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Titanium Dioxide and Its Effect on Human Health and Environment- An *in vitro* Study

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ABSTRACT

This work focuses on understanding the *in-vitro* effects of Titanium Dioxide (TiO_2) led in the environment on human health, algae, and agriculture. Human dermal fibroblasts (HDF) from juvenile foreskin and human red blood cells (HRBC) were taken as a model to study the effect of TiO_2 on human health *in vitro*. The study intended to define the toxic effect of TiO_2 at 50 ppm, 100 ppm, and 200 ppm. The effect of TiO_2 on the algae cyanobacterium as a model to analyze the algal toxicity effect i.e. was checked for freshwater toxicity at the concentration of TiO_2 at 100 and 200 ppm was studied. The effect of TiO_2 on the growth of microorganisms in wastewater was studied to determine its biodegradation *in-vitro* for 5 days using dissolved oxygen determination and the biological oxidation demand (BOD). Finally, phytotoxicity was monitored by observing the effect of TiO_2 on wheat seed germination. It was found that TiO_2 had no effect on HDF and HRBC at the tested concentrations as no cell death and hemolysis were observed when the cells were treated with TiO_2. However, a statistically significant algal toxicity of 32.14 % was observed at 100 ppm and a 42.86 % ($p<0.01$) decrease in biomass was observed at 200 ppm. Additionally, there was no effect found on BOD of wastewater in the presence or absence of TiO_2. The TiO_2 had a positive effect on wheat seed germination in a dose-dependent manner. There was an increase in root length from 3.4 cm to 4.3 cm and 4.6 cm at 100 and 200 ppm of TiO_2, respectively. Also, a slight increase in shoot length was observed at 100 ppm and 200 ppm. However, visible root thinning was a drawback observed. Hence, the present study gives an elaborative insight into the effects of Titanium Dioxide on human health and the environment.

Keywords: Algal Toxicity, dermal fibroblast, phytotoxicity, titanium dioxide.

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I. Introduction

Ultraviolet radiations, categorized as UVC (200-280nm), UVB (280-320 nm), and UVA (320-400 nm), are a part of the sun's spectral energy. Due to the ozone layer, only a part of UVB and complete UVA reaches earth (Mohania *et al*., 2017). UV radiation has a positive effect on humans as it promotes the natural synthesis of vitamin D and endorphins on the skin (D'Orazio *et al*., 2013). However, both UVA and UVB rays are considered to be environmental mutagens and are complete carcinogens, responsible for causing sun tanning, hyper-pigmentation, inducing inflammatory cascading reactions (Gasparro *et al*., 1998), and skin cancers (Mohania *et al*., 2017). For more than six decades, sunscreens have been used to protect the skin from harmful UV radiations (Schauder, 1997). Topical sunscreens can be broadly classified into two groups; chemical or organic sunscreens and physical or inorganic sunscreen agents. The most commonly utilized physical blockers are ZnO and TiO_2. Both reflect photons in the visible light range and work as mineral filters in the UV range where ZnO has

activity till 370 nm and TiO_2 till 400 nm, and both these SPF agents are considered to be safe (Cole *et al*., 2015).

TiO_2 is an oxide of the metal titanium, the ninth most galore element in the earth's crust. It belongs to the family of transition metal oxides and is insoluble in water. It is one of the widely studied metal oxides due to its interesting general properties, and its refractive index. Most of the TiO_2 has been synthesized from the mineral ilmenite. TiO_2 exists in three crystalline forms; anatase, rutile, and brookite, only the first two of which are of industrial interest (Haider *et al*., 2019). The worldwide production of TiO_2, a white powder, also termed titania powder, is estimated to be 4.5 million tons per year (McNulty, 2007).

TiO_2 is widely used due to its economic benefits, convenient chemical properties, and ease of mass production. It is used at a particle size of 150-300 nm, or in lower sizes of 20-150 nm also called micro- TiO_2. Such micronized TiO_2 has been known to have broad-spectrum UV protection against UVB and UVA2 wavelengths (315-340 nm). As per scientific evidence, TiO_2 concentration up to 25 % is considered safe on the human skin as TiO_2 does not penetrate through the skin (Trivedi & Murase, 2017). While titanium dioxide is nontoxic, titanium waste i.e., by-products formed in the production of TiO_2 are highly acidic and difficult to dispose of. Most plants dump diluted sulphuric acid (a by-product of TiO_2 extraction) into marine waterbodies, which reduces the pH of said water along with its oxygen content. Effluents from plants also include gaseous emission of sulphur and chlorine compounds into the air (Lane, 1991).

TiO_2 as an active ingredient in cosmetic sunscreens is a huge market and its production has been gunning to appear in various applications such as antibacterial, catalysis, paints, papers, etc. (Skocaj *et al*., 2011). Thus, the environmental fate of TiO_2 is a matter of concern. Also, its toxicity studies reported are contentious. Hence, the current research work focuses on the *in vitro* toxic effect of TiO_2 on human dermal fibroblast cells and human red blood cells. The paper also focuses on understanding the effect of TiO_2 on the environment by monitoring its effect on the dissolved oxygen content of water, and its effect on marine life by determining its activity on algal species in 1 week. Finally, the effect of titanium oxide on plant growth was measured by monitoring its effect on wheat germination for a period of 4 days. The results suggest that 200 ppm, 100 ppm, and 50 ppm had no statistically significant effect on the cell viability of human dermal fibroblasts and human red blood cells. The samples at the same concentration showed a slight effect on the BOD of wastewater. Similarly, titanium dioxide had a negative effect on algal biomass after a period of 7 days at 100 and 200 ppm. However, the sample showed a positive effect on root elongation and shoot elongation at higher concentrations of 100 and 200 ppm.

II. Material and Methods

A. Materials

Titanium dioxide (TiO_2) was obtained from Sigma Aldrich. Dulbecco's modified Eagle's medium (DMEM) was purchased from Himedia, Mumbai. Foetal bovine serum (FBS) was procured from Gibco, Thermo Fischer. Trypsin and Penicillin-streptomycin antibiotic solution were purchased from Himedia. Sulforhodamine B (SRB) dye was purchased from Sigma Aldrich. Trichloroacetic acid (TCA), Glacial acetic acid, and Tris Base used were of analytical grade. Wheat seeds, Sodium thiosulphate ($Na_2S_20_3$), starch powder, healthy human blood with anticoagulant, Phosphate buffer saline (PBS), Cyanobacteria spirulina media for algal growth.

B. Cytotoxic Effect of TiO_2

1) Sample Preparation

A stock solution of TiO_2 was prepared in DMEM cell culture medium at a concentration of 10 mg/ml. Further, working stocks were prepared in the same culture medium at final concentrations of 1, 0.5, 0.25, 0.125, 0.0625 mg/ml immediately before application.

2) Cell Line and Cytotoxicity Study

Human Dermal Fibroblast (HDF) cells were obtained from Himedia Laboratories. The cells were cultured in DMEM with 10% FBS, and 1 % antibiotic solution at 37 °C in a 5 % CO_2 humidified environment.

For cytotoxicity assay using SRB dye (Tolliver *et al*., 2020); cells were seeded in each well of 96-well plate at a seeding density of $0.5*10^4$ per well in a 100 µL culture medium. The plate was then incubated for 24 hours at 37 °C in a 5 % CO_2 incubator. On the second day, media was replaced by 100 ul of different concentrations of TiO_2 solutions, and the plate was incubated further for 24 hours. After overnight incubation, cells were fixed with 10 % TCA and later stained with SRB for 30 minutes, after which the excess unbound dye was removed by washing repeatedly with 10 % glacial acetic acid. The protein-bound dye was dissolved in a 10 mM Tris base solution for optical density determination at 530 nm using a microplate reader. Cells cultured in media without TiO_2 were used as a control with 100 % cell viability.

The percentage of cytotoxicity obtained was calculated as follows:

$$\% \text{ Cytotoxicity} = \{Absorbance\ of\ control - Absorbance\ of\ Test\ /Absorbance\ of\ control\} * 100$$

C. Phytotoxic Effect of TiO_2

In this section, we examined the effect of TiO_2 on seed germination, root and shoot length of wheat seedlings.

Different concentrations of TiO_2 (200, 100, and 50 ppm) were prepared by suspending 200, 100, and 50 mgL^{-1} in deionized water through ultra-sonication, immediately before use.

1) Seed Germination

Wheat seeds were rinsed twice thoroughly with deionized water. For each test concentration, uniformly sized 10 seeds were sown/ kept at equal distance on a petri dish lined with double folded tissue paper. Then 1 ml of TiO_2 suspensions of 50, 100 and 200 ppm were added to the corresponding Petri dishes, with deionized water for control. Each concentration was kept in triplicates including the control. Petri dishes were covered with aluminium foil to maintain a dark environment. Germination of seeds was observed after 4^{th} day of the experiment. Germination percentage was calculated with the help of related formula along with average root length and shoot length.

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D. Effect of TiO_2 on Biological Oxygen Demand (BOD)

In order to evaluate the effect of TiO_2 on dissolved oxygen in wastewater, 12.5, 25, and 50 mg of TiO_2 was added to amber-coloured bottles containing 250 ml of wastewater to get a final concentration of 50, 100, and 200 ppm. The bottles were incubated at 37 ^{0}C for 5 days. Control set, i.e. without TiO_2 was also maintained at the same condition. Experiments were carried out in duplicates. Dissolved oxygen (DO) content at day 0 and day 5 was performed using the standard titration method. The water sample was titrated against 0.0125 N Sodium thiosulphate, using starch as an indicator. Difference between day 0 and day 5 DO values provided with the BOD values for test samples and control which were then compared.

DO= {8*100* N of $Na_2S_20_3$* Burette reading/ volume of sample titrated}

BOD= DO (day0) - DO (day5)

E. Effect of TiO_2 on Haemolysis

Haemolytic activity is a parameter suggestive of toxicity in vivo. The assay was performed following previously described methods with minor modifications (Dhawal *et al*., 2017). Briefly, a blood sample from a healthy human was collected in a tube with an anticoagulant which was then centrifuged for 2 mins at 4 ˚C, further washed twice with 1 mM PBS to get rid of serum. Obtained RBCs pellet was then resuspended in PBS. An equal amount of blood solution was added in Eppendorf tubes containing different concentrations of TiO_2 (50, 100, 200 ppm) in saline. RBCs in distilled water were maintained as a negative control at the same experimental condition. All the tubes were incubated for 1h at 37 ˚C. After incubation tubes were centrifuged for 5 min at 4 ˚C. after which 100 µL of supernatant from each tube were added into wells of 96 well plate and the plate was read at 570 nm.

F. Effect of TiO_2 on Algal Growth

Cyanobacterial strain was provided by the Botany Research Department of KET's V.G.Vaze college, Mulund, Maharashtra, India. The growth inhibition test on cyanobacteria was conducted in Spirulina medium. TiO_2 was added to the flask containing culture medium to get a final concentration of 100 and 200 ppm. The control flask without TiO_2 was also maintained for comparison. Experiments were conducted in triplicates. Algae was inoculated in the flasks which were then kept on the shaker for continuous stirring with timely illumination. Algal biomass was weighed after 10 days of incubation which was then compared with the initial biomass.

G. Statistical Analysis

One-Way Analysis of variance (ANOVA) was calculated for all the tests using JMP Statistical Discovery from SAS Software. A p-value less than 0.05 was considered to be statistically significant.

III. Result and Discussion

A. Cytotoxic Effect of TiO_2 of Human Dermal Fibroblasts (HDF)

Result of the SRB assay was used to determine the percent cell death of treated cells with respect to control cells as a function of absorbance of dissolved bound dye. After 24 h of exposure, none of the investigated concentrations of TiO_2 significantly impaired the viability of the cells ($p<0.01$ using One-Way ANOVA), in fact, the lower concentration i.e., 0.0625 mg/ml showed almost 35 % proliferative effect on the cell line (Fig. 1). The only exception was, 3.5 % of cytotoxicity on HDF cells for 1 mg/ml concentration of TiO_2. A similar study, using trypan dye exclusion assay, conducted on human skin fibroblasts (HuDE) and mouse embryo fibroblasts (3T3) reported that the cell viability was always > 80 % even after exposure to the highest concentration of TiO_2 (Frenzilli *et al*., 2014) A study conducted to investigate the cytotoxic effect of TiO_2 on Syrian hamster embryo cells (SHE) revealed that the Nano-sized particles of TiO_2 showed a 2 to 3-fold higher cytotoxic effect than their micro-sized counterparts (Guichard *et al.*, 2012).

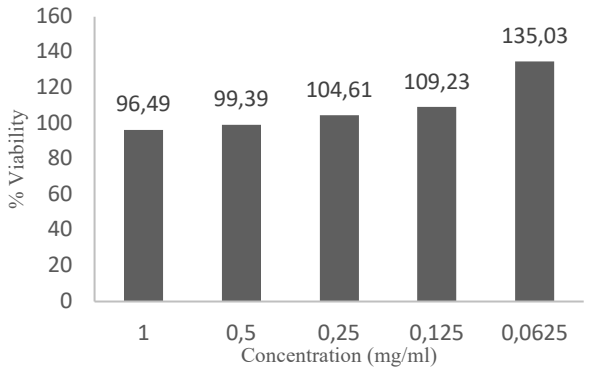

Fig. 1. Effect of TiO2 on HDF cell line.

B. Phytotoxicity of TiO_2

The effect of different concentrations of TiO_2 on the germination of wheat seeds compared to the control showed significant changes (Fig. 2). Out of 10 after 24 h of treatment, the average number of germinated seeds was 8 for control and 8, 8 and 7 for seeds treated with 50, 100 and 200 ppm of TiO_2, respectively ($p>0.05$ using One-Way ANOVA). On day 4, Control sets showed an average of 8 seeds germinated. The Average germinated seed number for seeds exposed at 50, 100, and 200 ppm of TiO_2 were 8, 9 and 10, respectively. Thus the result indicated a no effect of TiO_2 on wheat seed germination at concentrations 100 and 200 ppm with 90 and 100 % of germination.

Average root length and shoot height for control and exposed seeds were measured (Fig. 3). The average root length for control seeds was 3.4 cm and that for exposed seeds was 2.8 cm, 4.3 cm and 4.6 for 50, 100 and 200 ppm of TiO_2 respectively. There was a significant effect seen for 100 and 200 ppm on root length and shoot length ($p<0.01$ using One-Way ANOVA). However, root thinning was observed for exposed seeds at higher concentrations. The average shoot height for control seeds was 2.3 cm and that

for exposed seeds was 2 cm, 3.3 cm, and 3.3 cm for 50, 100 and 200 ppm of TiO_2, respectively.

Decrease in root length and shoot height at 50 ppm could possibly be attributed to the blocking of root pores and thus hindering the water and nutrient uptake. On the other hand, better root growth at higher concentrations could be because of the microbial resistance of TiO_2 which may help plants cope with stress conditions and improve their nutrient availability. Thus, results suggest that a higher concentration of TiO_2 could be beneficial for seed germination and growth but at the same time accompanied root thinning could be a drawback. On the other hand lower concentration could lead to some amount of phytotoxic effect.

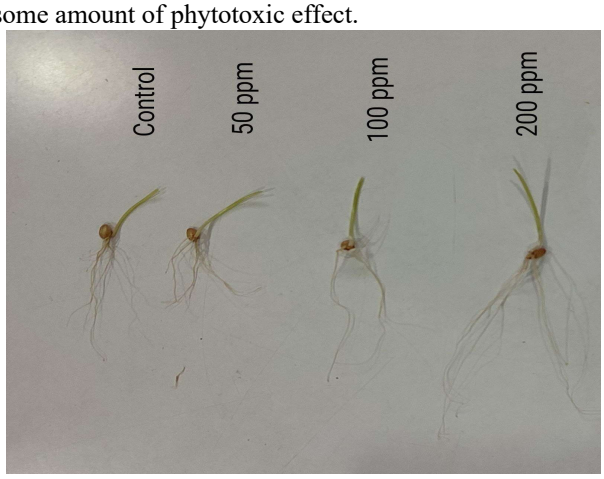

Fig. 2. Effect of Different concentrations of TiO2 on wheat seeds.

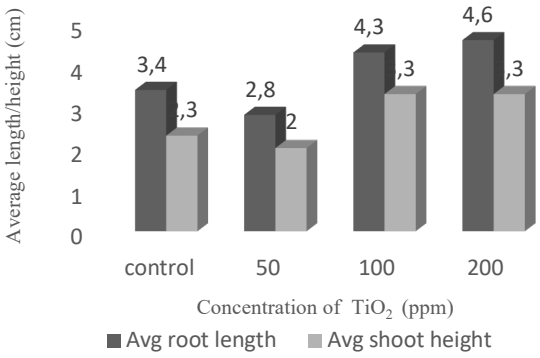

Fig. 3. Average shoot height and root length.

C. *Effect of TiO_2 on BOD*

Effect of applied concentrations of TiO_2 on BOD was investigated by comparing the values of the dissolved oxygen content of the water samples. The DO at day 0 was estimated to be 4.4 mg/L. DO after 5 days of incubation was found out to be 2.4 mg/L, 2.4 mg/L, and 2.8 mg/L for blank i.e., without TiO_2, 100 ppm of TiO_2, and 200 ppm of TiO_2, respectively. Thus, with the help of the formula, the BOD values were calculated to be 2, 2, and 1.6 mg/L, respectively. From the values, it is clear that a 200 ppm concentration of TiO_2 helped to improve the quality of the water sample under investigation by decreasing the oxygen demand caused by the presence of biological entities in the water sample. On the other hand, 100 ppm of TiO_2 showed no changes in the BOD value when compared with that of blank ($p>0.05$ using One-Way ANOVA).

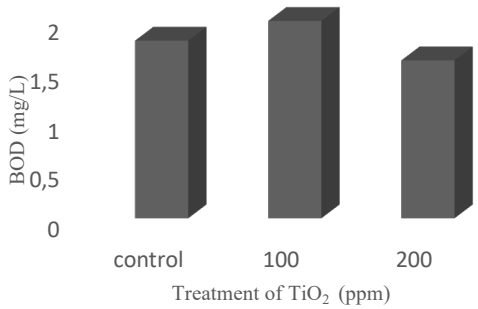

Fig. 4. Effect of TiO2 on BOD of waste water.

D. *Effect of TiO_2 on Haemolysis*

Haemolysis can be considered as a good endpoint to investigate pathogenicity of certain materials with biological membranes (Pavan *et al*., 2013). Thus, to assess the membrane interaction of TiO_2, different concentrations of TiO_2 were co-incubated with RBCs and the level of haemolysis was measured spectrophotometrically. The assay showed no visible haemolytic activity of TiO_2 at added concentrations when compared with negative control. Thus, TiO_2 possesses no toxicity towards RBCs. Surface charge of a material is considered an important contributing factor for heamolysis; with strongly positive surface charge leading to the disruption of the negatively charged RBCs (Lu et al., 2009). Therefore, the surface charge of TiO_2 could be the factor associated with no haemolysis.

E. *Effect of TiO_2 on Algal Biomass*

There are many Studies on algal growth inhibition in presence of TiO_2 nanoparticles but there are none that reported the toxic effect of TiO_2 powder as a bulk material, on algal growth.

Algal growth inhibition was observed when exposed to TiO_2. The initial weight of algal biomass i.e. at day 0 was 0.1 g/L. Control average biomass after 10 days of incubation weighed 0.28 g/L and exposed average biomass weighed 0.19 and 0.16 g/L, for 100 and 200 ppm of TiO_2 respectively. From the values, it can be concluded that the algal growth inhibition was TiO_2 concentration-dependent. 42.86 % growth inhibition by 200 ppm TiO_2, and 32.14 % inhibition by 100 ppm of TiO_2 ($p<0.01$ using One-Way ANOVA). TiO_2 occurs in two crystalline structures, Rutile and Anatase, and both of these are highly photoreactive substances (Skocaj et al., 2011). It is well known that photocatalytic materials generate high oxidative stress under both light and dark conditions (Augustynski, 1993). Thus, this oxidative stress could plausibly be the contributing factor for algal growth inhibition. However, this toxic effect could be species-specific and thus more elaborative experiments must be carried out in the future.

IV. Conclusion

The presence of TiO_2 in the environment can possibly cause a threat to organisms at a different level. In order to comprehensively evaluate the toxic effect of TiO_2, we considered four perspectives: Cytotoxicity, Phytotoxicity,

Algal toxicity, and Membrane toxicity. From the results, it is clear that there is no effect of TiO_2 on cells and on the membrane stability of RBCs. On wheat seeds germination TiO_2 showed a positive effect with an increase in shoot height and root length however root thinning was also accompanied. Some concentration-dependent toxic effect on algal growth was also observed. No effect or positive effect in the case of cells and seeds suggests that TiO_2 as a bulk counterpart is relatively safe. However, more in-depth experimental studies should be carried out to understand the toxicity at higher concentrations of TiO_2 as at high dosage or in different environmental conditions studied TiO_2 or its by-products, if any, could act as a Trojan horse.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

Augustynski, J. (1993). The role of the surface intermediates in the photoelectrochemical behaviour of anatase and rutile TiO2. *Electrochimica Acta*, *38*(1), 43–46.

Cole, C., Shyr, T., & Ou-Yang, H. (2015). Metal oxide sunscreens protect skin by absorption, not by reflection or scattering. *Photodermatology, Photoimmunology & Photomedicine*, *32*(1), 5–10.

Cytotoxicity and Genotoxicity of Nanosized and Microsized Titanium Dioxide and Iron Oxide Particles in Syrian Hamster Embryo Cells. (2012). *The Annals of Occupational Hygiene*.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV Radiation and the Skin. *International Journal of Molecular Sciences*, *14*(6), 12222–12248.

Dhawal, P., Satardekar, K., Hariharan, S., Barve, S. (2017). In-vitro analysis of cinnamomum verum for formulation of bioactive cosmetic gel. *International journal of pharmaceutical sciences and research*. *8(7),* 2988- 2995.

Frenzilli, G., Bernardeschi, M., Guidi, P., Scarcelli, V., Lucchesi, P., Marsili, L., et al. (2014). Effects of in vitro exposure to titanium dioxide on DNA integrity of bottlenose dolphin (Tursiops truncatus) fibroblasts and leukocytes. *Marine Environmental Research*, *100*, 68–73.

Gasparro, F. P., Mitchnick, M., & Nash, J. F. (1998). A Review of Sunscreen Safety and Efficacy. *Photochemistry and Photobiology*, *68*(3), 243–256.

Haider, A. J., Jameel, Z. N., & Al-Hussaini, I. H. (2019). Review on: Titanium Dioxide Applications. *Energy Procedia*, *157*, 17–29.

Lane, D.A., 1991. Pollution caused by waste from the titanium dioxide industry: Directive 89/428.

Lu, S., Duffin, R., Poland, C., Daly, P., Murphy, F., Drost, E., et al. (2009). Efficacy of Simple Short-Term in Vitro Assays for Predicting the Potential of Metal Oxide Nanoparticles to Cause Pulmonary Inflammation. *Environmental Health Perspectives*, *117*(2), 241–247.

Mcnulty, G.S., 2007. Production of titanium dioxide. In Proceedings of NORM V international conference, Seville, Spain (pp. 169-189).

Mohania, D., Chandel, S., Kumar, P., Verma, V., Digvijay, K., Tripathi, D., et al. (2017). Ultraviolet Radiations: Skin Defense-Damage Mechanism. *Advances in Experimental Medicine and Biology*, 71–87.

Pavan, C., Tomatis, M., Ghiazza, M., Rabolli, V., Bolis, V., Lison, D., et al. (2013). In Search of the Chemical Basis of the Hemolytic Potential of Silicas. *Chemical Research in Toxicology*, *26*(8), 1188–1198.

Schauder, S. (1997). Sunscreens in Europe: Recent Developments. *Skin Cancer and UV Radiation*, 276–295.

Skocaj, M., Filipic, M., Petkovic, J., & Novak, S. (2011). Titanium dioxide in our everyday life; is it safe? *Radiology and Oncology*, *45*(4).

Tolliver, L. M., Holl, N. J., Hou, F. Y. S., Lee, H. J., Cambre, M. H., & Huang, Y. W. (2020). Differential Cytotoxicity Induced by Transition Metal Oxide Nanoparticles is a Function of Cell Killing and Suppression of Cell Proliferation. *International Journal of Molecular Sciences*, *21*(5), 1731.

Trivedi, M., & Murase, J. (2017). Titanium Dioxide in Sunscreen. *Application of Titanium Dioxide*.