOPARTICLES

Wear particles are mostly generated at the articulating surfaces of artificial joints. The distribution of particle size and shape changes with the severity of wear (15): M-M articulation generates approximately $10^{12} - 10^{14}$ particles per year having on average a size of less than 50 nm. However, difficulties associated in isolating and characterizing small particles, smaller than 10 nm, suggest that the average size could be even less than 50 nm and that the number of particles actually produced could have been well underestimated. Studies have shown the majority of particles originating from wear of Co-Cr-Mo alloy implants to be often Co-deficient and frequently oxidized to hydroxides, phosphates, and oxides (16-18). This is in agreement with studies conducted on retrieved tissues (16, 19) and consistent with the fact that Cr tends rapidly to form an oxide.

In vitro and in vivo studies have shown that Co-Cr particles are the main stimulus for monocyte/macrophage activation and secretion of proinflammatory IL-1β, TNFα, IL-6 and IL-8 and can upregulate transcription factor NF-kB and downstream proinflammatory cytokine (20-24). Results from experiments with transgenic and knockout mice support the theory that wear debris particles stimulate osteolysis via NF-kB activation and TNFα production (25-27). Small particles are supposed to stimulate a continuous release of low levels of TNFα (28), which in synergy with the receptor activator of the NF-kB ligand (RANKL) stimulates bone resorption. It has been recently hypothesized that wear particles can induce macrophage response through the inflammasome multiprotein complex in a concentration-dependent manner (29). Cytokine induction via the NF-kB pathway can occur through a variety of proinflammatory receptors and pathways. However, inflammasome activation produces IL-1β that feeds back and activates NF-kB, resulting in the production of other proinflammatory cytokines. Nevertheless, it is still not clear to what degree metal implant debris activation of
the inflammasome results in NF-kB activation and TNFα release.

The toxicokinetics of metal wear nanoparticles and associated corrosion products remain unclear. However, it is becoming increasingly more evident that physicochemical features of nanoparticles have a major role in affecting their cellular uptake and subsequent physiological impact. To this regard, size has been shown to be critical. As the size of the particle decreases, its surface area increases allowing a greater proportion of atoms to be displayed on the surface rather than the interior. The increased surface area determines the potential number of reactive groups on the particle surface and ultimately could translate into greater sensitivity to corrosive environment. The small size and corresponding large specific surface area and consequent increased surface reactivity, predicts that metal nanoparticles exhibit a greater biological activity per given mass compared to larger particles. Recent in vitro studies have shown Co-Cr nanoparticles to be more biologically reactive than microparticles, inducing more DNA damage and cytotoxic effects and more free radicals in a cellular environment (33, 31).

Shape and morphology of the nanoparticulate also has an influence. Studies have shown spherical nanoparticles have a higher uptake compared to rod-shaped ones. However, internalization of rods is strongly influenced by the aspect ratio (AR). Thus, high AR particles are internalized considerably faster than those presenting a low AR and more symmetry (32-34).

Another important factor influencing the uptake of nanoparticulate is their agglomeration. Agglomeration could influence the cellular uptake because of the larger dimension that might no longer be in the nano size range. Consequently, the exposure-associated risks may be substantially reduced.

Surface charge and chemistry are also critical factors that play an important role governing cellular uptake. First, surface charge and chemistry govern the formation of agglomerates. Furthermore, since the phospholipids on the outer surface of the plasma membrane make it negatively charged, positively charged nanoparticles may be endocytosed at a greater rate than those that are negatively charged. Additionally, DNA is negatively charged, thus cationic nanoparticles may be more likely to interact with the genetic material.

Studies have shown that Co-Cr particles immersed in serum form a layer of calcium phosphate and a surface coating of protein, whereas particles immersed in synovial fluid are likely to be coated with proteoglycans (35, 36). It has been suggested that particles coated with proteins (for example albumin) are internalized at a lower rate because of their anionic nature (37).

There are different mechanisms by which metal nanoparticles can translocate across the plasma membrane. Diffusion (either directly or through membrane channels 10-30 nm wide) and endocytosis, particularly receptor-mediated endocytosis (RME), are the two main mechanisms. Clathrin- or caveolae-mediated endocytosis, result in the formation of pits in the region of 120 nm or up to 80 nm, respectively, that regulate the size of the particle they are able to enclose. Metal nanoparticles smaller than 200 nm are primarily internalized via RME and 50 nm particles are taken up faster and to a greater extent when compared to smaller (down to 14 nm) and larger (up to 500 nm) particles (32, 33, 38, 39). Another mechanism of internalization of small particles is via pinocytosis, a non-specific form of endocytosis.

IMMUNOLOGICAL REACTIVITY TO METAL IONS

The successful use of Co-Cr-Mo alloy in orthopedics is due to its high resistance to corrosion because of the formation of a thermodynamically stable oxide layer, highly enriched in Cr (40). This oxide layer acts as a kinetic barrier reducing the flow, both ways across the interface, of some species associated with corrosion (41, 42). When exposed to biological fluids and because of the synergistic effect of fretting or wear, this layer can be damaged or removed. Cr is rapidly transformed into Cr-oxide, while Co and Mo diffuse from the bulk metal into solution. Cycles of mechanical and/or chemical damage, reformation of the protective layer (cycles of activation/passivation), local corrosion due to the formation of cracks and scars (crevice and pitting) on the bearing surface (43), and the billions of particles of wear debris released (16), may contribute to a high concentration of metal ions in the surrounding environment.

In vitro corrosion models have shown that during activation/passivation cycles, Co dissolution is greater than predicted by the composition of the alloy (44). In an animal model, researchers have recently shown how loose implants led to a marked enrichment of Co in tissues (45). Routine analyses of blood and urine have shown increasing ion levels, particularly Co, associated with implant wear (14, 46, 47). The release of Cr(VI) from Co-Cr alloy remains controversial. The solution in contact with the implant and/or wear particles influences the formation of the protective layer and production of soluble metal ions. Adsorption of phosphate ions is thought to reduce the corrosion rate of the alloy by blocking the mass transport of oxygen (36). In contrast, the presence of protein complexing agents like fibrinogen, transferring, globulin, and albumin, with affinity for transition metals, produces increased dissolution of metal alloy and particles.

Cytotoxicity of Co and Cr on macrophages in vitro has been shown to be dose-dependent, as well as time-dependant (48). Co(II), which enters more readily into the intracellular environment, has been found to be more toxic than Cr(III) and at much lower concentration (20, 49). Additionally, it has been shown that Co-Cr alloy degra-
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Mention products in ionic and nanoparticulate form can act as haptons in the immune system. Binding to local proteins, they can induce protein conformational changes that result in neo-antigens, which can elicit an adaptive immune response analogous to the DTH reaction.

The combination of wear and corrosion of the bulk implant and of the released particles will significantly increase the ion levels in the synovial fluid, as well as in the phagocytic cells that ingest particles, possibly to toxic intracellular levels. Particles produced at the wearing surfaces can go either into the synovial fluid or into the tissues via the synovial fluid. Synovial fluid serves as a relatively large capacity environment so that if the particles corrode the ions that elute from them are released in a large volume. Thus, corrosion alone should not produce high concentration of ions in synovial fluid. Tissue however, is insoluble. Moreover, it acts as a sieve, entrapping the larger particles. Phagocytosed Co-rich particles will directly affect the cells via intra-cellular corrosion. Such a mechanism could lead to a vicious cycle of necrosis, recruitment of macrophages and rephagocytosis leading to an expanding necrotic zone. Moreover, studies have shown that internalized particles enter an acidified phagosomal microenvironment, with pH reaching values as low as 4.6 (37). Such a low pH is expected to favor accelerated corrosion of wear particles.

Necrosis is often extensive in the periprosthetic tissues, including pseudotumors, surrounding failed M-M implants (6). Co is more likely than Cr to cause necrosis, and in vitro studies support this view. At low concentrations of Co, apoptosis prevails, while higher concentrations produce cell necrosis (50, 51), and this is consistent with the lymphocytic inflammation seen in tissues. Unfortunately, cell toxicity studies have been typically performed using particles obtained with pin-on-flat testers (52, 53), commercially available nanoparticles (48, 54) or ionic salt solutions (51, 55). These forms of the metal substantially differ from the particles that initiate lesions in periprosthetic tissue.

Patients with Co-Cr hip replacements have shown increased levels of structural and numerical chromosomal aberration in peripheral blood lymphocytes (56, 57). In vitro studies have demonstrated that synovial fluid obtained from patients undergoing revision surgery induced double strand breaks in fibroblast cells (58) and chromosomal damage in primary amnion cells (56).

A key mechanism thought to be responsible for genotoxic effects involves oxidative stress, a redox imbalance within the cell usually due to increased intracellular reactive oxygen species (ROS) and decreased antioxidants. The high surface area of nanoparticles and the presence of transition metal ions (ie Co and Cr) can promote the generation of ROS. Oxidative stress has been shown to activate specific signal pathways including NK-kB, which together with the depletion of antioxidant defense, lead to the release of proinflammatory cytokines. This cascade of signal triggers inflammation, which leads to further ROS release from inflammatory cells resulting in a vicious circle.

It is still unclear if the cytotoxicity of Co-Cr nanoparticles is due to the nanoparticles themselves or mediated by metal ions in solution. Certainly, the size of the wear debris, the chemical composition, and the corrosion properties of the material pose new challenges in understanding and modeling in vitro the complex interaction between the metal wear particles, corrosion products and ions, and the biological milieu.

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