Commentary

The potential biohazards of nanosized wear particles at bone–prosthesis interface

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INTRODUCTION

Much attention has been paid to the nanoparticles (NPs) that are produced for applications in various areas, such as semiconductor or metallic NPs used in the fabrication of components, polymer additives in packaging, tires or NPs for catalysts or in sunscreen formulations, and oxide NPs applied in our daily life. These NPs are produced in the environment and may penetrate into the human body through outer-in routes, such as respiratory inhalation, gastrointestinal (GI) tract, or dermal and blood circulation.[1, 2]

However, there are some other ways in which NPs are generated in vivo and make the exposure of NPs different from the external environment and cause distinctive bioeffects. Less well realized by those interested in nanotoxicology is that there is also the possibility of an internal exposure to particles from surgical implants in the orthopedic field (Fig. 1). In this paper, we will mainly address wear debris continuously generated from motion taking place at the articulating surfaces.

Artificial hip joints have been used for endoprostheses since the middle of the last century. Approximately 1 000 000 joints are replaced annually worldwide, with a rising tendency. Metals, ultra high molecular weight polyethylene (UHMWPE), and ceramics are the three main materials used in the design of implant components for hip and knee replacement. In early 1976, Harris already reported the extensive localized bone resorption resulting in implant loosening without resulting in infection in hip arthroplasty. After that, aseptic loosening became the most common cause of revision of major arthroplasties. Among various causes resulting in aseptic loosening, wear particles is the major one considered by a majority of orthopedists. The formation and related biological responses of wear particles are major factors determining the longevity of hip and knee arthroplasties.[4]

Wear particles from the articulation interface dispersing into the joint fluid and directly coming in contact with the implant-bed and bone is the major cause leading to the alteration in osteoblast functions. It eventually results in abnormal bone formation and remodeling between implant and bone. Therefore, research in this area has largely focused on the properties of the retrieved implants. Periprosthetic tissues; in vivo and in vitro models of implant loosening; and the activities of major participating cells, such as osteoblasts, macrophages, and fibroblasts, also become the focus of the related studies.

Understanding the NP–cell interaction is critical for the safe development of nanomaterials, and the biological evaluation of NPs have been prone to be a necessity or a pioneering step in interdisciplinary nanotechnological fields. Much work has been performed to challenge the issue of how to assess the biosafety of these diverse chemicals that humans are potentially exposed to. In this review, we retrospect on the studies dedicated to biological response in joint tissues irritated by particulate debris that consist of metals, polyethylene (PE), and ceramics as the primary cause of periprosthetic osteolysis and the subsequent implant loosening in total joint replacements. Then we also survey the osteo-effects of nanosized wear particles and discuss the NPs’ biohazards when they are exposed within the privileged sites in the human body. We suggest potential future directions in biosafety evaluations of NPs with attention to nanotoxicology not only from the angle of environmental science but also based on the aspect of biomedical applications, especially in orthopedic surgeries involving metal implantations.
BONE CELLS BETWEEN THE BONE–PROSTHESIS INTERFACE

The current research focus is on the bone–prosthesis interface. Normal bone function relies on the equilibrium between bone formation and bone resorption. Over-stimulated bone resorption and/or inhibited bone formation cause the abnormal bone remodeling.

Many types of cells directly and indirectly encountering the wear particles influence bone remodeling. Within the interfacial granulomatous fibrous tissues, the dominant cell types are osteoblasts, osteoclasts, fibroblasts, macrophages, lymphocytes, bone marrow-derived mesenchymal stem cells (bMSCs), endothelial cells, and foreign-body giant cells. They are adjacent to prostheses, maintaining physiologic bone remodeling through the balanced coordination of bone formation and resorption.[5] The response of these cells to wear particles, including macrophages and fibroblasts, has been investigated extensively in the past.

In many situations, even the viability and proliferation of bone cells were not notably inhibited. Exposure to wear particles changes the osteoblasts’ function on bone remodeling process, resulting in osteolysis.[6–8] Studies showed that macrophages activated by wear particles released bone resorption mediators that triggered bone-resorbing activity.[9,10] Fibroblasts (fibrous tissue forming cells) cultured with wear particles demonstrated an increase in matrix metalloproteinase (MMP) synthesis, which was involved in periprosthetic osteolysis.[11,12] All these biological responses make the biosafety evaluation of NPs in wear particles different from that developed for traditional nanomaterials toxic study. First, it confines the cell types, osteoblasts, fibroblasts, and macrophages, to undergo the bioeffect evaluations. Second, not only the viability and proliferation of targeted cells need to be considered but the cellular functioning profiles should also be tested. Table 1 summarizes the major cell types and their characteristic mediators when they respond directly to particulate wear particles.

<table>
<thead>
<tr>
<th>Major cell types</th>
<th>Characteristic mediators</th>
</tr>
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<tbody>
<tr>
<td>Osteoblasts</td>
<td>Fibronectin (FN), type I and III collagen, osteoprotegerin, alkaline phosphatase, osteocalcin, IL-6, PGE2, RANKL</td>
</tr>
<tr>
<td>Macrophages</td>
<td>TNF-α, IL-1, IL-6, IL-8, M-CSF, GM-CSF, PGE2, MMP-1, MMP-2</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>MMP-2, type III collagen, M-CSF, OPG, RANKL</td>
</tr>
</tbody>
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biosynthesis of fibronectin (FN) and bone matrix collagen (types I and III), alkaline phosphatase (ALP) activity and osteocalcin production that reflects the osteoblast function, nuclear factor B ligand (RANKL) and local cytokine (prostaglandin-E2) production as the recruiters to osteoclasts are considered to be important indications of the functional modulation of osteoblasts. A decrease in bone formation may result from suppressed osteoblast function and reduced biosynthesis of bone matrix collagen and FN in response to wear particles.\cite{7-13}

In activated fibroblasts, enhanced expression of MMPs, collagenase, stromelysin, and, to a lesser extent, tissue inhibitors of MP have been found. Thus, to characterize the contribution of catabolic processes to tissue remodeling, \textit{in vitro} zymographic tests can be performed to evaluate the matrix activity of MMP-2 and MMP-9, the two proteolytic activities involved in the degradation of the organic matrix in the bone-implant bed.\cite{12,14}

Macrophages activated by phagocytosed particles produce inflammatory mediators, such as tumor necrosis factor $\alpha$ (TNF-$\alpha$), interleukin (IL)-1, and IL-6. These ‘bone-resorbing’ agents that eventually activate all cell types in the interface membrane in either a paracrine or an autocrine manner can be determined by enzyme-linked immunosorbent assay.\cite{10}

Only recently, attention has been paid to the effects of wear particles on progenitors of bone cells.\cite{15} Bone-resorbing osteoclasts are derived from the monocyte/macrophage hematopoietic cell lineage, whereas osteoblasts developed from MSCs.\cite{16} The alteration of differentiation, maturation, and function of osteoprogenitors profoundly contribute to the osteolytic process by decreasing bone formation. Orthopedic wear particles are continuously produced throughout the lifetime of the prosthesis; meanwhile, bone regeneration and remodeling around prosthetic implants is an ongoing process. Because many studies show that the viability, proliferation, and gene and protein expression of osteoblast progenitors are highly sensitive to the presence of orthopedic wear particles in different materials, it is also important to delineate the effects of orthopedic wear particles on osteoprogenitors.\cite{15}

In prosthesis-associated osteolysis, all of the various types of cells communicate via an intricate paracrine network of cytokines, chemokines, oxygen-containing radicals, and other molecules resulting in the undermining of the prosthetic bed.

**THE EFFECTS OF WEAR PARTICLES ON BONE CELLS**

Particles extracted from the tissues of failed arthroplasties display a wide distribution of wear particle sizes from 1 nm to 1000 $\mu$m.\cite{17,18} Previous studies mainly focused on wear particles with sizes ranging from submicron to micron meters, because the particle isolation and characterization methods in the past were relatively inaccurate. Later on, investigation of the biological response of bone cells to metallic, polymeric, and ceramic particles \textit{in vitro} were widely conducted to comprehend the relationship between characteristics of particles and cellular behaviors (Table 2).

Osteoblasts are used extensively to evaluate their responses to wear particles. Particles of commercially available pure titanium (CP Ti), Ti–6Al–4V alloy, chromium orthophosphate, medical grade UHMWPE (most of the particles $<5 \mu$m in diameter) have been exposed to MG 63 osteoblasts. All these types of particles significantly suppressed type I procollagen gene expression and increased the secretion of IL-6 and Transforming Growth Factor (TGF)-$\beta$1. However, they did not suppress other osteoblast-specific genes, including osteocalcin, osteopontin (OC), and ALP.\cite{19} When human osteoblasts were cultured with alumina (Al$_2$O$_3$, average diameter = 1 $\mu$m) or UHMWPE (size range = 0.1–1 $\mu$m) particles for 24 h, a reduced ratio of Osteoprotegerin (OPG) to receptor activator of NF-$\kappa$B ligand (RANKL) and induced-osteoclast formation from monocyte/macrophages were demonstrated, with alumina particles being more inert than PE particles.\cite{20} In the case of CP Ti, Ti–6Al–4V, and CoCr, particles approximately 1 $\mu$m in size were incubated with human bone cells up to 48 h, and it has been found that protein of osteopontin, OC, and bone sialoprotein

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Particles</th>
<th>Size ($\mu$m)</th>
<th>Responses of cell \textit{in vitro}</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblasts</td>
<td>CP Ti, Ti–6Al–4V, PE</td>
<td>$&lt;5 \mu$m</td>
<td>type I procollagen ↓</td>
<td>\cite{19}</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Al$_2$O$_3$</td>
<td>1 $\mu$m</td>
<td>IL-6 and TGF-$\beta$1 ↑</td>
<td>\cite{20}</td>
</tr>
<tr>
<td>Macrophages</td>
<td>PE</td>
<td>0.1–1 $\mu$m</td>
<td>OPG ↓</td>
<td>\cite{20}</td>
</tr>
<tr>
<td></td>
<td>CP Ti, Ti–6Al–4V and CoCr</td>
<td>1 $\mu$m</td>
<td>OPG ↓</td>
<td>\cite{21}</td>
</tr>
<tr>
<td></td>
<td>CP Ti</td>
<td>$&lt;5 \mu$m</td>
<td>Osteopontin, OC and bone sialoprotein (BSP) ↑</td>
<td>\cite{23}</td>
</tr>
<tr>
<td></td>
<td>CP Ti</td>
<td>$&lt;5 \mu$m</td>
<td>IL-6, RANKL ↑</td>
<td>\cite{10,12,14}</td>
</tr>
<tr>
<td></td>
<td>PE, CP Ti, CoCr</td>
<td>0.1–1 $\mu$m</td>
<td>MMP-2, MMP-9 ↑</td>
<td>\cite{10,12,14}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TNF-$\alpha$, IL-1, IL-6, GM-CSF, PGE$_2$ ↑</td>
<td>\cite{10,12,14}</td>
</tr>
</tbody>
</table>

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NANOSIZED WEAR PARTICLES OBSERVED FROM THE RETRIEVAL TISSUES

The description of nanometer-sized ceramic wear particles with a mean size of 24 ± 19 nm ranging from 5 to 90 nm in retrieval tissues was first described by Ingham’s research group, according to the advanced technologies in Periprosthetic Tissue Digestion. Wear debris extraction methods, metal NPs sized between 20 and 60 nm and PE NPs (the smallest identified was 30 nm) were also identified in samples retrieved in vivo. They also found that particle size was a crucial factor in the function, proliferation, and viability of the osteoblasts. A size of 1.5–4 µm had the greatest effects on the reduction of osteoblast proliferation and viability, and on enhancement of the expression of RANKL mRNA and matrix MP-2 and MP-9 in vitro. The size of the particles generated from the wear of a prosthetic device may be an important consideration in the development of superior implant technology. It can be seen that the size of the particles in the above studies is more than 100 nm, because isolation and scope of wear particles reported before are mostly in a range from submicrometers to several micrometers.

FUNCTIONAL DISTURBANCES OF CELLS ARE MORE CRITICAL IN NANOTOXICITY

As mentioned above, in the studies on wear particles, the evaluation of bone cell functional disturbances is the focus of our research. We know that most of the clinically used biomaterials or nanomaterials are screened using a series of toxic examinations so that they are less toxic or not toxic to cells. However, the lack of toxic effects does not necessarily mean that there is no influence on cells. In our recent studies, we used commercially available nanosized ceramic particles (alumina, zirconia, and silicon nitride) for assessing NPs’ biological effects on osteoblasts and macrophage-like cells in vitro. We found that NPs had differentiated effects on the same cell (viability and function); for instance, alumina NPs significantly promoted ALP activity and had no suppression on the viability of MG63 cells at low dose; silicon nitride and zirconia NPs could remarkably promote ALP activity of osteoblast-like MG63 cells and stimulate the secretion of TNF-α in RAW264.7 cells; alumina NPs promoted ALP activity of MG63 cells but had no irritation to RAW264.7 cells. In Melissa’s study, uptake of SiO2 and TiO2 NPs decreased the number of molecules released per granule in mast cells and particles. NPs appeared to disintegrate within the cells faster than microparticles with the creation of electron dense deposits in the cell, which were enriched in cobalt. But there seems to be some conflicting findings on particle behavior. In studies by Gutwein and Webster, they exposed human osteoblasts to conventional alumina (0.18 µm) and titania (4.12 µm) particles and nanosized particles of alumina (23 nm) and titania (32 nm) at concentrations of 100–10 000 mg/mL for up to 6 h. Compared to conventional particles, the nanosized particles were prone to increase cell viability and maintain more normal cellular morphology and spreading. They concluded that decrease in particle diameter to the nanometre range, which resulted in particles clumping into a nanophase, might facilitate osteblast survival. More and more studies indicated that wear particles with different sizes (from nanometers to submicrometers to micrometers) caused varying responses in vitro and in vivo.

With the increasing importance of nanotoxicity in the biomedical field, nanosized wear particles will be thoroughly studied for their bioeffects in the orthopedic implant field. Although wear debris of various compositions and wide range of sizes have been well studied for their osteo-related bioactivity in previous work, researches on wear particles below 100 nm are lacking. Furthermore, unlike the commercially available nanomaterials, the morphology of nanosized wear particles is greatly heterogeneous. This implies that the studies of nanosized wear particles must be clinically orientated.
Future field of nanotoxicology. When co-cultured with Au NPs, exocytosis of serotonin from mast cells increased in the earlier 24-h exposure while there was no decrease in viability. However, 72-h exposure showed decreased secretion of serotonin with Au NP exposure and a slight viability decrease. These results show that the critical changes in cell behavior happen even when viability is unaffected. The bioeffects of NPs are far more than the toxicity on cells. Their disturbance on cellular function may be critical in biomedical uses. To illustrate the dynamic nature of NPs, cell interactions may become the core area in the future field of nanotoxicology.

FUTURE DIRECTION IN NPS’ BIOSAFETY EVALUATIONS

The evaluation of the toxicity of NPs should be a basic step, no matter whether it is for industrial or medical use. Yet the importance of safety issues regarding NPs was recognized only recently, and no general regulations were setup for the biosafety evaluation of NPs. The ISO 10993 may be considered only as a suggestion rather than a standard of biocompatibility of NPs. We should understand how the NPs react with a cell (in vitro) or body (in vivo); that is what we call the basic phase in which efforts are mostly dedicated to cytotoxic testing. In this stage, NPs are evaluated by incubating with several kinds of cell types and using multiple cytotoxicity assays that reflect different aspects of cellular physiology considering that human bodies would be exposed to NPs through many pathways and have different tissues in contact with NPs. With respect to the particle size, composition, and surface decorations, the high throughput ‘predictive toxicity’ strategies have emerged as possible solutions to deal with issue of how to assess the safety of the diverse chemicals.

In cytotoxicity assays, changes in cellular, nuclear morphology or permeability of cell membrane, viability measuring, special enzyme release monitoring, DNA damage detecting, etc. are available. It is hoped that these multidimensional data clustering could identify NPs with similar patterns of biological activity across a broad sampling of cellular contexts, as opposed to extrapolating from results of a single in vitro assay, and develop predictive models for in vivo animal studies which are not too expensive and labor-costly to make it impractical for widespread testing. That is called the high throughput testing methodology; it sorts out safe NPs from the remarkable diversity of NPs with more consideration of the complexity involved in the in vitro toxicity assay than a tailor-made test case. Many toxicologists around the world are purchasing the high-speed assays for testing the toxicity of even thousands of different NPs at the same time.

The major concerns are whether the NPs are harmful to a cell or many cells and how serious the harm may be. Owing to the exposure routes such as the respiratory tract, GL tract, and dermal and blood circulation, the cells to be chosen could be fibroblast cells, epithelial cells, endothelial cells, lymphocytes, macrophages, MSCs, etc. These objects are individual; in other words, there is no relation to each other and they are representative at cell levels.

However, different cells may react to the same type of NPs with corresponding biological responses. This means that if NPs travel to a restricted site or tissue in the human body, we should notice that the NPs’ discriminatory impact on cells may rearrange the integrated behavior of these cells and lead to new tissue composition and function. So we call it the second phase, in which the single cell’s fate is integrated into both the structure and the function of the tissue or organ.

With an increasing application of nanotechnology in life sciences and medicine, e.g. as drug-delivery agents, biosensors, imaging contrast agents, and nanocomposite materials used as orthopedic implant or intravascular stent, NPs’ exposure to living organisms would be more common. This retention would lead to tissue-specific exposure of NPs according to the NP-specific targeting applications such as in the bone or brain for therapeutic purposes. It is suggested that an evaluation of the safety of NPs should rely on the specific tissue or organ which they apply or relate to, and that correlated pivotal cells and assays should be chosen to evaluate the NPs’ biological effects.

How to evaluate NPs as potential human health risks has been a continuing debate over the years. Our review provides a retrospection on the comprehension of how to evaluate the biohazards of NPs, especially when they are exposed in privileged sites within the human body. It does not focus on the mechanism of interaction between cell and NPs or on the technological aspects of nanotoxicity testing. However, how the NPs affect cells still remains an essential question to be addressed in the future, i.e. it could be a direct interference or otherwise in a particle—cell—cell pattern. With regard to the vast physiochemical characteristics of NPs, the effects on various biological tissues require further exploration.

Acknowledgements

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