Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (5):1244-1248

(International Peer Reviewed Journal)

Nanostructure of Titanium Dioxide (TiO_2) and its Correlation with Human Health and Safety

Shailesh Sharma[1*], Deepak Sinha[2], D. K. Sharma[3] and Reena Nashine[4]

1. Department of Chemistry, Disha Institute Of Management And Technology, Raipur (CG), **INDIA**
2. Department of Chemistry, Govt. Nagarjuna PG College of Science Raipur CG, **INDIA**
3. Department of Anatomy, All India Institute of Medical Sciences, Raipur, CG, **INDIA**
4. Department of Chemistry, Chouksey Engineering College Bilaspur CG, **INDIA**

Email: Shailesh.chemist@gmail.com

Received on 9th August and finalized on 20th August 2013.

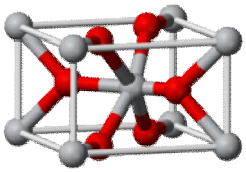
ABSTRACT

The titanium dioxide with its chemical formula TiO_2 is a naturally occurring oxide of titanium. The synonyms are titanium (IV) oxide, titania, titanium white and pigment white 6. It has a wide range of applications and is employed as a pigment to provide whiteness and opacity in paints, coatings, plastics, papers, inks, sunscreens, foods and eatables, medicinal pills and tablets, toothpastes etc. TiO_2 is now one of the most fascinating materials in the modern era which is vastly capturing the attention of physical chemists, physicists, scientists, engineers, doctors and social workers in exploring its distinctive properties. As the cytotoxicity of the nanoparticles of this compound is not clearly understood, the aims of this study was to assess the cytotoxicity of TiO_2 nanopowder and to exhibit the slow poisonous/harmful effects of TiO_2 being consumed in our body by the contact, ingestion or inhalation in daily routine life. The cytotoxicity was examined in human skin fibroblasts using the colorimetric MTS in vitro assay. This study found the human skin fibroblasts sensitive to TiO_2 nanoparticles and correlated with the available in vivo and in vitro toxicity data. Accurately assessing the toxicity and safety of the nanomaterials to human health is of upmost importance and though this study will simply draw the attention but it will definitely form a base for undertaking further experimental works on the nanomaterials of other compounds and their correlation with human health safety and welfare.

Keywords: Nanostructure, Titanium Dioxide, Synonym, Pigment, Health.

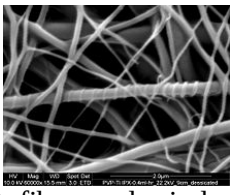
INTRODUCTION

The titanium dioxide with its chemical formula TiO_2 is a naturally occurring oxide of titanium. The synonyms are titanium oxide and titania. As a pigment it is called Titanium White or Pigment White 6. Its sources are ores like Ilmenite, Rutile, Anatase and Brookite and it has a wide range of applications from paint and sunscreen to food coloring. Titanium dioxide has eight modifications: in addition to Rutile, Anatase, and Brookite three metastable phases can be produced synthetically (monoclinic, tetragonal and orthorhombic) and five high pressure forms (α-PbO_2-like, baddeleyite-like, cotunnite-like, orthorhombic OI, and cubic phases [1].



TiO_2 unit cell

TiO_2 powder



TiO_2 fibers and spirals

Titanium dioxide is found as a mineral in magmatic rocks and hydrothermal veins as well as weathering rims on perovskite. Anatase can be converted by hydrothermal synthesis to delaminated anatase inorganic nanotubes and titanate nanoribbons which are of potential interest as catalytic supports and photocatalysts. A higher reaction temperature (170 °C) and less reaction volume give the corresponding nanowires [2, 3]. Titanium dioxide is the most widely used white pigment because of its brightness and very high refractive index and approximately 4.6 million tons of pigmentary TiO_2 are consumed annually worldwide and this number is expected to increase as consumption continues to rise [4]. Titanium dioxide has been shown statistically to increase skimmed milk's whiteness, increasing skimmed milk's sensory acceptance score. Titanium dioxide is used to mark the white lines of some tennis courts [5, 6]. TiO_2 is being used from drugs to doughnuts, cosmetics to catalysts, paints to pharmaceuticals, and sunscreens to solar cells etc. The U.S. Food and Drug Administration permits up to 1% TiO_2 as an inactive ingredient in food products. While there are no known health effects, a recent study found 3–6 year old children are the most affected group of people that consume TiO_2 particles from food products [7]. TiO_2 offers great potential as an industrial technology for detoxification or remediation of wastewater [8]. On the other hand, practical use of TiO_2 for remediation of chemical contaminants from wastewater remains a challenge because of adverse catalyst poisoning effects [9, 10]. Titanium dioxide has potential for use in energy production, as a photocatalyst, it can carry out hydrolysis by breaking water into hydrogen and oxygen and the hydrogen collected, can be used as a fuel. Titanium dioxide can also produce electricity when in nanoparticles form. Research suggests that the nanoparticles in pixels of a screen generate electricity when transparent and under the influence of light. If subjected to electricity on the other hand, the nanoparticles blacken, forming the basic characteristics of a LCD screen. According to creator Zoran Radivojevic, Nokia has already built a functional 200-by-200-pixel monochromatic screen which is energetically self-sufficient. TiO_2 can also oxidize oxygen or an organic material directly and thus added to paints, cements, windows, tiles, or other products for its sterilizing, deodorizing and anti-fouling properties and is used as a hydrolysis catalyst. It is also used in dye-sensitized solar cells, which are a type of chemical solar cell. Many sunscreens use nanoparticles titanium dioxide and zinc oxide, despite reports of potential health risks, is not actually absorbed through the skin [11]. The effects of titanium dioxide nanoparticles on human health are not well understood. Nevertheless, allergy to topical application has been confirmed [12].

Though it helps protect the skin from ultraviolet light but TiO_2 creates the radicals in the photocatalytic reaction which are carcinogenic, and could damage the skin. Titanium dioxide dust, when inhaled, has been classified by the International Agency for Research on Cancer (IARC) as an IARC Group 2B carcinogen, meaning it is possibly carcinogenic to humans. The findings of the IARC are based on the discovery that high concentrations of pigment-grade (powdered) and ultrafine titanium dioxide dust caused respiratory tract cancer in rats exposed by inhalation and intratracheal instillation [13]. The series of biological events or steps that produce the rat lung cancers (e.g. particle deposition, impaired lung clearance, cell injury, fibrosis, mutations and ultimately cancer) have also been seen in people working in dusty environments. Therefore, the observations of cancer in animals were considered, by IARC, as relevant to people doing jobs with exposures to titanium dioxide dust. For example, titanium dioxide production workers may be exposed to high dust concentrations during packing, milling, site cleaning and maintenance, if there are insufficient dust control measures in place. However, the human studies conducted so far do not suggest an association between occupational exposure to titanium dioxide and an increased risk for cancer. The safety of the use of nano-particle sized titanium dioxide, which can penetrate

the body and reach internal organs, has been criticized. Studies have also found that titanium dioxide nanoparticles cause inflammatory response and genetic damage in mice [14]. The mechanism by which TiO_2 may cause cancer is unclear. Molecular research suggests that cell cytotoxicity due to TiO_2 results from the interaction between TiO_2 nanoparticles and the lysosomal compartment, independently of the known apoptotic signaling pathways [15]. There is some evidence the rare disease 'yellow nail syndrome' may be caused by titanium, either implanted for medical reasons or through eating various foods containing titanium dioxide [16]. Titanium, used in orthopaedic devices and oral implants, although considered an inert material, can actually induce toxicity or allergy and be responsible for successive unexplained cases of failure of dental implants in some patients known as 'cluster patients' [17].

MATERIALS AND METHODS

Cytotoxicity testing and evaluation method were adopted from previously published article by Fin Dechsakulthorn et al. 2007 [18]. TiO_2 powder suspended in a culture medium at the concentration of about 5000 ppm was dispersed by ultrasonic vibration for 20 minutes. Before every use this suspension was stirred on vortex agitation for about 1 minute in order to get its uniformity.
Human skin fibroblasts were collected from fresh skin biopsies of healthy individuals in research lab. All cultures were maintained in a phenol red free culture medium DMEM/F12 (Dulbecco's modified essential medium/Ham12 nutrient mixture) supplemented with 5% fetal calf serum and 1% antibiotic (2mM L-glutamine, 100 U ml^{-1} Penicillin and 0.1 mg ml^{-1} Streptomycin). Cultured cells were put at 37ºC in a humidified 5% CO_2 incubator. Once the cells reached confluence, the culture medium was removed from the flask and the cells were rinsed three times with sterile HBSS (Hank's Balanced Salt Solution). The confluent cell layers were enzymatically removed, using Trypsin/EDTA, and resuspended in culture medium. Cell viability was assessed by vital staining with Trypan blue (0.4% (w/v)), and cell number was determined using a light microscope.

Nanoparticles were suspended in culture media, serially diluted across microtiter plates (100µL), and incubated at 37ºC with 5% CO_2. Two sets of exposure times were carried. These included 6 hours and 24 hours exposure periods. Six hours prior to the end of each exposure period a MTS mixture (20 µL) was added. After the completion of exposure period, the plates were then placed on a microwell plate reader, shaken for 10 seconds and the absorbance of the formazan product was read at 492 nm. Each experiment was repeated on three separate occasions. Two internal controls were set up for each experiment: (1) an IC_0 consisting of cells only and (2) IC_{100} consisting of medium only. Background absorbance due to the non-specific reaction between test compounds and the MTS reagent was deducted from exposed cell values [19]. Dose response curves were plotted for the test chemicals after correction by subtracting the background absorbance from the controls. NOAEC (No Observable Adverse Effect Concentration), IC_{50} (50% inhibitory concentration) and TLC (Total Lethal Concentration) values were extrapolated graphically from the plotted absorbance data.

RESULTS AND DISCUSSION

Relationship between test chemical TiO_2 concentration (ppm) and cell viability (%) after 6 and 24 hours exposures using the MTS assay are presented in fig. 1. Each experimental curve represents the average of a series of three different experiments. Cell viability is significantly reduced in a dose-dependent manner after exposure of human fibroblasts to nanoparticles using the MTS assay. The experimental data for NOAEC, IC_{50}, and TLC values on the cells are summarised in table 1 with data presented as mean values ± standard deviations. The experiment is conducted following 6 and 24 hours exposure time. Slightly Reduced cell viability is observed after 6 hours exposure time but, by increasing the exposure time to 24 hours the cytotoxicity of nanoparticles gets increased substantially. Following 24 hours exposure time for TiO_2 the IC_{50} was 3,234 ± 556 ppm.

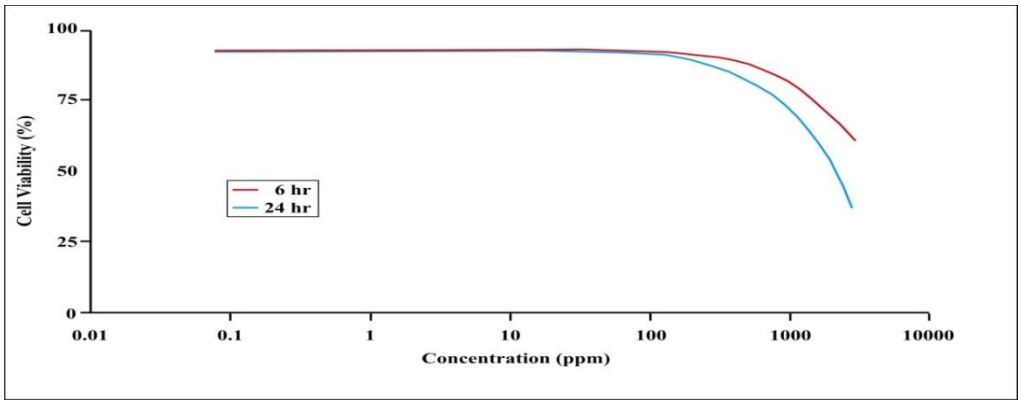


Fig. 1: Concentration–cell viability curves of titanium dioxide nanoparticle following 6 and 24 hours exposure on human skin fibroblasts using the MTS assay

Table 1: Toxicity values of titanium dioxide

Toxicity Endpoints	Titanium Dioxide (TiO_2)	
(ppm)	6 h	24 h
NOAEC	270.5 ± 70.5	88.34 ± 14.44
IC_{50}	n/a	3234 ± 556
TLC	n/a	125862 ± 3,188

TiO_2 nanoparticles exposure indicated a range of responses of cytotoxicity to human skin fibroblast cells. NOAEC values at 6 hours and 24 hours exposure using the MTS assay showed the distinct toxicity. However, other toxic endpoints of IC_{50} and TLC could not be measured due to incomplete dose response curves. These results indicate that at 6 hours exposure the nanoparticles present a slight adverse effect to human cell fibroblasts but when the exposure time was increased to 24 hours, the nanoparticles had a substantial toxic impact to cells. These results are supported by previous published results that indicated that longer (3 day) exposure generated a greater toxicity to human bronchial epithelial cells (IC_{50} (TiO_2) 6.5 μg mL^{-1}) using the MTT assay [20].

Results of this study demonstrated that the MTS assay could be implemented as an effective and sensitive tool to assess cytotoxicity of TiO_2 nanoparticles on human skin fibroblasts. Present study showed the cell viability significantly reduced in a dose-dependent manner after exposure of human fibroblasts to TiO_2 nanoparticles. Recent published in vivo studies have shown significant adverse effects to mice using nano-scale Zn metal powder and TiO_2 at 5 g kg^{-1} body weight [21, 22]. Pulmonary toxicity has also been observed in rats after 24 hours exposure to low concentration (5 mg kg^{-1}) of nano-scale TiO_2 particles [23]. In vitro toxicity assessment has become widely used for recent toxicity studies. Such assays provide rapid, cost effective and reliable results [24]. Toxicity results of ZnO nanoparticles in Chinese hamster ovary cells indicated a NOAEC and IC_{50} at 54 and 340 μg mL^{-1}, respectively [25].

Nanomaterials usage is continuously being increased rapidly and widely in areas such as cosmetics, eatables, pharmaceuticals and other industrial applications. Accurately assessing the toxicity and safety of these nanomaterials to human health is of upmost importance and though this study will simply draw the attention but it will definitely form a base for undertaking further experimental works towards nanomaterials of other compounds and human welfare.

APPLICATIONS

This study is useful to know about whether the human skin fibroblasts sensitive to TiO_2 nanoparticles or not.

CONCLUSIONS

TiO_2 has emerged as one of the most fascinating materials in the modern era. It is vastly capturing the attention of physical chemists, physicists, scientists, engineers, doctors and social workers in exploring its distinctive properties. This study found the human skin fibroblasts sensitive to TiO_2 nanoparticles. Accurately assessing the toxicity and safety of the nanomaterials to human health is of upmost importance and undertaking further experimental works towards nanomaterials of other compounds and their correlation with human health safety and welfare is a necessity.

REFERENCES

[1] R Marchand, L. Brohan, M. Tournoux, *Materials Research Bulletin*, **1980**, 15 (8), 1129–1133.
[2] G. Mogilevsky, Q. Chen, A. Kleinhammes, Y. Wu, *Chemical Physics Letters*, **2008**, 460 (4–6), 517–520.
[3] A. Graham, A. Robert, C. Jesús, B. Peter, *Chemical Communications* (19): 2454.
[4] J. Winkler, *Vincentz Network*, **2003**, 5.
[5] L. Phillips, G. Barbano, M. David, *Journal of Dairy Science*, 80 (11), 2726.
[6] Les, Caren B, (November 2008) Light spells doom for bacteria, Photonics.com.
[7] A. Weir, P. Westerhoff, L. Fabricius, K. Hristovski, N. von Goetz, *Environ. Sci. Technol*, **2012**, 46, 2242-2250.
[8] Konstantinou, K. Ioannis, T. Albanis, *Applied Catalysis B: Environmental*, **2004**, 49: 1.
[9] K. Maeda, K. Domen *J. Phys. Chem. Lett*, **2010**, 1, 2655–2661.
[10] P. Kamat, *J. Phys. Chem. Lett*, **2012**, 3, 663–672.
[11] N. Sadrieh, A. Wokovich, N. Gopee, *Toxicol. Sci*, **2011**, 115 (1), 156–66.
[12] T. Shaw, B. Simpson, B. Wilson, H. Oostman, D. Rainey, F. Storrs, *Dermatitis*, **2010,** 21 (4), 185–98.
[13] N. Serpone, C. Kutal, *American Chemical Society*.**1993**.
[14] A. Yazdi, G. Guarda, N. Riteau, *Proc. Natl. Acad. Sci. U.S.A.***2010**, 107 (45), 19449–54.
[15] Y. Zhu, J. Eaton, C, Li, PLoS ONE 7, **2012**, (11): e50607.
[16] F. Berglund, B. Carlmark, *Biol Trace Elem Res*, **2011,**143 (1), 1–7.
[17] E. Laurence, **2011**, 531-544 Published online.
[18] D. Fin, H. Amanda, B. Shahnaz, J. Lucky, W, *AATEX 14*, Special Issue, **2007** 397-400.
[19] AJ. Hayes, B. Markovic, *Food and Chemical Toxicology*, **2002**, 40, 535-543.
[20] JR. Gurr, ASS. Wang, CH. Chen, KY. *Jan, Toxicology*, **2005**, 213, 66-73.
[21] B. Wang, WY. Feng, TC. Wang, G. Jia, M. Wang, JW Shi, F. Zhang, YL. Zhao, ZF. Chai, *Toxicology Letters*, **2006**, 161, 115-123.
[22] J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li, J. Sun, Y. Li, F. Jiao, Y. Zhao, Z. Chai, *Toxicology Letters*, **2007,** 168, 176-185.
[23] DB. Warheit, TR. Webb, KL. Reed, S. Frerichs, CM. Sayes, *Toxicology*, **2007,** 230, 90-104.
[24] A. Hayes, B. Markovic, Cosmetics, Aerosols & Toiletries in Australia, 12, 24-30.
[25] EK. Dufour, T. Kumaravel, GJ. Nohynek, D. Kirkland, H. Toutain, *Mutation Research*, **2006,** 607, 215-224.