# The Cytotoxic Effects of Titanium Oxide Nanoparticle on MDA-MB–231 AND MCF–7 Cells

Serap Sahin Bolukbasi
Faculty of Pharmacy, Department of Biochemistry, Cumhuriyet University, Sivas, Turkey.
E-mail: wserap@yahoo.com

Nese Keklikcioglu Cakmak
Faculty of Engineering, Department of Chemical Engineering, Cumhuriyet University, Sivas, Turkey
E-mail: nkeklikcioglu@gmail.com

Ayca Tas (Corresponding author)
Faculty of Health Sciences, Department of Nutrition and Diet, Cumhuriyet University, Sivas, Turkey
E-mail: aycatas@cumhuriyet.edu.tr

Esma Ozmen
Faculty of Medicine, Department of Medical Biochemistry, Cumhuriyet University, Sivas, Turkey
E-mail: ozmenesma07@gmail.com

Esranur Cevik
Faculty of Pharmacy, Biochemistry Department, Cumhuriyet University, Sivas, Turkey.
E-mail: esra_cvk@hotmail.com

Erkan Gumus
Faculty of Medicine, Department of Histology and Embryology, Cumhuriyet University, Sivas, Turkey
E-mail: erkangumus@gmail.com

Yavuz Silig
Faculty of Medicine, Department of Medical Biochemistry, Cumhuriyet University, Sivas, Turkey
E-mail: ysilig@cumhuriyet.edu.tr

*This study was carried out at Cumhuriyet University's Advanced Technology Application and Research Center (CUTAM).*

**Abstract**
Breast cancer is one of the leading causes of mortality among women around the world due to various factors such as aggressive invasion, early metastasis, resistance to existing chemotherapeutic drugs, and high mortality. Because traditional chemotherapeutic agents affect the whole body system through the blood, there are too many systematic side effects such as tissue damage, gastrointestinal stress. In recent years, scientists have focused on research on nanotechnology molecules that exhibit anti-cancer activity, and progress has been made to the relevant pharmacotherapeutic field. Nanotechnology is one of the fastest developing research fields in the world and in our country. In this study, Titanium oxide ($TiO_2$) nanoparticle was synthesized. The formation of $TiO_2$ nanoparticles was characterized by UV–vis spectrophotometry and zeta potential measurements. X-ray diffractometer (XRD) spectrum of the nanoparticles confirmed the formation of $TiO_2$ nanoparticles. The synthesized $TiO_2$ were applied to the MDA-MB-231 and MCF-7 cancer cell line ($1x10^5$ cells/well, 96 well plates in DMEM supplemented with 10% FBS and 1% penicillin) and cytotoxic effect was determined. Cytotoxicity was carried out using (3- (4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT). MDA-MB-231 and MCF-7 cells were treated with different concentrations of $TiO_2$ for 24h, 48h and 72 h. The spectrophotometric readings at 570 nm were analyzed by the *Graphpad Prism7* program. MTT test results showed that $TiO_2$ significantly reduced cell viability by compared to control.

**Key words:** $TiO_2$, MDA-MB-231, MCF-7, Breast cancer, Nanoparticle

## 1. Inroduction

Breast cancer is one of the most prevalent and lethal malignancies in women worldwide. Statistically, 1.5 million females were diagnosed with breast cancer in 2016. Breast cancer accounts for almost 29 % of all incident cases of cancer in women (Siegel, 2016). The treatment of cancer involves different therapies based on alkylating agents, antimetabolites, biological agents, etc.; but one of the principal problems is the side effects due to difficulties in differentiating between cancerous and normal cells, which produces systemic toxicity (Stewart, 2014). When exploring new strategies for the treatment of cancer, one possibility is the use of nanomaterials. For more than 30 years, nanomaterials have been used as pharmaceutical carriers to enhance the in vivo antitumor efficacy of drugs. The development of nanostructured devices for drug delivery and controlled release constituted new antitumor chemotherapies (Rasmussen, 2010).

Nanotechnology is one of the fastest growing sectors of the high-tech economy. There are more than 200 separate consumer products alone using nanomaterials with personal, commercial, medical, and military uses (Brumfiel, 2006; Griffitt, 2007). Metal oxide nanoparticles are finding a variety of applications in catalysis, drug delivery, cosmetics, clothing, energy conversion, water disinfection, lubrication, home appliances, and environmental remediation (Aitken, 2004; Hoet, 2004a; Hoet, 2004b; Nohynek, 2008; Akimov, 2010; Kumar, 2011; Kumar, 2013). $TiO_2$ nanoparticles have the second highest annual production (estimated 3000 tons), with major applications as a photocatalyst for bacterial disinfection and as a component of sunscreens in the cosmetics industry (Bondarenko, 2013). $TiO_2$ nanoparticles are also used in products such as food packaging, therapeutics, biosensors and surface cleaning agents, among others (Shukla, 2013; Shukla, 2014). $TiO_2$ nanoparticles are environment friendly, relatively stable, have excellent biocompatibility with being smaller than cellular organelles or no toxicity, and low cast (Thurn, 2011; Takaki, 2013; Mossesson, 2008; Kansara, 2015). These properties make $TiO_2$ nanoparticles an excellent candidate for biomedical applications such as drug delivery and cancer therapy (Tomasina, 2013; Schilling, 2010). In the present study, we aimed to investigate the cytotoxic effects of $TiO_2$ nanoparticles on MDA-MB–231 and MCF–7 cancer cell line.

## 2. Materials and methods

### *Preparation and Characterization of $TiO_2$ nanoparticles*

Nanoparticles are particles between 1 and 100 nanometers in size. A nanoparticle is a small object that behaves as a whole unit in terms of its transport and properties. They are currently the most sought after and studied field of science, and have become the materials of choice in various nanomedicine applications. In this study, $TiO_2$ were used as the nanoparticles and deionize water (DIW) were chosen as the base fluid. $TiO_2$ nanoparticles were produced by a sol-gel process (Mahbubul, 2017). Titanium iso-propoxide (TIP) was used as starting precursor for preparing $TiO_2$-nanoparticles using the sol-gel method. TIP was added to n-propanol and the mixture was stirred for 5 min using a magnetic stirrer at 500 rpm. After stirring, a mixture of HCl and n-propanol was added at the rate of 1 ml/min (molar ratio of HCl/TIP was 0.2). The mixture was stirred for 30 min. After stirring, a mixture of water and n-propanol was added at a rate of 1 ml/min. The molar ratio of $H_2O$/TIP was 4. Then, the mixture was stirred for 24 h at room temperature and the gel was allowed to dry overnight. Subsequently, the gel was heated at 300 $^0$C for 1 h in a muffle furnace. Thus, ($TiO_2$-300℃) nanoparticles were obtained. $TiO_2$ nanoparticles were synthesized by hydrolysis of titanium (IV)-iso-propoxide with water in n-propanol with HCl as catalyst. Hydrolysis products were thermally treated at 300 0C, 1 h after drying at room temperature (Mahbubul, 2017). The morphologies of $TiO_2$ were characterised by the XRD pattern. X-ray diffraction (XRD) patterns of powder and oriented aggregates of the fraction were recorded using a Rigaku DMAX IIIC diffractometer, with graphite monochromator, slits (DS and 1º, RS 0.30 mm, RSM 0.80 mm), CuKα radiation (1.541871 Å), a Ni filter or a diffracted-beam monochromator, accelerating voltage of 35 kV, beam current of 20 mA, and scan speeds of 2º 2θ/min in the ranges of 5-65º 2θ at Department of Geological Engineering of Cumhuriyet University in Sivas, Turkey. The nanofluids were prepared through two-step method, no surfactants were added during the sample preparation. The $TiO_2$–$H_2O$ nanofluid sample with a nanoparticle fraction of 0.5% ($\varphi$ = 0.5 vol.%) were prepared with ultrasonication (amplitude: 50%, with 2 s ON and 2 s OFF pulses) to study the effect of the duration for ultrasonication on nanoparticles stability within the base fluid (Mahbubul, 2017; Rajesh, 2017). The mixture was shacked and stirred by hand for 10 minutes and then ultrasonicated to break any possible aggregation of nanoparticles in a Probe Sonicator and ultrasonication periods 30, 60, 90, 120, 150, and 180 min. is applied with nanofluids. As a vital operation in the sample preparation, ultrasonication conceivably may have an impact on the total volume and the concentration of nanofluids, since temperature of the

ultrasonicated sample increases with agitation by 10 °C/min, initially (Mahbubul, 2017). To avoid this negative outcome, cold water bath is used for temperature control (Figure 1). Stability of the nanofluids was determined by measuring their zeta potantial values (malvern Zetasizer Nano Z). Zeta potential analyses were conducted for the evaluation of their stability deterioration may be a critical constraint for potential applications of nanofluids. Proper ultrasonication can improve the stability, and possibility of the safe use of nanofluids in different applications.

**Figure 1.a.** Preparation of nanoparticles **b.** cold water bath is used for temperature control.

***Cell Culture***

Cell lines including MCF-7 and MDA-MB-231 cells were maintained in DMEM medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in at 37 °C, 5% $CO_2$ and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

***Cytotoxic effect of $TiO_2$ nanoparticles in MCF-7 and MDA-MB-231 cells***

The in vitro cytotoxicity of the $TiO_2$ against MCF-7 and MDA-MB-231 cell lines was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method (Skehan, 1990). Briefly, cells were trypsinized and plated into 96-well plates (Corning, USA) in 0.1 mL of complete culture medium at a density of $1 \times 10^5$ cells per well and allowed to attach for 24 h. 1 µL of test substance at concentrations ranging between 0.1-30 µM were added into each well containing the cells. Test substance was diluted with sterilized water into the desired concentrations from the stock. The plates were incubated at 37°C with an internal atmosphere of 5% $CO_2$. After 24h, 48h and 72 h incubation, with different concentrations of compounds, MTT (5 mg/ml dissolved in PBS) 10 µl/well was added directly to all the wells and incubated for 2 hours at 37°C. The supernatant was carefully removed from each well and 100 mL of DMSO was added to each well to dissolve the formazan crystals. After mixing with a mechanical plate mixer for 15min, the absorbance of plates were recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were parallel tested in triplicate and were performed at least 3 times; control samples were run with 1% sterilized water.

## 3. Results and Discussion

XRD in figure 2 illustrated that the as-prepared $TiO_2$ sample was in the anatase phase. XRD spectra indicated the presence of the main peaks at 2θ values of $25.42^0$ (101), $37.89^0$, $48.12^0$, $55.16^0$, $62.79^0$ that are typical of the anatase phase of $TiO_2$ (Vlazan, 2015) Zeta potential is defined as the electrical potential existing between the nanoparticle surface and the base fluid, and the zeta potential absolute value is related to the nanoparticle stability. If the measured zeta potential absolute value is greater than 25 mV, it can be said that the produced nanofluid is stable. The zeta potential values of the nanofluids produced in this study were measured with the Malvern Zetasizer Nano Z. Results showed the positive impact of ultrasonication on nanofluid dispersion properties up to some extent. As can be seen in Table 1, ultrasonication for 150 min was the optimum period yielding highest stability. Ultrasonication longer than 150 min resulted in re-agglomeration of nanoparticles. The nanofluid maintained its stability for

 

days. The zeta potential is directly related to stability period of nanofluids; higher the absolute value of zeta potential, higher the stability period. That is, the produced nanofluid is quite. When the change in measured potential values is analyzed over time, zeta potential values remain stable for 5 days (Table 2). We have successfully synthesized and prepared stable $TiO_2$ nanoparticle, environmentally friendly, cost effective, and rapid method for synthesis of $TiO_2$ nanoparticles.

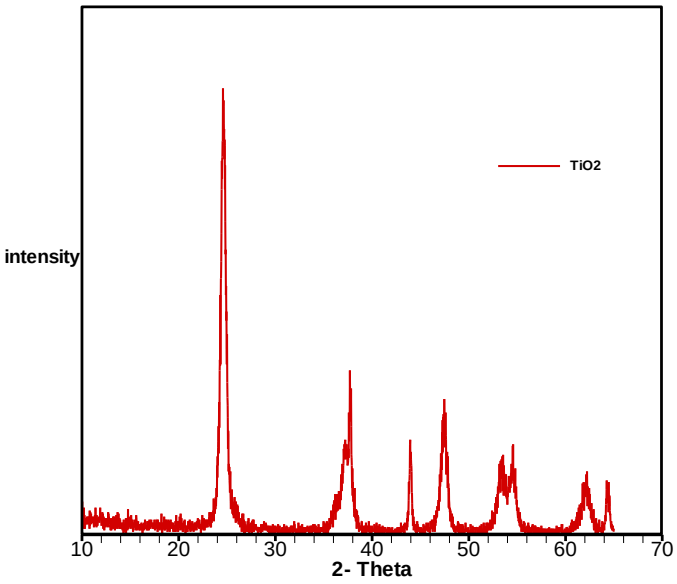


**Figure 2.** XRD pattern of $TiO_2$

**Table 1.** Zeta potential change with ultrasonication time

| Ultrasonication time (minutes) | 0.5 %$TiO_2$ |
|---|---|
| 0 | 36.8 |
| 30 | 37,1 |
| 60 | 32,9 |
| 90 | 33,2 |
| 120 | 33,5 |
| 150 | 32,4 |

**Table 2.** Zeta potential change with time.

| Measuring time (day) | 0.5 % $TiO_2$ |
|---|---|
| 1 | 34,5 |
| 2 | 34,8 |
| 3 | 37,4 |
| 4 | 34,8 |
| 5 | 35,6 |

Titanium dioxide serves as a photocatalyst against air and water pollution, and is widely used in paints, pigments, cosmetics and skin care products. It is an active ingredient in sunscreens, absorbs ultraviolet (UV) light and effectively protects skin against harmful UV light (Fujishima, 2000; Chen, 2007). $TiO_2$ has been classified as a biologically inert substance when studied on humans and animals (Chen, 2008;

Gurr, 2005).
$TiO_2$ nanoparticles have been shown to induce cytotoxicity in organ specific cell lines. These effects have been attributed to reactive oxygen species (ROS) generation, pulmonary inflammation, oxidative stress, and fibrosis (Borm, 2006; Nel, 2006; Singh, 2007; Jin , 2008; Shukla, 2011; Pfuhler, 2013). The DNA damaging potential of the $TiO_2$ nanoparticles and their correlation with ROS and oxidative stress is also well documented (Shukla, 2013; Shukla, 2011). Recently, Prasad et al. (2013) demonstrated the genotoxic potential of $TiO_2$ nanoparticles in human BEAS-2B lung cells (Prasad, 2013). However, micronucleus formation induced by TiO2 nanoparticles were dependent on serum concentration and the type of cell culture media used to grow the BEAS-2B cells. It is also known that the genotoxicity induced by $TiO_2$ nanoparticles in cells relies on a variety of cell cycle checkpoints and DNA repair pathways (Prasad, 2013; Arenz, 2006; Cui, 2012). Genotoxic studies of $TiO_2$ nanoparticles revealed dose-dependent DNA damage, chromosomal aberrations and errors in chromosome segregation (Wang, 2007), and formation of sister chromatic exchanges (Lu, 1998).
The cytotoxicity was estimated by MTT assay against both cell lines, since MTT assay can accurately measure metabolic activity of living cells via MTT reaction with mitochondrial dehydrogenases (M. B. Hansen, 1989) Figure 3 shows changes in cell inhibition for 24, 48 and 72 hours versus increasing concentrations of MCF-7 and MDA-MB-231 cell lines. x-axis shows cell types and varying time points, while the y-axis shows the inhibition rates of cancer cells relative to the control. As you can see in Fig. 3 in parallel with the increase in $TiO_2$ concentration, there has been an increase also in the mortality rates of MCF-7 and MDA-MB-231 breast cancer cells. The low $IC_{50}$ value " concentration of complex required for killing 50% of breast cancer cells" indicates that high cytotoxicity. Despite the time and dose dependent increase in the cytotoxicity of $TiO_2$ in MDA-MB-231 cells, $IC_{50}$ values was not observed for 24, 48 and 72 hours in the working range. Dose dependently increased cytotoxicity was also observed in MCF-7 cells and $IC_{50}$ value was determined 30 µM for 72 h. Our data showed that, $TiO_2$ nanoparticles have cytotoxic effects on MCF-7 and MDA-MB-231 the cells. This effect is more pronounced on the MCF-7 cells which have $IC_{50}$ value for 72 hour.

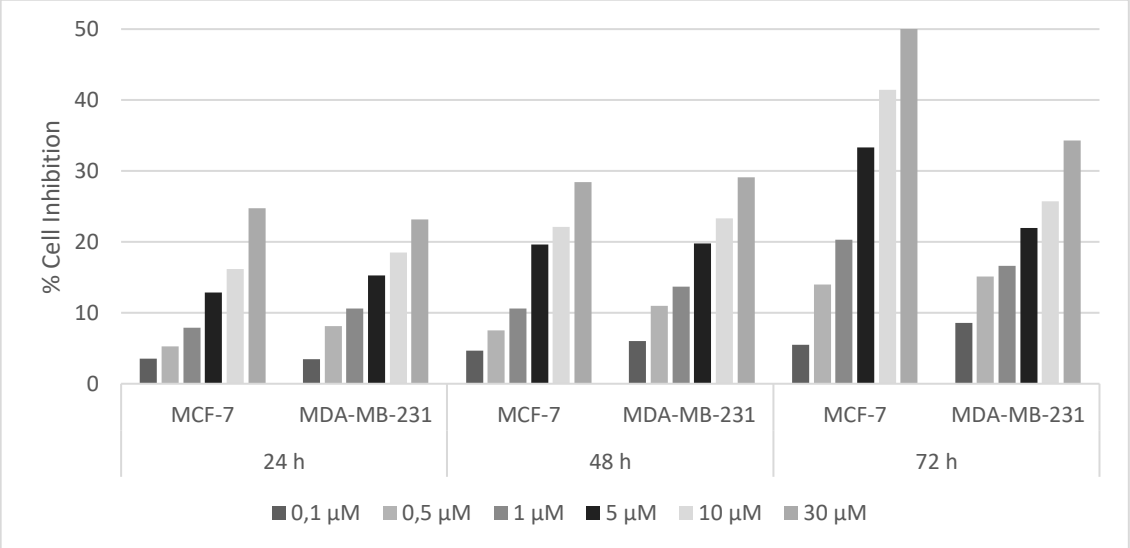


Figure 3: Cytotoxic effects of $TiO_2$ on MCF-7 and MDA-MB-231 cells

## 4. Conclusions

This study demonstrates the possibility of using $TiO_2$ nanoparticles to inhibit the growth of breast cancer (MDA-MB–231 and MCF–7) and their cytotoxicity for potential therapeutic treatments and offers a new method to develop molecule for cancer therapy. Finally, the present results showed that $TiO_2$ nanoparticles might be a potential alternative agent for human breast cancer therapy.

**Referances**

Aitken RJ et al. (2004). Seeds of concern. *Nature* 432: 48–52.

Akimov YA et al. (2010). Resonant and nonresonant plasmonic nanoparticle enhancement for thin-film silicon solar cells. *Nanotechnology* 21:235201.

Arenz A et al. (2006). Gene expression modulation in A549 human lung cells in response to combustion-generated nano-sized particles. *Ann N Y Acad Sci* 170–183.

Bondarenko O et al. (2013). Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: A critical review. *Arch Toxicol* 87:1181–200.

Borm PJ. et al. (2006). The potential risks of nanomaterials: a review carried out for ECETOC. *Part Fibre Toxicol* 3:11.

Brumfiel G. et al. (2006). Consumer products leap aboard the nano bandwagon. *Nature*; 440:262. DOI: 10.1038/440262b

Chen, X et al. (2007). Titanium dioxide nanomaterials: Synthesis, properties, modification and applications. *Chem. Rev.* 107, 2891–2959

Chen E et al. (2008). Ultrafine titanium dioxide nanoparticles induce cell death in human bronchial epithelial cells. *J. Exp. Nanosci.* 3, 171–183.

Cui Y et al. (2012). Gene expression in liver injury caused by long-term exposure to titanium dioxide nanoparticles in mice. *Toxicol Sci* 128:171–185.

Fujishima A et al. (2000). Titanium dioxide photocatalysis. *J. Photochem. Photobiol. C* 1, 1–21.

Griffitt RJ et al. (2007). Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (Danio rerio). *Environ Sci Technol*; 41:8178–86.

Gurr, J.R et al. (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology.* 213, 66–73.

Hoet PH et al. (2004). Nanoparticles – known and unknown health risks. J *Nanobiotechnol* 2:12.

Hoet PH et al. (2004). Health impact of nanomaterials? *Nat Biotechnol* 22:19.

Jin C et al. (2008). Paclitaxel-loaded poly(D,L-lactide-co-glycolide) nanoparticles for radiotherapy in hypoxic human tumor cells in vitro. *Cancer Biol Therapy* 7:729–734.

Kansara K1 et al. (2015). TiO2 nanoparticles induce DNA double strand breaks and cell cycle arrest in human alveolar cells. *Environ Mol Mutagen*. 56(2):204-17

Kumar A et al. (2011). Engineered ZnO and $TiO_2$ nanoparticles induce oxidative stress and DNA damage leading to reduced viability of Escherichia coli. *Free Radic Biol Med* 51:1872–1881.

Kumar A, et al. (2013). Nano-safety, Standardization and Certification. In Manual on Critical Issues in Nanotechnology R&D Management, *An Asia-Pacific Perspective* 1–40.

Lu et al. (1998) "Induction of sister chromatid exchanges and micronuclei by titanium dioxide in Chinese hamster ovary-K1 cells," *Mutation Research*, vol. 414, no. 1–3, pp.15–20.

Mahbubul I.M et al. (2017). "Optimization of ultrasonication period for better dispersion and stability of TiO2–water nanofluid" *Ultrasonics Sonochemistry*, 37, 360–367.

M. B. Hansen et al. (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Meth.*119, 203–210.

Mossesson Y et al. (2008). Detailed endocytosis: An emerging feature of cancer. *Nat Rev Cancer* 8: 835-850.

Nel A. et al. (2006). Toxic potential of materials at the nanolevel. *Science* 311:622–627.

Nohynek GJ et al. (2008). Nanotechnology, cosmetics and the skin: Is there a health risk? *Skin Pharmacol Physiol* 21: 136–149.

Pfuhler S. et al. (2013). Genotoxicity of nanomaterials: Rrefining strategies and tests for hazard identification. *Environ Mol Mutagen* 54:229–239.

Prasad RY et al. (2013). Titanium dioxide nanoparticles activate the ATM-Chk2 DNA damage response in human dermal fibroblasts. *Nanotoxicology* 7:1111–1119.

Rajesh C et al. (2017). "Stability analysis of $Al2O_3$/water nanofluids", *Journal of Experimental Nanoscience*,1-12.

Rasmussen, J.W. et al. (2010). Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Exp. Opin. Drug Deliv.* 7, 1063–1077. doi: 10.1517/17425247.2010.502560.

Schilling K et al. (2010). Human safety review of 'nano' titanium dioxide and zinc oxide. *Photochem Photobiol Sci* 9: 495-509.

Siegel, R. et al. (2016). Cancer statistics, 2016. CA: a cancer journal for clinicians, 66(1), 7-30. doi: 10.3322/caac.21332. Epub 2016 Jan 7

Singh P et al. (2007). Bio-distribution, toxicity and pathology of cowpea mosaic virus nanoparticles in vivo. *J Control Release* 120:41–50.

Shukla RK et al. (2011). ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicol In Vitro* 25:231–241.

Shukla RK et al. (2011). Titanium dioxide nanoparticles induce oxidative stress-mediated apoptosis in human keratinocyte cells. *J Biomed Nanotechnol* 7:100–101.

Shukla RK et al. (2013). $TiO_2$ nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. *Nanotoxicology* 7:48–60.

Shukla RK et al. (2014). Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. *Nanomedicine (Lond)* 9:1423–1434.

Skehan P et al. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.*82(13):1107-1112.

Stewart, B et al. (2014); ISBN 978-92-832-0429-9; International Agency for Research on Cancer World Health Organization: Lyon, France, World Cancer Report 2014. Available online: http://www.iarc.fr/en/publications/books/wcr/wcr-order.php (accessed on 24 March 2015).

Takaki K et al. (2013). Induction of apoptosis associated with chromosomal DNA fragmentation and caspase-3 activation in leukemia L1210 cells by $TiO_2$ nanoparticles. *J Biosci Bioeng* 13: 1389-1723.

Thurn KT et al. (2011). Endocytosis of tianium dioxide nanoparticles in prostate cancer PC-3M cells. *Nanomedicine* 7: 123-130.

Tomasina J et al. (2013). Rapid and soft formulation of folate-functionalized nanoparticles for the targeted delivery of tripentone in ovarian carcinoma. *Int J Pharm* 13.

Vlazan P et al. (2015). Structural and electrical properties of $TiO_2$/ZnO core-shell nanoparticles synthesized by hydrothermal method. *J Mater Characterization.* 101:153-158.

Wang et al. (2007) "Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration," *Toxicology Letters*, vol. 168, no. 2, pp. 176–185.