Review

Health Effects and Safety Assurance of Nanoparticles in Vulnerable Generations

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Nanoparticles have a variety of useful functions. They have already been put to practical use in products in many industrial arenas, such as the cosmetics and food fields. Therefore, we cannot avoid the unintentional nanoparticle exposure of vulnerable people such as pregnant women and infants, and the importance of evaluating the safety of such vulnerable generations, who are highly sensitive to chemical substances, has been pointed out worldwide. However, it is still difficult to determine the hazards posed by nanoparticle exposure in everyday life. From this perspective, to analyze the risk from nanoparticles to vulnerable generations, nano-safety science research has been conducted through the collection of toxicity information on nanoparticles based on their physicochemical properties and kinetics *via* the association analysis of physicochemical properties, kinetics, and toxicity. The results of this nano-safety science research have been used in nano-safety design research to develop safer forms of nanoparticles. The findings of these studies will not only provide insights that will help us to formulate new policies for the risk management of nanoparticles; they will also lead directly to the development of sustainable nanotechnology (nanotechnology that can be safely, usefully, and sustainably used). These developments will contribute not only to the development of the nano-industry and the promotion of its social acceptance, but also to future developments in the field of health science.

Key words nanoparticle; vulnerable generation; nano-safety science; nano-safety design

1. INTRODUCTION

In recent years, there has been progress in the development of nanoparticles in the form of new materials such as fullerenes and carbon nanotubes, as well as improved materials such as metal nanoparticles.[1] Nanoparticles are generally defined as materials manufactured with a one-dimensional size of 100 nm or less.[2,3] As particles become smaller, their specific surface area increases. Nanoparticles therefore exhibit a number of useful functions not performed by conventional, larger, materials. For example, tissue permeability, electron reactivity, and interfacial reactivity are improved.[4] As a result, nanoparticles are used in various fields such as medicine (in contrast agents or as carriers in drug delivery systems),[5] cosmetics (in foundations or sunscreens),[6] or the food industry (in anti-caking agents).[7] Nanoparticles have become indispensable and are important for improving our QOL.

However, despite the usefulness of nanoparticles, there are now safety concerns about their use.[8] There is an urgent need for safety evaluation to enable us to use nanoparticle-containing products safely and securely, but research on safety assessment and safety assurance is still lagging. In this regard, considering the likelihood that opportunities for exposure to nanoparticles will increase as the amounts used and the types developed increase,[9] to ensure safety it is indispensable to analyze the relationships of physical properties, such as size and surface texture, to biological responses. Various nanoparticles have been administered to cells or animals and their responses analyzed, but the focus has been on hazard identification. For the comprehensive risk analysis of nanoparticles, we need to evaluate biological and cellular responses to real-world exposures. In other words, we need to collect information that will contribute to risk analysis by qualitatively and quantitatively analyzing not only hazard information but also toxicokinetic information, such as absorption, distribution, metabolism, and excretion, and clarifying the actual exposure level.

Promoting such nano-safety science research should make it possible, on the basis of risk analysis, to make appropriate decisions on which items are safe and on how to regulate items for which there are safety concerns. If there are concerns about the safety of nanoparticles, then the promotion of nano-safety design research aimed at ensuring safety by processing items of concern into safe nanoparticles is also an important issue. By using the two wheels of nano-safety science and nano-safety design research to develop a nanotechnology that will enable the sustainable use of nanoparticles and promote their social acceptance, it will be possible to create a society in which men and women of all ages can enjoy the use of products containing highly safe nanoparticles and the environment will be protected from adverse effects (Fig. 1).

2. NANO-SAFETY SCIENCE RESEARCH BY LINKAGE ANALYSIS OF THE PHYSICAL CHARACTERISTICS, KINETICS, AND TOXICITY OF NANOPARTICLES, AND DEVELOPMENT OF NANO-SAFETY DESIGN RESEARCH

As part of our original "physical characteristics–kinetics–

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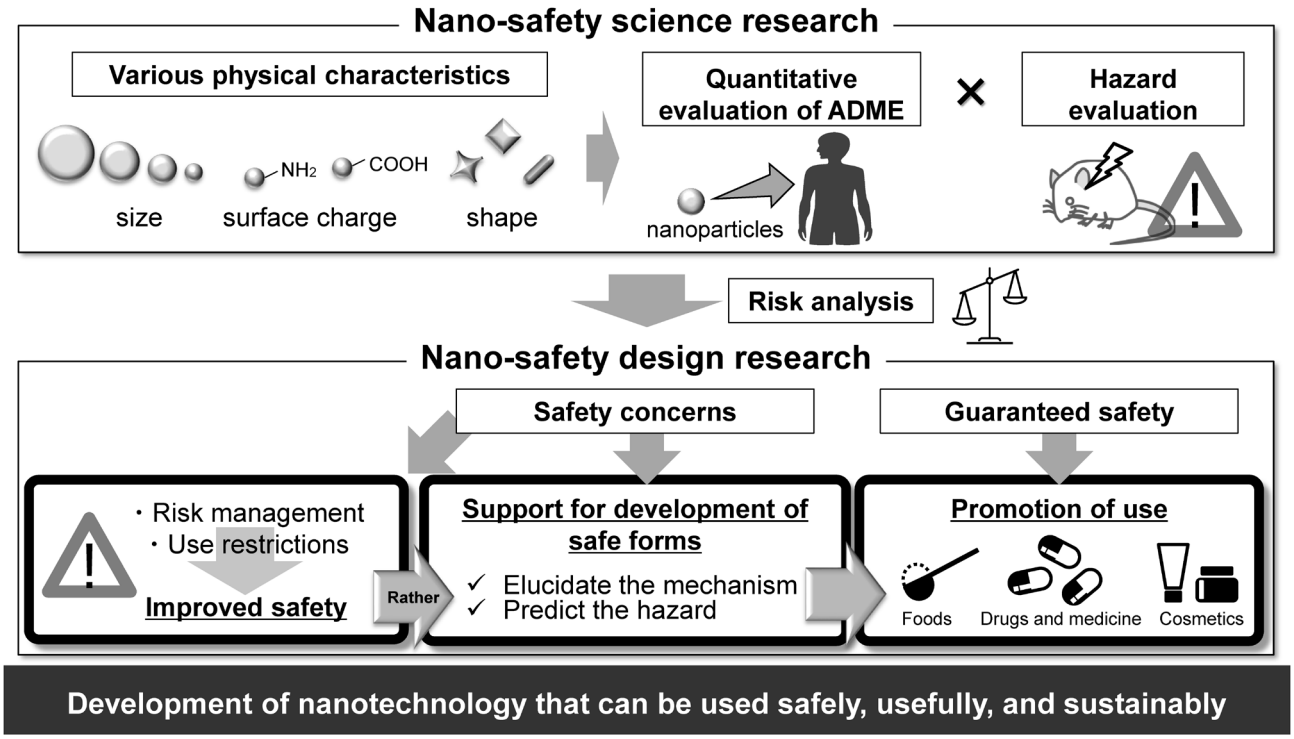

Fig. 1. Nano-safety Science Research and Nano-safety Design Research

toxicity" linkage analysis, our group has been promoting nano-safety science research to collect toxicity information on nanoparticles that is based on physical properties and kinetics. For example, as part of our effort to quantify exposure to nanoparticles (exposure amounts, toxicokinetic, intracellular behavior), we have found that the physical characteristics of nanoparticles are intimately associated with the particles' intracellular dynamics and behavior.[10] We have also identified new hazards, such as the fact that silver nanoparticles can trigger the onset or exacerbation of metal allergies[11] and that amorphous silica nanoparticles can induce disseminated intravascular coagulation syndrome.[12] In addition, in exploring the molecular mechanisms of hazard expression, we have identified key molecules (biomarkers) related to hazard expression[13–15] and have elucidated the mechanism of epigenetic control of hazard expression.[16] What is important here is that the hazards posed by, and exposure to, nanoparticles are greatly affected by physical properties such as the size, shape, and surface charge of the nanoparticles. This means that it is possible to design safe and useful nanoparticles, as well as to develop nano-safety design research for sustainable nanoparticle use.[17,18]

2.1. Toxicokinetic and Reproductive Toxicity of Amorphous Silica Nanoparticles Children are not just small adults: Fetuses and infants have underdeveloped biological defense mechanisms, such as the blood–brain barrier and the immune system.[19,20] Therefore, exposure to chemical substances has the potential to induce fatal adverse events in children, even if the exposure level has no biological effect in adults. Furthermore, the fetal period is extremely vulnerable to chemical substances, and the effects can remain for a lifetime after birth.[21,22] Therefore, health impact assessment of environmental factors such as chemical substances on vulnerable people such as pregnant women and infants is of great interest, and risk assessment of new materials such as nanoparticles is an urgent issue.

From this perspective, by using amorphous silica nanoparticles as model nanoparticles, our group has analyzed their biodistribution to the placenta and fetus and the subsequent biological effects in mice.[23] People are frequently and repeatedly exposed to amorphous silica nanoparticles because they are components of products that are used in daily life such as foods and cosmetics.[24] First, to collect *in vivo* kinetics

Biography

Kazuma Higashisaka, Ph.D., has been an Associate Professor at the Institute for Advanced Co-Creation Studies, Osaka University, since 2021. He graduated from Osaka University in 2009 and received his doctoral degree from that university in 2016. He has been a Research Fellow of the Japan Society for the Promotion of Science (2012); an Assistant Professor at the Graduate School of Pharmaceutical Sciences, Osaka University (October 2012 to September 2017); and a Specially Appointed Lecturer in the Graduate School of Medicine, Osaka University (October 2017 to March 2021). He has been awarded the Pharmaceutical Society of Japan, Kinki Branch Award for Young Scientists (2013) and the Pharmaceutical Society of Japan Award for Young Scientists (2022).

Kazuma Higashisaka

information in pregnant mice and their placentas, we exposed pregnant mice to very high doses of silica nanoparticles with a diameter of 70 nm (nSP70) and submicron-sized silica particles with a diameter of 300 or 1000 nm and evaluated the distribution to the placenta and fetus. We found that only nSP70 crossed the blood–placental barrier and were transferred to the fetal liver and brain. In addition, in a hazard analysis of the silica particles after their transfer to the mouse placenta and fetus, we observed barely any effect on the fetus in the group given submicron-sized silica particles, whereas nSP70 caused reproductive toxicity, including reduced uterine weight with increased fetal death and fetal growth restriction. Therefore, amorphous silica can cross the mouse blood–placental barrier and cause reproductive toxicity to an extent that depends on its physical characteristics.

Moreover, we showed that, in the placentas of pregnant mice given nSP70, decreased blood flow and increased apoptosis in placental cells. In addition, a decrease in serum sFlt-1, a factor that regulates maternal angiogenesis during pregnancy,[25] suggested that placental function was impaired.[23] However, we also revealed that modification of the surface of nSP70 with functional groups such as amino groups and carboxyl groups as part of nano-safety design research has potential to avoid increases in fetal resorption rate and decreases in fetal weight due to nSP70.[23] Therefore, by using the findings obtained from nano-safety science research, we can help to develop safe forms of nanoparticles.

2.2. Contribution of Neutrophils to Reproductive Toxicity Induced by nSP70 To maximize the usefulness of nanoparticles, we need not only to establish usage standards within a safe range based on hazard information, but also to elucidate the mechanism of hazard expression so as to construct a method for coping with hazards. We found that nSP70 can induce reproductive toxicity in mice, but the detailed mechanism by which this occurs has not been clarified. Neutrophils are the most abundant leukocytes in humans and contribute to the failure of pregnancy maintenance: An increase in neutrophil abundance, together with neutrophil activation, damages endothelial and epithelial cells, causing signs such as fetal growth restriction and preeclampsia.[26,27] Therefore, we considered it possible to clarify part of the mechanism of hazard expression by performing a linkage analysis of the contribution of neutrophils to the induction of reproductive toxicity by nSP70 administration. The overall aim of the analysis was to collect safety information.[28]

First, we performed a flow cytometric analysis of changes in the peripheral blood neutrophil fraction in pregnant mice after intravenous nSP70 administration. nSP70 significantly increased the neutrophil fraction compared with that in an untreated control group. Then, to analyze the relationship between reproductive toxicity and the increase in the neutrophil fraction due to nSP70 administration, pregnant mice were either pretreated with anti-Ly-6G antibody to deplete their neutrophils or treated with isotype-matched control antibodies. They were then exposed to nSP70 and their body weights monitored. nSP70 significantly reduced the body weights of the isotype control pregnant mice compared with those in mice not exposed to nSP70, as we had shown in a previous study.[23] In the mice that received intravenous nSP70 after neutrophil depletion, the decrease in maternal body weight was enhanced, suggesting that neutrophil depletion exacerbated the reproductive toxicity of nSP70 administration.

We had also shown previously that nSP70 induced collapse of the placental structure by damaging the trophoblast layer of the placenta.[23] Therefore, we analyzed how neutrophils are involved in placental function during pregnancy maintenance.[28] Analysis by hematoxylin and eosin staining confirmed the presence of marked marginal congestion in the placentas of nSP70-administered pregnant mice pretreated with anti-Ly-6G antibody. The degree of congestion was enhanced compared with that in mice given nSP70 after isotype control pretreatment. In addition, in the mice given intravenous nSP70 after neutrophil depletion, the injury caused by nSP70 in the trophoblast layer was increased. Moreover, administration of nSP70 in the absence of neutrophils increased angiopathy and the abundance of apoptotic cells in the placenta. Together, these results suggest that neutrophil depletion enhanced the placental damage induced by nSP70 and led to worsening of the subsequent reproductive toxicity.

Given that neutrophils are involved in the mechanism of metabolism and excretion of nanoparticles,[29] we consider that nSP70-induced increases in the abundance of peripheral blood neutrophils could control the pharmacokinetics of nSP70 and act as a defense against the onset of placental injury. In other words, it is possible that, in the absence of neutrophils, placental injury is exacerbated by an increase in the amount of nSP70 transferred to the placenta (Fig. 2). Collecting quantitative dynamic information on the placenta is a topic for future study.

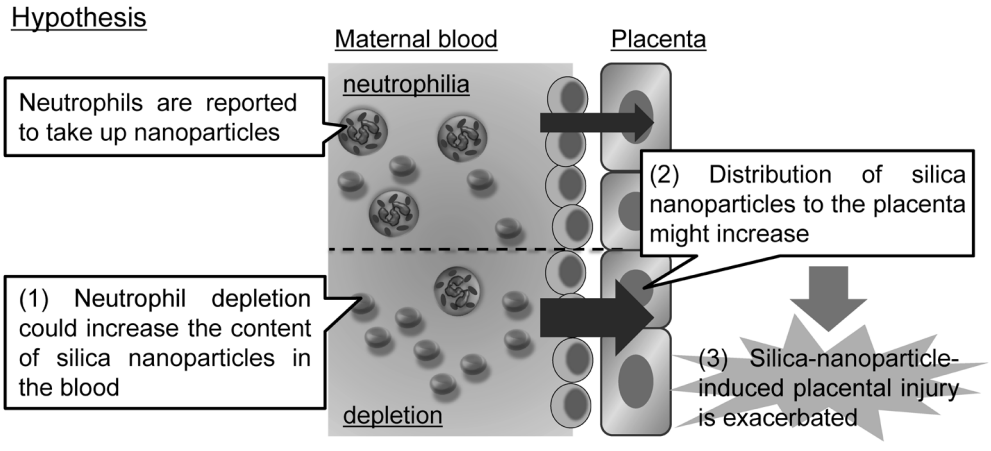

Fig. 2. Contribution of Neutrophils to the Reproductive Toxicity Induced by nSP70

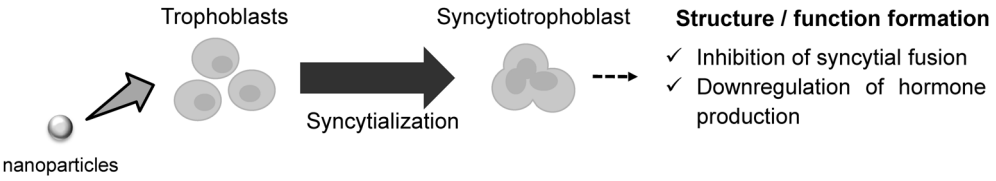

Fig. 3. Effects of Nanoparticles on Placentation

In addition, silica nanoparticles have been shown to induce pregnancy complications in mice by inducing the NLRP3 (nucleotide-binding oligomerization domain-like receptor family, pyrin domain-containing 3) inflammasome.[30] Moreover, injected silver nanoparticles and gold nanoparticles can increase the permeability of vascular endothelial cells in mice,[31] thus enhancing the permeability of biological barriers such as the blood–placental barrier. The accumulation of data on these issues, including the data gathered by our own group's efforts, should provide important insights into the mechanism of nanoparticle-induced reproductive developmental toxicity.

2.3. Effects of Silica Nanoparticles on the Biological Defense Mechanism After demonstrating the importance of the protective function of neutrophils against the reproductive toxicity of nSP70, we attempted to collect information on the fluctuation of neutrophil counts in non-pregnant mice exposed to nSP70.[32] First, we analyzed changes in the peripheral blood neutrophil fraction after intravenous nSP70 administration. The neutrophil fraction was significantly greater in mice given nSP70 than in those given saline. In addition, plasma levels of granulocyte-colony-stimulating factor (G-CSF), an essential factor for neutrophil proliferation and differentiation,[33] was significantly increased in the nSP70-treated mice, and pretreatment with anti-G-CSF antibody significantly suppressed the increase in the neutrophil fraction caused by nSP70 administration.[32] These results indicated that the neutrophilia induced by nSP70 was caused by an increase in G-CSF.

Neutrophils release myeloperoxidase (MPO) by activating and causing degranulation,[34] which acts in the decomposition of carbon-based nanomaterials.[35] Neutrophils thus play an important role in the decomposition and exclusion of foreign substances such as nanoparticles. In this regard, enzyme-linked immunosorbent assay analysis found that blood MPO levels increased significantly in the mice given nSP70, and neutrophil depletion partially, but significantly, suppressed the increase in blood MPO levels due to nSP70 administration.[32] These results suggest that nSP70 induces neutrophil activation, with an increase in the neutrophil fraction.

By acting on macrophages, serum amyloid A (SAA), an acute-phase protein, is involved in the mechanism of neutrophil production *via* G-CSF.[36] In this regard, through a proteomics analysis of mouse blood after administration of nSP70, we have found that nSP70 induces an increase in blood SAA levels.[13] We hope that promoting analyses of the association between the increase in the neutrophil count and the increase in abundance of SAA in the acute phase will help to elucidate the mechanism of neutrophilia in response to nSP70 administration.

3. UNDERSTANDING THE MECHANISM OF PLACENTAL TOXICITY TO ENSURE NANOPARTICLE SAFETY

As the placenta has important functions, including the supply of nutrients and oxygen to the fetus, excretion of waste products, provision of reproductive immunity, and elimination of foreign substances, collapse of the placental structure and the resulting dysfunction lead to fetal death and fetal growth restriction.[37] Therefore, normal placental maintenance and development are essential for the establishment and maintenance of pregnancy and healthy fetal growth. Given that many of the functions are maintained mainly by syncytiotrophoblasts in the placenta,[38] the process of syncytiotrophoblast formation is important throughout the whole pregnancy. Syncytiotrophoblasts are formed through polynuclearization and the fusion of mononuclear trophoblasts.[39] This cell fusion process is called syncytialization. As the syncytium progresses, the placenta matures in terms of both structure and function. Therefore, if there is an abnormality in syncytialization during placentation, the placenta may not form well and may not acquire function, thus adversely affecting the pregnancy.

For these reasons, our group has been evaluating the effects of nanoparticles by focusing on syncytialization. We used immunostaining to determine the nucleus size in BeWo cells, a human villous cancer cell line, that had been treated for 48 h with forskolin (an inducer of syncytium formation[40]) and silver nanoparticles with a diameter of 10 nm (nAg10). The nucleus was larger in the forskolin-treated group than in the forskolin-free group, but co-treatment with forskolin and nAg10 reduced the size of the nucleus. Furthermore, the fusion ratio (number of nuclei in syncytiotrophoblasts/total number of nuclei) increased with the addition of forskolin and decreased with nAg10 treatment, suggesting that nAg10 could result in inadequate syncytial cell formation (Fig. 3).

In general, the progression of syncytialization is regulated by various pathways centered on the cyclic adenosine monophosphate signal.[41] In addition, the silver nanoparticles used in our above-described yet-to-be published study are hazardous to multiple organelles, including mitochondria.[42] Given the complexity of the intracellular dynamics of silver nanoparticles and the diversity of syncytialization-promoting signals, we can expect the mechanisms of hazard expression by silver nanoparticles to be diverse. Therefore, we are currently trying to accumulate knowledge that can be applied to future toxicity evaluation by narrowing down the pathways involved in syncytialization suppression.

4. RISK ANALYSIS OF NANOPARTICLES IN HUMANS

Recent observational studies have found black carbon particles in the placentas of women exposed to air pollution[43] and have determined that microplastics can reach the placenta.[44] Moreover, carbon nanotubes have been found in the lungs of some pediatric asthma patients, and the causal relationship between tissue persistence and respiratory diseases, including respiratory diseases, has been investigated.[45] Asbestos,

which, like nanoparticles, was expected to be a revolutionary new material, has caused serious health damage to our living environment after a latent period of several decades since exposure.[46] Given the permeating nature of nanoparticles and today's increased awareness of the need to safeguard human health and the environment, collecting information on the real-world status of nanoparticle exposure and on the effects of nanoparticles on human health is an urgent issue.

Nanoparticles' dynamics *in vivo*, the quality of their effects, and their strengths and weaknesses are intimately associated with their physical characteristics, as we have described.[18,47,48] Therefore, the risk analysis of nanoparticles does not end with hazard research and needs to encompass the kinds of nanoparticles and our degree of exposure. It is important to understand our exposure from two perspectives, namely the physical properties and the dynamics of nanoparticles. However, with conventional general evaluation methods it is difficult to simultaneously analyze the types, abundances, and modes of existence of the items being evaluated. A new basis for such evaluations is indispensable. Therefore, we have established a method that can identify nanoparticles, detect their abundance, and determine their mode of existence in the living body.[49,50] In this way, by constructing an analytical platform with the aim of analyzing the real-life nanoparticle exposure status of humans, we are trying to understand the kinds, and loads, of nanoparticles in humans and the actual status of exposure.

5. AIMING TO ACHIEVE CONTROL OVER HEALTH EFFECTS IN VULNERABLE GENERATIONS

In recent years, in many developed countries, including Japan, rates of miscarriage and premature birth have been increasing, as is the frequency of low birth weight in infants. Low-birth-weight infants have higher prenatal and postnatal illness rates and mortality rates than normal infants.[51] Moreover, mental developmental disorders and metabolic dysfunction occur frequently in these children, as is predicted by the developmental origins of health and disease hypothesis.[52] Therefore, low-birth-weight infants are regarded as a major issue not only in perinatal medical care but also for health throughout life. In fact, in the National Survey on Children's Health and Environment, although developmental abnormalities of the fetus and newborn are mentioned as the central hypothesis to be elucidated, there are still many cases in which the causes of such abnormalities have not been identified. Analysis from a new perspective is therefore essential to ensure the health of the next generation.

From this point of view, recent epidemiological studies have investigated the association between maternal exposure to fine particles in the environment and the birth or premature birth of low-birth-weight infants. For example, a cohort study in the United States has suggested that exposure during pregnancy to airborne particulate matter in amounts above the environmental quality standards set by the United States Environmental Protection Agency increases the risk of preterm birth by approximately 19%.[53] In addition, exposure to atmospheric particulate matter during pregnancy and lactation has been reported to increase the risk of autism in children,[54] raising concerns about the impact of the particles on pregnancy outcomes in humans. Therefore, investigating biological responses to fine particles as environmental factors, as well as determining their mechanisms of action, is key to deepening our understanding of life phenomena and in turn helping to improve the quality of human health and medical care. In this regard, humans are at increased risk of exposure to not only natural fine particles but also synthetic fine particles such as nanoparticles. The amounts of nanoparticles to which humans, including vulnerable generations, are exposed are likely to increase further in the future, regardless of their impacts on our health, and we can no longer avoid unintentional exposure. Therefore, from the perspective of the exposure of vulnerable generations to nanoparticles, it is important that we unravel the relationship between the kinetics of nanoparticles and biological responses in terms of maternal and child health by gaining an understanding of the molecular pathology of health issues and the early detection of health disorders. Such an understanding would help to build preventive measures and is important to future developments in the field of health science.

6. CONCLUSION

In the future, analyzing the health effects of nanoparticle exposure on vulnerable generations will help us to elucidate new biological response mechanisms and explore unknown pathological conditions, thus improving the quality of human health and medical care. Such research could help to build a safe and prosperous society in which human health and sustainable industry are assured through the development of useful and safe forms of nanoparticles.

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Conflict of Interest The author declares no conflict of interest.

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