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Toxicity of Titanate Nanosheets on Human Immune Cells

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Additional information is available at the end of the chapter

Abstract

Titanium oxide is regarded as a bio-inert material, but studies concerning the toxic effects of titanium dioxide (TiO_2), particularly nano-scaled TiO_2 particles, have been accumulating that indicate nano-scaled TiO_2 particles show more harm and cause greater alteration of immune functions compared with large particles. Inorganic nanosheets have been the focus of increasing interest because of their ultrathin structure, as well as diversity of compounds and structures leading to various functions. Oxide nanosheets are included in the group comprising inorganic nanosheets, and titanate nanosheets (TiNSs) represent a form of oxide nanosheets. We therefore examined the toxicity of nano-scaled 2D materials of TiNSs on human immune cells. Our study revealed that TiNSs have the potential to cause harm through caspase-dependent apoptosis of human peripheral blood mononuclear cells (PBMCs) to the same degree as asbestos. Furthermore, isolated monocytes developed marked vacuoles prior to cell death upon exposure to TiNSs, which were found in the vacuoles and indicated engulfment of TiNSs. A consideration of these findings with the co-localization of vacuoles with endocytosed fluorescence-labeled dextran indicates that TiNSs entered the endosomal pathway, leading to the formation of vacuoles in monocytes and subsequent cell death. TiNSs might therefore affect immune functions through interference of endo-lysosomal functions.

Keywords: titanate nanosheets, apoptosis, vacuole formation, endosome

1. Introduction

Titanium oxide is used broadly in industrial production for ceramics, materials containing composite oxides and photocatalysts, and even as a food additive. In addition, titanium and its

alloy are used for various kinds of biomaterials such as artificial joints and dental implants, where generation of titanium oxide (titania) film is beneficial because of its bio-inertness [1–3]. Titanium oxide was therefore regarded as a harmless material. However, studies detailing the toxic effects of titanium oxide have been accumulating recently as shown in the next section. The International Agency for Research on Cancer (IARC) decided in 2010 to change its categorization of titanium dioxide (TiO_2) from "Group 3: Not classifiable as to carcinogenicity to humans" to "Group 2B: Possibly carcinogenic to humans". This conclusion resulted from sufficient evidence in experimental animals and inadequate evidence from epidemiological studies. The carcinogenicity of titanium oxide was evaluated by examining the relationship between exposure to titanium oxide and the risk of lung cancer in two previously conducted case-control studies, which showed no detectable excess risk of lung cancer [4]. In contrast, two studies using animal experiments demonstrated elevated lung cancer in rats exposed to fine or ultrafine TiO_2 [5, 6]. Additionally, it is estimated that the total production of nano-TiO_2 would reach approximately 2.5 million metric tons (MT) per year in 2025 from 40,000 MT in 2006 in the US [7], which means we would become more exposed to nano-scaled materials of titanium oxide in the future, thereby motivating us to better clarify the toxicological effects of this material. The various forms of titanium oxide are known and include spheres, rods, needles and fibers, as well as sheets. Titanate nanosheets (TiNSs) are crystalline materials composed of titanium and oxygen with a very thin and flat structure representing 2D materials. TiNSs are expected to be valuable materials in industry for production of UV- or corrosion-resistant films, dielectric thin films and catalysts. Therefore, we recently examined the effects of exposure to TiNSs on human immune cells (manuscript of an original article under preparation). Here, we would like to review the progress of studies regarding the toxicity of titanium oxides, summarize our recent study concerning the toxicity of TiNSs and finally discuss the findings obtained from that study.

2. Toxicity of titanium dioxide materials

The following two studies form the basis for the decision to reappraise the carcinogenicity of TiO_2. In 1985, Lee et al. conducted *in vivo* experiments with rats and reported the occurrence of bronchioalveolar adenomas carcinomas and squamous cell carcinomas in a portion of both sexes exposed by inhalation to fine TiO_2, which possesses a micro-scaled diameter [5]. The study by Heinrich et al. demonstrated that exposure to ultrafine TiO_2 nano-scaled particles caused tumors in comprising squamous cell carcinomas, adenocarcinomas and benign squamous cell tumors in female rats [6]. In addition, Schins et al. evaluated data in the literature and reported that tumorigenesis by TiO_2 involves a mechanism of genetic damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) that were produced by a TiO_2 exposure-induced inflammatory response [8]. Naturally occurring TiO_2 is distinguished as rutile and anatase due to the difference in crystal structure. It has been reported that an anatase type of nano-TiO_2 showed higher production of ROS and more toxic characteristics compared with a rutile type of the material in an *in vitro* experiment with human fibroblasts and lung epithelial cells [9]. In addition, it has been demonstrated that TiO_2 nanoparticles caused high production of 8-hydroxyl-2′-deoxyguanosine (8-OHdG), a DNA adduct, which contributed to the development of tumor, whereas the nanoparticles did not cause DNA breakage in human

lung fibroblasts of IMR-90 [10]. Chen et al. demonstrated that single intratracheal instillation with TiO_2 nanoparticles caused altered expression of mouse lung-tissue genes involved in pathways associated with the cell cycle, apoptosis, chemokines and the complement system [11]. TiO_2 nanoparticles increased expression of placenta growth factor (PIGF), CXCL1, CXCL5 and CCL3, and results were similar to those observed in an *in vitro* experiment with human THP-1 cells. Warheit et al. demonstrated the difference in effects resulting from exposure to nano-TiO_2 rods and dots [12]. Moreover, their study compared various kinds of TiO_2 and demonstrated that the pulmonary effects caused by exposure to ultrafine TiO_2 differ based on the crystal structure and composition of TiO_2 [13]. These findings show evidence for the toxicity of titanium oxide, but the toxicity of nano-scaled materials needs to be examined further, particularly the crucial role played by the size, crystal structure and composition of TiO_2 in regard to toxicity.

3. Studies concerning nano-scaled titanium dioxide particles

Before examining TiNSs, previous studies regarding the toxicity of nano-scaled TiO_2 materials should be reviewed. The toxicological effect of TiO_2 nanoparticles on skin and in dermal tissue is of interest to medical science and manufacturers because TiO_2 is used as a physical photoprotective agent in sunscreen and as a whitening agent in cosmetics. It is unlikely that TiO_2 nanoparticles easily reach dermal tissue. Experiments using human skin-transplanted mice show that TiO_2 nanoparticles did not penetrate the barrier of an intact epidermis [14]. Sadrieh et al. [15] and Newman et al. [16] also demonstrated no significant penetration of TiO_2 nanoparticles through the epidermis. However, this does not eliminate concern regarding its toxicity on skin because an *in vitro* experiment using cell lines of keratinocytes, sebocytes, fibroblasts and melanocytes showed decreases in viable cells, an increase in apoptosis and decreases in the differentiation markers of those cells [14]. Moreover, several studies have shown actual penetration of TiO_2 nanoparticles through skin. Bennat and Muller-Goymann demonstrated that TiO_2 nanoparticles are able to pass through skin using an oil-in-water emulsion [17]. Another study demonstrated that TiO_2 nanoparticles reached the deep area of the epidermis in the pig ear after topical skin exposure to the nanoparticles, some of which even reached the brain, whereas there was no penetration in an *in vitro* experiment using isolated porcine skin [18]. Once TiO_2 nanoparticles pass through the epidermis due to the broken or unhealthy status of the epidermal barrier, they produce harmful effects on the tissue. A study utilizing *in vitro* experiments confirmed the phototoxicity of nano-sized TiO_2 in an experiment with human skin keratinocytes of HaCaT under irradiation of UVA, which is mainly dependent on the ROS production level [19]. Furthermore, a study investigating the mechanism of toxicity of TiO_2 nanoparticles upon exposure to UVA found that exposure to TiO_2 caused decreases in the mitochondrial membrane potential and ATP level and an increase in caspase 3 activity [20]. It has also been shown that subcutaneous injection with TiO_2 nanoparticles promoted dermal sensitization induced by dinitrochlorobenzene (DNCB) [21]. Moreover, intradermal administration with TiO_2 nanoparticles resulted in aggravated skin lesions such as those of atopic dermatitis related to mite antigen in NC/Nga mice [22]. These findings indicate that TiO_2 nanoparticles do not penetrate through the epidermis easily as long as the barrier is healthy, but these nanoparticles have the potential to cause toxic

effects on dermal tissue when that barrier is broken. Pulmonary exposure to TiO_2 nanoparticles is expected to result in these toxic effects because a barrier comprising pulmonary tissue is not tough. In addition, alveolar macrophages are always ready to engulf particles in the region, leading to inflammatory responses. Sager et al. used F344 rats to examine the pulmonary response to intratracheal instillation of nano-sized ultrafine TiO_2 in comparison to fine TiO_2 particles. Administration with ultrafine TiO_2 caused higher levels of polymorphonuclear neutrophil (PMN) cell number, lactate dehydrogenase (LDH) activity, albumin and inflammatory cytokines compared with fine TiO_2 [23]. Interestingly, they also examined the amounts of TiO_2 in trachea-bronchial and thymic lymph nodes at days 7 and 42 after administration and then measured alterations in those tissues during that period. Ultrafine TiO_2 showed a faster decline of the remaining amount in trachea-bronchial nodes than fine TiO_2. In addition, although both ultrafine and fine TiO_2 were translocated to lymph nodes, the amount was higher for ultrafine TiO_2 than fine TiO_2. There was also a striking difference in the amounts of lavagable and non-lavagable components between fine and ultrafine TiO_2 in the lungs. Eight-one percent of ultrafine TiO_2 was non-lavagable, suggesting migration to the interstitium, even at day 7 post-exposure, whereas 91% of fine TiO_2 was still lavagable at this stage. Shinohara et al. investigated pulmonary clearance kinetics of TiO_2 nano- and sub-micron particles by intratracheal administration to male F344 rats [24], and results confirmed the translocation of administered TiO_2 to the thoracic lymph nodes that increased in a time- and dose-dependent manner. van Ravenzwaay also observed that inhaled TiO_2 nanoparticles reached the lungs and draining lymph nodes [25]. These findings indicate that it is easier for TiO_2 nanoparticles to migrate from bronchoalveolar to interstitial tissue or be cleared by alveolar macrophages, leading to increased inflammatory responses, and that TiO_2 nanoparticles have a greater potential to influence the function of immune cells. Actually, several studies have reported alteration of immune functions following administration with TiO_2 through the respiratory pathway. Chang et al. demonstrated that intratracheal instillation with TiO_2 nanoparticles caused an increase in GATA-3 and decrease in T-bet mRNA levels, which are master genes for Th2 and Th1 cell development, respectively [26]. Mishra et al. examined the effect of administration with TiO_2 nanoparticles as an adjuvant in an experiment utilizing a murine asthma model. It was found that TiO_2 nanoparticles augmented airway hyper-responsiveness, lung damage and a mixed Th2/Th1 dependent immune response, associated with increases in Stat3, Socs3, NF-κB, IL-6 and TNF-α [27]. The effect of intradermal administration with TiO_2 nanoparticles as mentioned above should also be understood in relation to acquired immunity. NC/Nga mice treated with TiO_2 nanoparticles showed over production of IL-4 in the skin, IgE and histamine levels in serum, as well as aggravation of skin lesions [22]. Studies using intraperitoneal administration of TiO_2 nanoparticles are valuable for an understanding of the immunological influences of these particles. Larsen et al. showed that mice receiving intraperitoneal treatment with TiO_2 nanoparticles and OVA and subsequently challenged with aerosols of OVA responded with high production of IgE and IgG1 antibodies specific for OVA in serum with increases in eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage fluid (BALF), which suggests induction of a Th2-dominant immune response [28]. Moreover, Moon et al. demonstrated that intraperitoneal injection with TiO_2 nanoparticles results in the damaged development and proliferation of B and T cells, a decreased cytokine production by LPS-stimulated peritoneal macrophages and a decreased

percentage of NK cells in spleenocytes, leading to an increased tumor growth of implanted B16F10 melanoma cells [29]. Several *in vitro* studies also provide further information regarding the direct effects of TiO_2 nanoparticles on immune cells. Munidasa et al. reported that TiO_2 nanoparticles showed greater influence on the antigen presenting activity of monocytes and alveolar macrophages [30]. In addition, Moon et al. showed a reduction in lymphocyte proliferation induced by lipopolysaccharide (LPS) or concanavalin A (Con A) upon exposure to TiO_2 nanoparticles [29]. The overall results of these *in vivo* or *in vitro* studies indicate that nano-scaled TiO_2 particles have the potential to cause harmful outcomes if they enter the body. In addition, it is also clear that nano-scaled TiO_2 particles cause greater alteration of immune functions compared with large particles. Therefore, we planned to examine the toxicity of nano-scaled 2D materials composed of titanium and oxygen (TiNSs) on human immune cells.

4. The characteristics of titanate nanosheets (TiNSs)

Techniques to synthesize nano-scaled 2D materials have been studied recently. The basis of this field is derived from the development of methods for manipulating graphene, a carbon nanosheet with a thickness of one atom, which triggered the subsequent development of various 2D nanomaterials [31–33]. It is against this background that inorganic nanosheets have acquired greater interest because they have an ultrathin structure as well as a diversity of compounds and structures leading to various functions [34–36]. Oxide nanosheets are included in the group comprising inorganic nanosheets, and titanate nanosheets (TiNSs) represent a form of oxide nanosheets. Although TiNSs are composed of a TiO_6 octahedron as the particles of TiO_2, TiNSs have the unique crystal structure of lepidocrocite, differing from anatase or rutile, which results in a shape having an ultralow thickness and high aspect ratio [34]. In the 1990s, Sasaki et al. first succeeded in delaminating layered titanate into single titanate nanosheets [37, 38], and TiNSs are now incorporated into useful applications such as photocatalysts, semiconductors and dielectric materials [39–42]. However, the following characteristics suggest possible harmful effects of TiNSs. First, it is noteworthy that TiNSs have a very large surface area per gram due to their ultralow thickness, which is generated from the limited height of one and a half of a sideways TiO_6 octahedron together with the repeated linkage of the octahedron horizontally [34]. Such a large surface of TiNSs might enhance the toxic machinery of bulk titanium particles. Second, the large surface of TiNSs is known to be negatively charged due to oxygen atoms existing at the edges of the octahedron, and this suggests the possible influence of TiNSs through a cationic interaction. In addition to TiNSs delaminated from layered titanate, it has been reported that TiNSs with a small diamond shape and crystal structure of lepidocrocite can be synthesized in a bottom-up manner [43, 44], which allows TiNSs to be synthesized at a small scale. Dr. Yoshioka, one of our colleagues, has modified that method to synthesize TiNSs in our group. **Figure 1** shows images of TiNSs taken by transmission electron microscopy (TEM). The TiNSs showed a diamond shape with about 20- and 30-nm diagonals, which is almost the same as that reported in a previous study [43]. Additionally, the TiNSs showed the characteristic peaks of a lepidocrocite structure confirmed by X-ray diffraction analysis. Since it was verified that TiNSs could be synthesized, we therefore started to examine the effect of TiNSs on human immune cells using *in vitro* experiments.
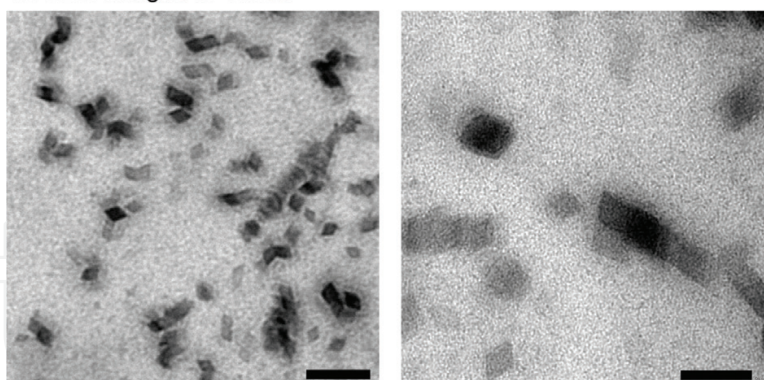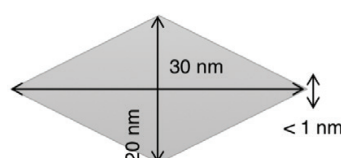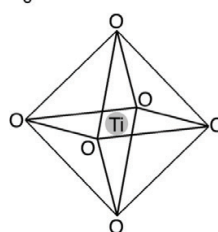
A TEM images of TiNSs

B Single TiNS

30 nm

20 nm

< 1 nm

C TiO₆ octahedron

Figure 1. TEM images of TiNSs used in the present study and illustrations of a single TiNS and TiO$_6$ octahedron. (A) TiNSs were synthesized by Dr. Yoshioka and observed by TEM. These images show the uniform diamond shapes of TiNSs in our present study. Some TiNSs appear to be piled up showing darker diamonds. Scale bars of 100 (left) and 50 nm (right) are shown. (B) Illustration of single TiNSs having a diamond shape with almost 20- and 30-nm diagonals, based on TEM images. (C) Illustration of a TiO$_6$ octahedron in the orthostatic position, of which TiNSs are composed. TiNSs have the depth of one and a half of a sideways TiO$_6$ octahedron [34].

5. TiNSs-caused cell death and vacuole formation

Before the culture experiments, the diameters of TiNSs in water and in culture media supplemented with 10% of FBS were measured using a Zetasizer. The results showed that the diameter of about 25 nm in water is consistent with the size estimated by TEM images, while that in the media was about 422 nm, indicating induced agglomeration of TiNSs in the culture media. In addition, TiNSs in water showed a zeta potential of −22.1 mV. To examine the effects of exposure to TiNSs on human immune cells, peripheral blood mononuclear cells (PBMCs) were prepared and cultured with various doses of TiNSs, or with asbestos as a control cytotoxic material, for 7 days. Exposure to asbestos caused an increase in annexin V (Anx)-positive apoptosis of cells when exposed to more than a dose of 1 μg/ml at day 2 after culture, whereas apoptosis was not observed in the culture exposed to TiNSs even at the maximum dose of 10 μg/ml. However, exposure to TiNSs caused dose-dependent apoptosis to the same degree as asbestos at day 7 of the culture. The effects of exposure to bulk TiO$_2$ particles and crystalline silica were compared with TiNSs, but they did not cause apoptosis of PBMCs. The cell death induced by exposure to TiNSs or asbestos, but not to TiO$_2$ or silica, was also confirmed by measuring the sub-G1 population, apoptotic cells with a low DNA content, using flow cytometry. Interestingly, marked formation of vacuoles was observed in the culture of PBMCs exposed to TiNSs but not the other materials. It was confirmed by staining with fluorescence-labeled antibodies to CD14 that

vacuole formation was present in monocytes but not in lymphocytes. Q-VD-OPh, a pan-caspase inhibitor, suppressed the increase in Anx$^+$ cells induced by TiNSs as well as asbestos. The increase in apoptotic cells caused by TiNS exposure was also observed in the culture of isolated CD14$^+$ monocytes as well as CD4$^+$ lymphocytes. These findings indicate that TiNSs have the potential to cause caspase-dependent apoptosis in immune cells, particularly where monocytes show the formation of large vacuoles prior to apoptosis upon exposure to TiNSs.

6. Identification of intra-vacuolar TiNSs in monocytes

The results obtained from the cell cultures demonstrated the characteristic toxicity of TiNSs for monocytes, comprising apoptosis associated with the striking formation of vacuoles. Therefore, we investigated the presence of intracellular microstructures in monocytes exposed to TiNSs. Monocytes were isolated from human PBMCs, cultured with TiNSs at 10 μg/ml and then harvested at day 1 or 2 after the culture for subsequent TEM observations. The TEM images showed rapid formation of vacuoles in monocytes even at day 1, and the number and size of vacuoles increased at day 2. It is noteworthy that nano-scaled materials with TiNS-like shapes were found within the vacuoles of the monocytes and that most of the material was located near the inner surface of the vacuolar membrane (**Figure 2**). In order to confirm whether these intra-vacuolar nano-scaled materials were TiNSs, we observed the inner surface of the vacuolar membrane in monocytes using scanning electron microscopy (SEM), followed by energy dispersion X-ray (EDX) analysis for titanium. SEM observations showed that there was a rough area in the inner surface of the vacuolar membrane in monocytes harvested at day 1 after the culture with TiNSs. The rough area of the vacuolar membrane was also seen in other monocytes exposed to TiNSs. Analysis of the rough area by EDX confirmed the presence of titanium, in contrast to results for the cytosolic region in TiNS-exposed or control monocytes. These overall findings indicate that TiNSs were actually engulfed by monocytes and included in the vacuoles.
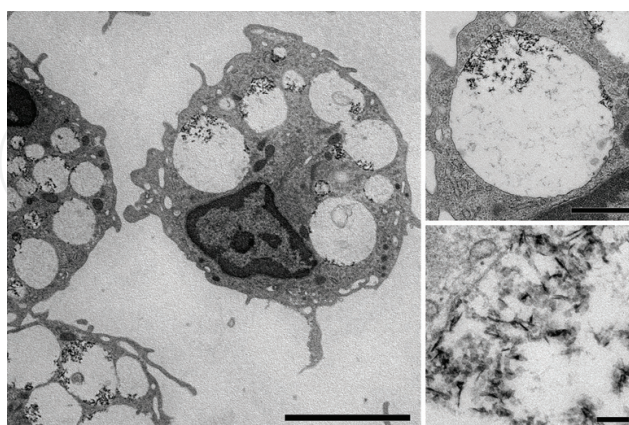

Figure 2. Observation of microstructures in TiNS-exposed monocytes by transmission electron microscope (TEM). The images are taken at day 1 after culture with TiNSs. It can be seen that monocytes have obvious vacuoles even at this early time. Additionally, nano-scaled materials with TiNS-like shapes can be seen inside the vacuoles, and most of the nano-scaled material is located near the vacuolar membrane. Finally, it was confirmed by scanning electron microscopy (SEM) with energy dispersion X-ray (EDX) analysis that these materials included titanium, indicating that the material contained TiNSs. Scale bars of 5 μm (left), 1 μm (upper right) and 100 nm (bottom right) are shown.

7. Relation between TiNSs-caused vacuoles and endosomal pathway

Previous studies have reported formation of vacuoles originating from endocytic organelles, where the vacuolization of late endosomes was induced by the inhibition of kinases for regulation of vesicular transport and sorting [45–47], and several bacterial toxins induced vacuoles having an endosomal/lysosomal origin [48–50]. To examine the relation between TiNS-induced vacuoles and the endosomal pathway, monocyte-derived adherent cells were incubated with fluorescence-dye-labeled dextran to visualize endosome structures. The adherent cells were prepared using a pre-culture of PBMCs. TiNSs were then added to the culture of endosome-visualized adherent cells at 10 μg/ml, and observations were then made of vacuoles using the fluorescence derived from dextran. In the control culture without TiNSs, a diffuse fluorescence was observed. However, exposure to TiNSs induced vacuoles in the adherent cells, where most of the vacuoles showed co-localization with the fluorescence derived from endocytosed dextran. These observations indicate that TiNS-induced formation of vacuoles is related with some part of the endosomal pathway.

8. Discussion

Our study revealed the unique toxicity of TiNSs. This 2D nano-scaled material has the harmful potential to cause caspase-dependent apoptosis of immune cells to the same degree as asbestos. In particular, monocytes showed formation of marked vacuoles prior to cell death upon exposure to TiNSs, which were later found in the vacuoles and suggest the actual engulfment of TiNSs by monocytes. A consideration of these findings with the observation regarding co-localization of vacuoles with endosomal dextran indicates that engulfed TiNSs entered the endosomal pathway, leading to the formation of vacuoles in monocytes and subsequent cell death. As mentioned previously, TiNSs have a very large surface area per gram. **Figure 3** represents an illustration showing the large surface area of 2D nano-materials. Nano-scaled spheres with a diameter of 20 nm have a volume of 4189 nm^3 and surface area of 1257 nm^2. Diamond-shaped nanosheets with diagonals of 20 and 30 nm, a depth of 1 nm and resulting volume of 300 nm^3 have the same density as a nanosphere. The total volume of 14 nanosheets (4200 nm^3) is almost equivalent to the volume of one nanosphere (4189 nm^3), but those nanosheets result in a total surface area of 8400 nm^2 as the sum of both sides, which is 6.68 times as large as the surface area of the nanosphere. Thus, nanosheets have an extremely large surface that probably enhances the chemical activities of titanium oxide, which might contribute to the toxicity of TiNSs. In addition, TiNSs are negatively charged on the surface due to the presence of the oxygen atom, which might cause cationic interference in endo-lysosomes that leads to the formation of vacuoles. Various kinds of stimulation such as oxidative stress disrupt the integrity of the lysosomal membrane and cause lysosomal-membrane permeabilization (LMP), which triggers cell death including caspase-dependent apoptosis [51, 52]. Autophagy is the machinery of the intracellular degradation process, which is also the part of the intracellular membrane system and is linked to the endo-lysosomal pathway [51, 53]. Furthermore, recent studies have been accumulating concerning a new type of cell death associated with large vacuoles, named methuosis, although it is thought
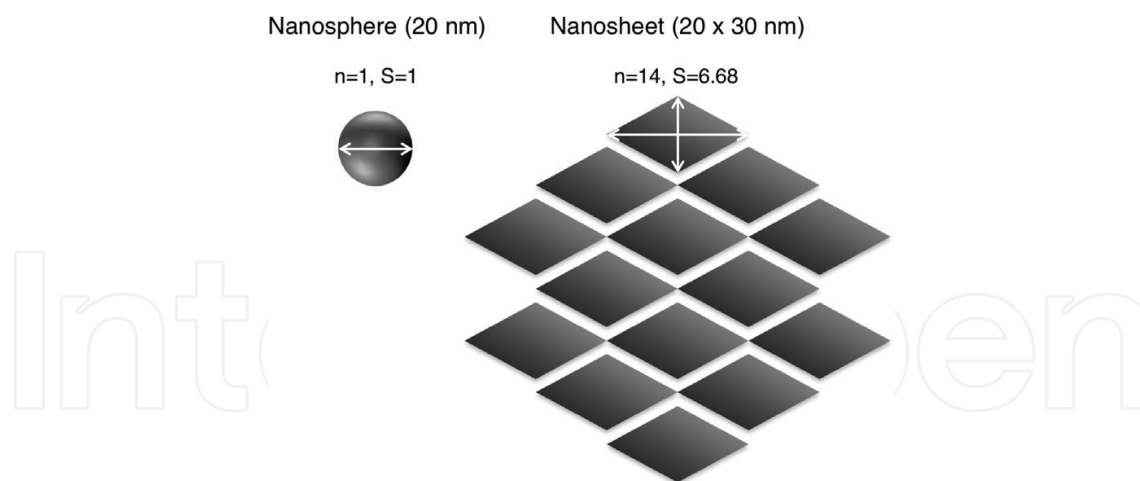
Figure 3. Illustration of a comparison of volume and surface area between nanosphere and nanosheet. A nanosphere with a diameter of 20 nm has a volume of 4189 nm^3 and surface area 1257 nm^2. A diamond-shaped nanosheet with 20- and 30-nm diagonals and depth of 1 nm has the same density as a nanosphere. Fourteen nanosheets have almost the same volume as a nanosphere but possess a total surface area of 8400 nm^2 as the sum of both sides, which are 6.68 times as large as the surface area of the nanosphere.

that this type of cell death does not require caspases [54, 55]. Some of the machineries mentioned above might be related to the cell death caused by TiNSs. TiNSs in the endosomal pathway might affect immune functions executed by cell surface receptors through interference of endosomal trafficking. Additionally, CD4+ lymphocytes were also damaged by exposure to TiNSs, which suggests possible alteration of immune responses in a direct manner caused by TiNSs. Further investigation concerning these issues will contribute to a clarification of the toxic machinery of TiNSs and the immunotoxicological effects of TiNSs.

Acknowledgements

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References

[1]   Okido M, Nishikawa K, Kuroda K, Ichino R, Zhao ZW, Takai O. Evaluation of the hydroxyapatite film coating on titanium cathode by QCM. Materials Transactions. 2002; **43**(12):3010-3014

[2]   Yamamoto D, Iida T, Kuroda K, Ichino R, Okido M, Seki A. Formation of amorphous TiO_2 film on Ti using anodizing in concentrated H_3PO_4 aqueous solution and its osteoconductivity. Materials Transactions. 2012;**53**(3):508-512

[3]   Yamamoto D, Kawai I, Kuroda K, Ichino R, Okido M, Seki A. Osteoconductivity of anodized titanium with controlled micron-level surface roughness. Materials Transactions. 2011;**52**(8):1650-1654

[4]   Ramanakumar AV, Parent ME, Latreille B, Siemiatycki J. Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: Results from two case-control studies in Montreal. International Journal of Cancer. 2008;**122**(1):183-189

[5]   Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicology and Applied Pharmacology. 1985;**79**(2):179-192

[6]   Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, et al. Chronic inhalation exposure of wistar rats and 2 different strains of mice to diesel-engine exhaust, carbon-black, and titanium-dioxide. Inhalation Toxicology. 1995;**7**(4):533-556

[7]   Robichaud CO, Uyar AE, Darby MR, Zucker LG, Wiesner MR. Estimates of upper bounds and trends in nano-TiO_2 production as a basis for exposure assessment. Environmental Science & Technology. 2009;**43**(12):4227-4233

[8]   Schins RP, Knaapen AM. Genotoxicity of poorly soluble particles. Inhalation Toxicology. 2007;**19**(Suppl 1):189-198

[9]   Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, et al. Correlating nanoscale titania structure with toxicity: A cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicological Sciences. 2006; **92**(1):174-185

[10]   Bhattacharya K, Davoren M, Boertz J, Schins RP, Hoffmann E, Dopp E. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells. Particle and Fibre Toxicology. 2009;**6**:17

[11]   Chen HW, SF S, Chien CT, Lin WH, SL Y, Chou CC, et al. Titanium dioxide nanoparticles induce emphysema-like lung injury in mice. The FASEB Journal. 2006;**20**(13):2393-2395

[12]   Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL. Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: Toxicity is not dependent upon particle size and surface area. Toxicological Sciences. 2006;**91**(1):227-236

[13]   Warheit DB, Webb TR, Reed KL, Frerichs S, Sayes CM. Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: Differential responses related to surface properties. Toxicology. 2007;**230**(1):90-104

[14] Kiss B, Biro T, Czifra G, Toth BI, Kertesz Z, Szikszai Z, et al. Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Experimental Dermatology. 2008;**17**(8):659-667

[15] Sadrieh N, Wokovich AM, Gopee NV, Zheng J, Haines D, Parmiter D, et al. Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO_2 particles. Toxicological Sciences. 2010;**115**(1):156-166

[16] Newman MD, Stotland M, Ellis JI. The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. Journal of the American Academy of Dermatology. 2009; **61**(4):685-692

[17] Bennat C, Muller-Goymann CC. Skin penetration and stabilization of formulations containing microfine titanium dioxide as physical UV filter. International Journal of Cosmetic Science. 2000;**22**(4):271-283

[18] Wu J, Liu W, Xue C, Zhou S, Lan F, Bi L, et al. Toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. Toxicology Letters. 2009;**191**(1):1-8

[19] Yin JJ, Liu J, Ehrenshaft M, Roberts JE, PP F, Mason RP, et al. Phototoxicity of nano titanium dioxides in HaCaT keratinocytes—Generation of reactive oxygen species and cell damage. Toxicology and Applied Pharmacology. 2012;**263**(1):81-88

[20] Xue C, Luo W, Yang XL. A mechanism for nano-titanium dioxide-induced cytotoxicity in HaCaT cells under UVA irradiation. Bioscience, Biotechnology, and Biochemistry. 2015; **79**(8):1384-1390

[21] Hussain S, Smulders S, De Vooght V, Ectors B, Boland S, Marano F, et al. Nano-titanium dioxide modulates the dermal sensitization potency of DNCB. Particle and Fibre Toxicology. 2012;**9**:15

[22] Yanagisawa R, Takano H, Inoue K, Koike E, Kamachi T, Sadakane K, et al. Titanium dioxide nanoparticles aggravate atopic dermatitis-like skin lesions in NC/Nga mice. Experimental Biology and Medicine (Maywood, N.J.). 2009;**234**(3):314-322

[23] Sager TM, Kommineni C, Castranova V. Pulmonary response to intratracheal instillation of ultrafine versus fine titanium dioxide: Role of particle surface area. Particle and Fibre Toxicology. 2008;**5**:17

[24] Shinohara N, Oshima Y, Kobayashi T, Imatanaka N, Nakai M, Ichinose T, et al. Pulmonary clearance kinetics and extrapulmonary translocation of seven titanium dioxide nano- and submicron materials following intratracheal administration in rats. Nanotoxicology. 2015;**9**(8):1050-1058

[25] van Ravenzwaay B, Landsiedel R, Fabian E, Burkhardt S, Strauss V, Ma-Hock L. Comparing fate and effects of three particles of different surface properties: nano-TiO_2, pigmentary TiO_2 and quartz. Toxicology Letters. 2009;**186**(3):152-159

[26] Chang X, Fu Y, Zhang Y, Tang M, Wang B. Effects of Th1 and Th2 cells balance in pulmonary injury induced by nano titanium dioxide. Environmental Toxicology and Pharmacology. 2014;**37**(1):275-283

[27] Mishra V, Baranwal V, Mishra RK, Sharma S, Paul B, Pandey AC. Titanium dioxide nanoparticles augment allergic airway inflammation and Socs3 expression via NF-kappaB pathway in murine model of asthma. Biomaterials. 2016;**92**:90-102

[28] Larsen ST, Roursgaard M, Jensen KA, Nielsen GD. Nano titanium dioxide particles promote allergic sensitization and lung inflammation in mice. Basic & Clinical Pharmacology & Toxicology. 2010;**106**(2):114-117

[29] Moon EY, Yi GH, Kang JS, Lim JS, Kim HM, Pyo S. An increase in mouse tumor growth by an in vivo immunomodulating effect of titanium dioxide nanoparticles. Journal of Immunotoxicology. 2011;**8**(1):56-67

[30] Munidasa DT, Koike E, Kobayashi T. An in vitro study of the effect of size and timing of administration of titanium dioxide particles on antigen presenting activity of alveolar macrophages and peripheral blood monocytes. Inhalation Toxicology. 2009;**21**(10):849-856

[31] Geim AK. Graphene: Status and prospects. Science. 2009;**324**(5934):1530-1534

[32] Geim AK, Novoselov KS. The rise of graphene. Nature Materials. 2007;**6**(3):183-191

[33] Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, et al. Electric field effect in atomically thin carbon films. Science. 2004;**306**(5696):666-669

[34] Osada M, Sasaki T. Two-dimensional dielectric nanosheets: Novel nanoelectronics from nanocrystal building blocks. Advanced Materials. 2012;**24**(2):210-228

[35] Ma R, Sasaki T. Nanosheets of oxides and hydroxides: Ultimate 2D charge-bearing functional crystallites. Advanced Materials. 2010;**22**(45):5082-5104

[36] Chhowalla M, Shin HS, Eda G, Li LJ, Loh KP, Zhang H. The chemistry of two-dimensional layered transition metal dichalcogenide nanosheets. Nature Chemistry. 2013;**5**(4):263-275

[37] Sasaki T, Watanabe M. Osmotic swelling to exfoliation. Exceptionally high degrees of hydration of a layered titanate. Journal of the American Chemical Society. 1998;**120**(19):4682-4689

[38] Sasaki T, Watanabe M, Hashizume H, Yamada H, Nakazawa H. Macromolecule-like aspects for a colloidal suspension of an exfoliated titanate. Pairwise association of nanosheets and dynamic reassembling process initiated from it. Journal of the American Chemical Society. 1996;**118**(35):8329-8335

[39] Shibata T, Sakai N, Fukuda K, Ebina Y, Sasaki T. Photocatalytic properties of titania nanostructured films fabricated from titania nanosheets. Physical Chemistry Chemical Physics. 2007;**9**(19):2413-2420

[40] Osada M, Ebina Y, Funakubo H, Yokoyama S, Kiguchi T, Takada K, et al. High-κ dielectric nanofilms fabricated from titania nanosheets. Advanced Materials. 2006;**18**(8):1023-1027

[41] Sakai N, Ebina Y, Takada K, Sasaki T. Electronic band structure of titania semiconductor nanosheets revealed by electrochemical and photoelectrochemical studies. Journal of the American Chemical Society. 2004;**126**(18):5851-5858

[42] Sasaki T, Watanabe M. Semiconductor nanosheet crystallites of quasi-TiO$_2$ and their optical properties. The Journal of Physical Chemistry B. 1997;**101**(49):10159-10161

[43] Tae EL, Lee KE, Jeong JS, Yoon KB. Synthesis of diamond-shape titanate molecular sheets with different sizes and realization of quantum confinement effect during dimensionality reduction from two to zero. Journal of the American Chemical Society. 2008;**130**(20):6534-6543

[44] Ohya T, Nakayama A, Shibata Y, Ban T, Ohya Y, Takahashi Y. Preparation and characterization of titania thin films from aqueous solutions. Journal of Sol-Gel Science and Technology. 2003;**26**(1):799-802

[45] Johnson EE, Overmeyer JH, Gunning WT, Maltese WA. Gene silencing reveals a specific function of hVps34 phosphatidylinositol 3-kinase in late versus early endosomes. Journal of Cell Science. 2006;**119**(Pt 7):1219-1232

[46] Jefferies HB, Cooke FT, Jat P, Boucheron C, Koizumi T, Hayakawa M, et al. A selective PIKfyve inhibitor blocks PtdIns(3,5)P(2) production and disrupts endomembrane transport and retroviral budding. EMBO Reports. 2008;**9**(2):164-170

[47] Cuesta-Geijo MA, Galindo I, Hernaez B, Quetglas JI, Dalmau-Mena I, Alonso C. Endosomal maturation, Rab7 GTPase and phosphoinositides in African swine fever virus entry. PLoS One. 2012;**7**(11):e48853

[48] Papini E, Satin B, Bucci C, de Bernard M, Telford JL, Manetti R, et al. The small GTP binding protein rab7 is essential for cellular vacuolation induced by Helicobacter pylori cytotoxin. The EMBO Journal. 1997;**16**(1):15-24

[49] Nagahama M, Itohayashi Y, Hara H, Higashihara M, Fukatani Y, Takagishi T, et al. Cellular vacuolation induced by *Clostridium perfringens* epsilon-toxin. The FEBS Journal. 2011;**278**(18):3395-3407

[50] Johnson C, Kannan TR, Baseman JB. Cellular vacuoles induced by *Mycoplasma pneumoniae* CARDS toxin originate from Rab9-associated compartments. PLoS One. 2011;**6**(7):e22877

[51] Kroemer G, Jaattela M. Lysosomes and autophagy in cell death control. Nature Reviews Cancer. 2005;**5**(11):886-897

[52] Settembre C, Fraldi A, Medina DL, Ballabio A. Signals from the lysosome: A control centre for cellular clearance and energy metabolism. Nature Reviews. Molecular Cell Biology. 2013;**14**(5):283-296

[53] Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature. 2011;**469**(7330):323-335

[54] Maltese WA, Overmeyer JH. Methuosis: Nonapoptotic cell death associated with vacuolization of macropinosome and endosome compartments. American Journal of Pathology. 2014;**184**(6):1630-1642

[55] Mbah NE, Overmeyer JH, Maltese WA. Disruption of endolysosomal trafficking pathways in glioma cells by methuosis-inducing indole-based chalcones. Cell Biology and Toxicology. 2017;**33**(3):263-282