Cytotoxicity Studies of TiO_2/ZnO Nanocomposites on Cervical Cancer Cells

Ayca Tas[1*], Nese Keklikcioglu Cakmak[2], Yavuz Silig[3]

[1]*Department of Nutrition and Dietetics, Faculty of Health Sciences, Sivas Cumhuriyet University, 58140, Sivas, Turkey*

[2]*Department of Chemical Engineering, Faculty of Engineering, Sivas Cumhuriyet University, 58140, Sivas, Turkey*

[3]*Department of Biochemistry, Faculty of Medicine, Sivas Cumhuriyet University, 58140, Sivas, Turkey*

Abstract

Objective: Cervical cancer (CC) is one of the leading causes of cancer-related deaths among women worldwide.Human papillomavirus (HPV) is the most important element in this disease.The aim of this study is to prepare TiO_2/ZnO nanocomposite (NC), titanium dioxide (TiO_2) and zinc oxide (ZnO)nanoparticles (NPs) to determine the anticancer activity on human CC cell line (HeLa) and healthy mouse fibroblast cell line (L-929).

Materials&Methods: ZnO, TiO_2 NPs and NC were prepared by a solution combustion synthesis method. The samples were characterized by ultraviolet–visible spectroscopy. Stability analysis was performed with zeta potential. The synthesized NC and NPs were permormed to the HeLa and L-929 cell lines and anticancer activity of these NC and NPs were determined by using MTT method. The HeLa and L-929 cells were treated with different concentrations of these NC and NPs (0,5-100 μg/ml) for 24, 48 and 72 hours. The spectrophotometric readings at 570 nm were recorded and analysed with *Graphpad Prism7*.

Results: NC and NPs were successfully synthesized**.** The effects of these NC and NPs on the HeLa and L-929 cells were compared with the control group and IC_{50} values were determined for 24, 48 and 72 hours. Then we compared the effects of these molecules on the L-929 cell line with the HeLa cell line and founded more active is on HeLa cells.

Conclusion: There are many drugs used in CC treatment. However, undesirable toxicity and drug resistance of these drugs negatively affect treatment.We have synthesized NC and NPs in order to formulate basis of a new drug in this study and have identified anti-cancer activity.As a result, we found that NC and NPs anti-cancer activity was higher in HeLa cells than in L-929.

Key Words: Cervical cancer, HeLa, L-929, TiO_2, ZnO

I. Introduction

Cervical cancer is one of the common cancers in the reproductive age women worldwide [1].This type of cancer is the most common cause of any cancer-related deaths in developing countries, and their number is ten fold higher than in developed countries [2].It has been shown that persistent infection with CC, high-risk human papillomavirus (HPV) types may cause development of this cancer [3].HPV is usually a sexually transmitted infection during sexual activity [4].High-risk rate HPV have been detected in nearly all CCs, and HPV onco-proteins must be expressed to protect the cancer phenotype.The International Agency for Research on Cancer has identified 12 HPV types carcinogenic in humans [5].Chemotherapy, surgery and radiotherapy treatment metods are available for CC treatment. However, none of these methods is very effective in preventing CCs [6].Antitumor agents such as doxorubicin, docetaxel, cisplatin and methotrexate are chemotherapy treatment methods that are commonly used in cancer therapy.However, there are serious side effects, especially due to

nonspecific drug distribution and rapid excretion from circulation [7,8].In addition, the anticancer effects of current therapies are restricted by a high degree of cancer clonal heterogeneity and development of drug resistance [9].Recently, nanotechnology has attracted attention with various potential applications.Nanotechnological methods offer great opportunity to improve drug solubility and stability, increase drug half life in plasma, minimize target effects, concentrate drugs in the target area and increase cytotoxicity in cancer cells [10,11].

In the age of technology, the development of NPs has become one of the basic needs. These nanoparticles have the potential to be used for a wide variety of applications, so that improvements in the quality of the NPs are being investigated, and their use in the field of further application is under research. In the studies with NPs, especially in the medicine and pharmaceutical industry, applications have gained a great speed over the last decade. Semiconductor ZnO and TiO_2 are commonly used in many fields due to their functional properties. ZnO is a semiconductor material that has gained considerable scientific interest due to its applications in a wide range of fields such as biomedical, optical, electronic and optoelectronics. ZnO NPs offer the advantages of high colloidal dispersibility in water, environmental friendly and biocompatibility advantages for use in biomedical applications where nanomaterials prepared using toxic chemicals are not suitable [12]. Owing to the anti-bacterial and disinfecting properties of ZnO nanoparticles, it is also used in the production of different kinds of medicine like dermatological substances for curing inflammation and itching. In earlier ages, epilepsy and diarrhoea were treated using ZnO [13]. TiO_2 has drawn much attention due to photocatalytic activity, optical and electronic properties, non-toxicity, chemical stability, and and low cost [14]. Recently, unlike single-phase NPs, the use of NCs has shown a significant improvement in opportunities to produce new products in areas such as antibacterial, tissue engineering (TE), cancer treatment, drug delivery, medical imaging, dental practices. NCs are a class of materials characterized by superior properties compared to macro and micro composites [15].

II. Materials and methods

2.1 Chemicals

Zinc nitrate hexahydrate, ascorbic acid, titanium isopropoxide and glycine were used as the precursors for the preparation of NCs. Distilled water was used as the solvent all of the experimental stages. All the chemicals were used in their original state and were not subjected to additional purification steps. No surfactants or additives have been used.

2.2 Preparation of TiO_2 *and* ZnO *NPs*

In this study, ZnO and TiO_2 NPs were prepared by a combustion synthesis process. In a typical synthesis step to prepare zinc oxide NPs, zinc nitrate hexahydrate and ascorbic acid were dissolved in distilled water at 1:0.3 molar ratios and heated on a hot plate at 300°C until a brown precipitate formed. The precipitate was crushed finely and calcined at 400°C [16]. The resulting powder was white and consisted of ZnO NPs. TiO_2 NPs were prepared using a similar process using titanium isopropoxide and glycine as the precursors [17].

2.3. Preparation of the TiO_2*/ZnO NCs*

A suspension with water was prepared in a 1:1 weight ratio from the previously prepared ZnO and TiO_2 particles. To prepare a very good suspension, the solution was sonicated for 1 hour with the probe sonicator. Afterwards, solution was heated at 100 ° C to obtain a dry powder, which is the composite of the samples.

2.4 Characterization of TiO_2*/ZnO NCs,* TiO_2 *and ZnO NPs*

All samples were ultrasonicated 60 minutes to break any possible aggregation of nanoparticles in a Probe Sonicator(Sonics & materials INC, USA). Stability of the samples was determined by measuring their zeta potantial values (Malvern Zetasizer Nano Z). The UV–Vis optical absorption analysis made by UV-vis analysis (UV-1280, Shimadzu, Japan).

2.5 Cell culture and Treatment of HeLa and L-929 cells with TiO_2*/ZnO nanocomposite,* TiO_2*and ZnOnanoparticles*

HeLa and L-929 cell lines were cultured in fluids in DMEM medium containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). This cells were grown at 37 °C in a 5% CO_2 incubator. For each cell line, 70-80% cell growth was then typsinized in culture fluxes and the cells were plated on 96-well plates. The in vitro cytotoxicity measurement of TiO_2/ZnO NCs, TiO_2 and ZnO NPson HeLa and L-929 cell lines was performed by Skehan's MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric

assay method [18]. The trypsinized cells were plated in 0.1 mL 96 well plates (Corning, USA) at a density of $1x10^5$ cells per well and allowed to incubate for 24 hours. Which is in the range of 0,5-100µg/ml, 1µl of TiO_2/ZnO NCs, TiO_2 and ZnO NPs was added to the cells in each well. Plates were incubated at 37 °C in a 5% CO_2 incubator.After 24, 48 and 72 hours incubation with different concentrations of compound, MTT (5 mg/ml dissolved in PBS) was added per 10µl wells and incubated for 2 hours at 37 °C. The supernatant in each well was then carefully aspirated. 100µl of DMSO was added to each well to dissolve Formazan crystals. The absorbance of plates were recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were tested in parallel in three replicates.

III. Results and Discussion

3.1 Stability of TiO_2/ZnO NCs, TiO_2 and ZnO NPs suspensions

In recent years, there has been a steadily growing interest in using nanosystems in different biomedical applications such as targeted drug delivery, hyperthermia, photoablation therapy, bioimaging and biosensors [19,20]. TiO_2 is a material that is highly discoursed and widely studied. TiO_2 has many unique features that make it an attractive material in the biomedical industry. Until recently, there was not much work with ZnO, but the interest in ZnO over the last few years has increased considerably due to the use of biomedical applications. These two NPs and their composite, which attracted considerable attention in the medical field, were studied in this study. In characterization analysis, zeta potential analysis of the prepared samples, all of the solutions were slightly higher than 32 mV, all samples were moderately stable [21]. The UV Vis optical absorption analysis of solutions is represented in Figure 1. Fig. 1 showed the presence of a broad UV–Vis optical absorption band at 375 nm, which indicated the formation of ZnO NPs. From the UV/Vis spectra, the TiO_2 nanoparticle can absorb most light with wavelengths less than 400 nm. The results are in accordance with the literature [22,23].

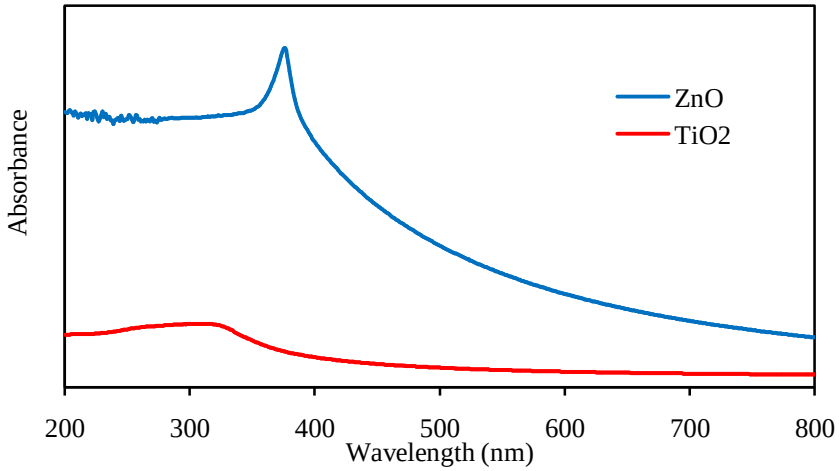

Figure 1. UV-VIS spectrum of TiO_2 and ZnO NPsnanoparticles in water

3.2 Cytotoxic activity of TiO_2/ZnO NCs, TiO_2 and ZnO NPs on HeLa and L-929 cell lines

As nanotechnological methods progress, NPs, nanotechnology and biomedical common studies promise new prospects in cancer treatment [24-26].The idea that nanometers may cause autophagy in cancer cells, especially in the studies on NPs, has attracted attention since it may be a new and useful approach to treatment cancer [27-29]. Also, NPs can be modified to minimize unwanted systemic side effects, increase bioavailability, promote delivery to the targeted site, and increase tumor penetration and cellular uptake [30]. Certain sized NPs can reach and collect the tumor site by passive targeting strategy, thereby reducing the side effects of drugs and improving treatment efficacy [31]. In particular, studies in the field of nanotechnology can change many aspects of CC diagnosis and treatment [32]. Nanotechnology can help overcome the barrier for drug delivery and can achieve higher efficacy in CC

treatment [33]. Several toxicity studies have shown that the toxicity of NPs is significantly related to the unique physicochemical properties of NPs and different mechanisms for cancer cells [34]. In this study, we determined the cytotoxic effects of 12 different doses (0.5-100 µg /mL) of each of TiO_2/ZnO NCs, TiO_2 and ZnO NPs to HeLa and L-929 cells by MTT metod (Fig 2. and Fig 3.) Time-dependent cell viability was determined after 24, 48 and 72 hours incubation. Compared to the control group, HeLa cells treated with TiO_2/ZnO NCs, TiO_2 and ZnO NPs showed a marked reduction after 24 hours, 48 hours and 72 hours of incubation.Among these three molecules, TiO_2/ZnO NCs was the most active on HeLa cells.The TiO_2/ZnO, ZnO and TiO_2 nanoparticles on HeLa cells showed the highest activity after 72 hours of incubation. In addition, 72 hours post IC_{50} values of TiO_2/ZnO, ZnO and TiO_2 were 2,19±0,34µg/ ml, 34,71±2,91µg / ml and 8,06±0,38 µg/ml, respectively (Table 1). In addition, we determined the cytotoxicity of TiO_2/ZnO NCs, TiO_2 and ZnO NPs after incubation in L-929 cells for 24, 48 and 72 hours.We have found that these NPs are more active in the L-929 cell line than in the control group.IC_{50} values of TiO_2/ZnO NCs, ZnO and TiO_2 NPs after 72 h incubation were 18,47±1,11µg/ml, 54,12±1,17µg /ml and 16,51±1,31µg /ml in L-929 cells, respectively(Table 1).However, in L-929 cells, we found that TiO_2 NPs were more active than other molecules.When we compared HeLa cells with L-929 cells, TiO_2/ZnO NCs, ZnO and TiO_2 NPs were more active in Hela cells.

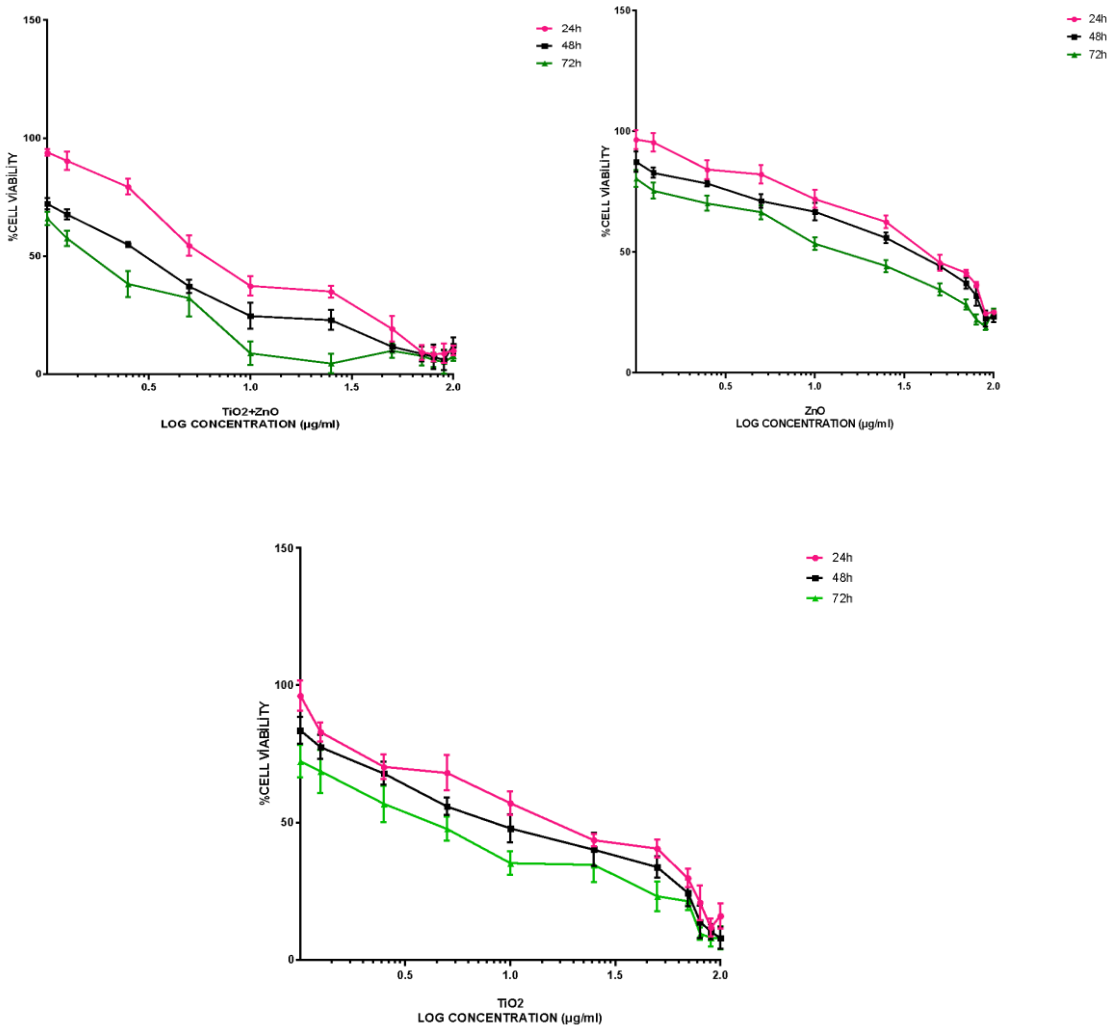

Figure 2. Anti-cancer activity ofTiO_2/ZnO nanocomposite, ZnOand TiO_2 nanoparticles on HeLa cell lines

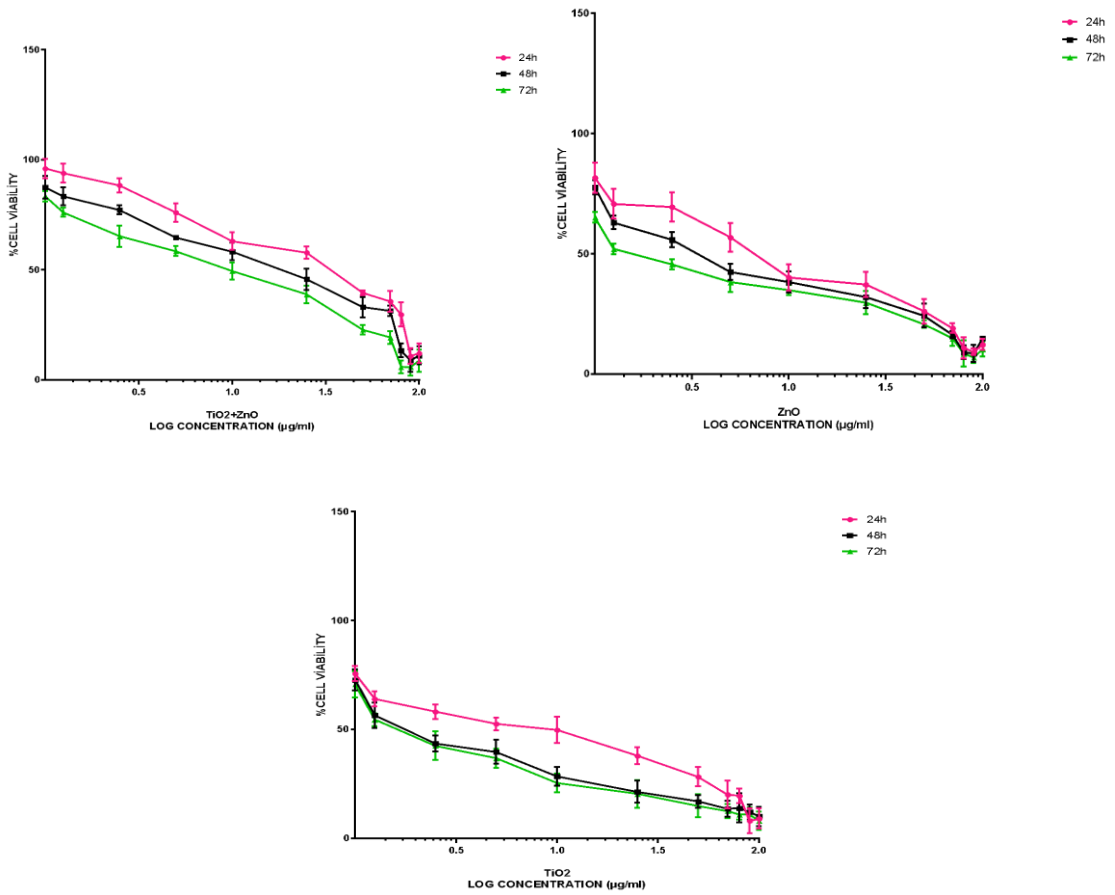

Figure 3. Anti-cancer activity ofTiO_2/ZnO nanocomposite, ZnOand TiO_2 nanoparticles on L-929 cell lines

Table 1. Comparison of IC_{50} values between TiO_2/ZnO NCs, ZnOand TiO_2 NPs on HeLa and L-929 cells after 24 h, 48 h and 72 h of incubation.

	HeLa			**L-929**		
	IC_{50} (µg/ml±SD*)					
NC and NPs	24h	48h	72h	24h	48h	72h
TiO_2/ZnO	3,64±0,30	2,52±0,18	2,19±0,34	25,74±1,08	21,77±1,06	18,47±1,11
ZnO	51,50±2,08	41,61±0,83	34,71±2,91	68,81±2,12	62,55±0,25	54,12±1,17
TiO_2	16,63±1,41	15,09±0,27	8,06±0,38	20,83±0,55	17,88±0,66	16,51±1,31

* Mean standard deviation values of IC_{50} obtained from three independent experimental repetitions after 24 h, 48 h and 72 h incubation for HeLa and L-929 cell lines.

Furthermore, in this study we evaluated cell morphologies after 24 h incubation after applying 50µg/ml TiO_2/ZnO NCs, TiO_2 and ZnO NPs to Hela and L-929 cells. After treatment with HeLa and L-929 cells, we were visualized with a 20X magnification on a microscope (Figure 4).As shown in Figure 4, TiO_2/ZnO NCs

have been found to be more active on HeLa cells over 24 hours compared to control and other nanoparticles (TiO2 and ZnO NPs).In addition, TiO_2 NPs on L-929 cells were more active morphologically determined than TiO_2/ZnO NCs.However, when we compared HeLa and L-929 cells, we observed that the TiO_2/ZnO NCs further disrupted the morphology of the HeLa cell. In addition, we found that the TiO_2 nanoparticle acts more than ZnOon both cell lines. As a result, TiO_2 NPs were more toxic in cancer cells when they produced a synergistic effect with ZnO NPs.In addition, these NPs tend to aggregate in aqueous media and cause undesirable problems in biological systems. One of the most common methods used to prevent aggregation is to cover them with a hydrophilic polymer [35].To overcome this problem, the well-known polymer is PEG. By plating the surface of NPs with PEG, the biocompatibility of NPs will be increased.In addition, PEGylated NPs can escape from the reticulo-endothelial system (RES) and the residence time in the biological system increases [36].

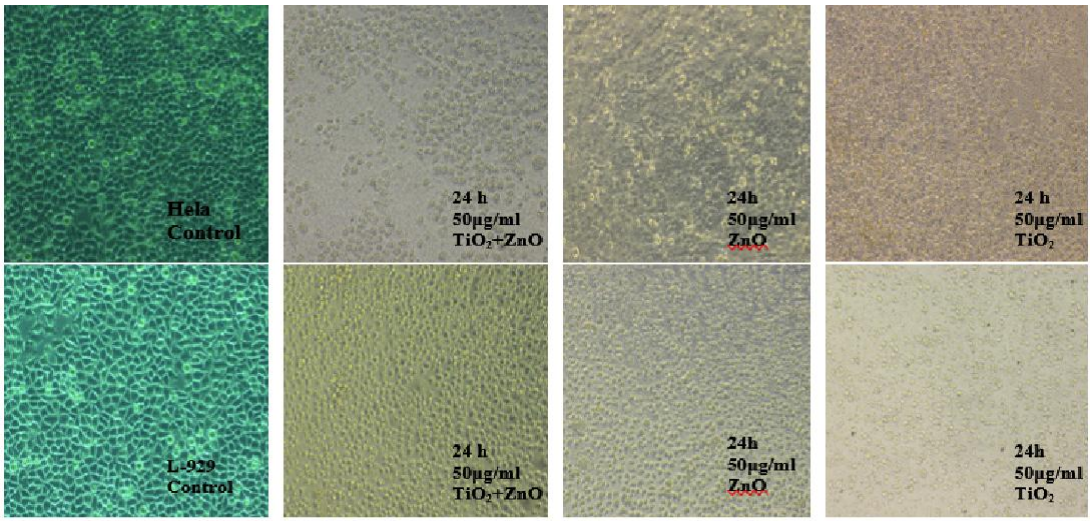

Figure 4. Morphological changes of HeLa and L-929 cells after 24 hour of incubation with concentrations (50 µg/ml) of TiO_2/ZnO NCs, ZnOand TiO_2 NPs the results presented are from that were carried out and photographed microscopically

IV. Conclusions

Nanomaterials are currently affecting most of our daily lives. Of these, inorganic NPs consist of those who do not directly affect our interaction with the world around us indirectly. Although inorganic NPs do not directly affect, it affects us indirectly because of our interaction with our environment. Inorganic NCs are obtained by preparation of doping or substitution of different concentrations of synthesized NPs alone. These new particles retain both the properties of each particle and have the new properties. This feature enabled the use of two separate NPs to combine their properties to enhance their properties, thus making it of great use in the medical field. Synergistic application of more than one drug is common in the medical field when combined drugs do not produce undesirable effects. Because of this in this study, NCs of ZnO and TiO_2 NPs were made and tested for anticancer properties. According to our results, TiO_2 NPs were determined to be more active in cancer cells than in healthy cells when composite was formed with ZnO. However, it is undesirable for TiO_2 to be toxic alone with more active in healthy cells. To reduce the TiO_2 toxicity can be pegylated with poly ethylene glycol (PEG).

ACKNOWLEDGEMENTS

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

References

[1.] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. *IARC monographs on the evaluation of carcinogenic risks to humans*, 2010, 94: v.

[2.] Aggarwal, P., Batra, S., Gandhi, G., & Zutshi, V. (2011). Can visual inspection with acetic acid under magnification substitute colposcopy in detecting cervical intraepithelial neoplasia in low-resource settings. *Archives of gynecology and obstetrics*, *284*(2), 397-403.

[3.] Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A., Shah, K. V., ... & Muñoz, N. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*, *189*(1), 12-19.

[4.] Schiffman, M., Castle, P. E., Jeronimo, J., Rodriguez, A. C., & Wacholder, S. (2007). Human papillomavirus and cervical cancer. *The Lancet*, *370*(9590), 890-907.

[5.] IARC (2012). Biological agents. IARC Monogr Eval Carcinog Risks Hum. 100B:1–441.

[6.] Schiller, J. T., Castellsagué, X., Villa, L. L., & Hildesheim, A. (2008). An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. *Vaccine*, *26*, K53-K61.

[7.] Markman, J. L., Rekechenetskiy, A., Holler, E., & Ljubimova, J. Y. (2013). Nanomedicine therapeutic approaches to overcome cancer drug resistance. *Advanced drug delivery reviews*, *65*(13-14), 1866-1879.

[8.] Mondal, J.; Pangrahi, A. K.; Khuda-Bukhsh, A. R. (2014).ConventionalChemotherapy: Problems and Scope for Combined Therapies with Certain Herbal Products and Dietary Supplements. Austin Journal of Molecular and Cellular Biology, 1, 1−10.

[9.] Li, F., Zhao, C., & Wang, L. (2014). Molecular- targeted agents combination therapy for cancer: developments and potentials. *International journal of cancer*, *134*(6), 1257-1269.

[10.] Zhang, X. F., & Gurunathan, S. (2016). Combination of salinomycin and silver nanoparticles enhances apoptosis and autophagy in human ovarian cancer cells: an effective anticancer therapy. *International journal of nanomedicine*, *11*, 3655.

[11.] Harris TJ, von Maltzahn G, Bhatia SN. Multifunctional nanoparticles for cancer therapy. In: Amiji MM, editor. Nanotechnology for Cancer Therapy. Boca Raton (FL): CRC Press; 2006:59–75.

[12.] Zhao, C., Zhang, X., & Zheng, Y. (2018). Biosynthesis of polyphenols functionalized ZnO nanoparticles: Characterization and their effect on human pancreatic cancer cell line. Journal of Photochemistry and Photobiology B: Biology, 183, 142-146.

[13.] Carp, O., Huisman, C. L., & Reller, A. (2004). Photoinduced reactivity of titanium dioxide. Progress in solid state chemistry, 32(1-2), 33-177.

[14.] Kolodziejczak-Radzimska, A., & Jesionowski, T. (2014). Zinc oxide-from synthesis to application: a review. Materials, 7(4), 2833-2881.

[15.] Aguilar, Z. (2012). Nanomaterials for medical applications. Newnes.

[16.] Rrajendar, V., Raghu, Y., Rajitha, B., Chakra, C. S., Rao, K. V., & Park, S. H. (2017). Synthesis, characterization, and photocatalytic behaviour of nanocrystalline ZnO, $T\dot{I}O_2$ and $ZnO/T\dot{I}O_2$ nanocomposites. *Journal of ovonic research*, *13*(3).

[17.] Bolukbasi Sahin, S., Keklikcioglu Cakmak, N., Tas, A., Ozmen, E., Cevik, E., Gumus, E., Silig Y.,2018, The Cytotoxic Effects of Titanium Oxide Nanoparticle on MDA-MB–231 and MCF–7 Cells, International Journal of Scientific and Technological Research, 4, 8.

[18.] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., ... & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107-1112.

[19.] McNamara, K., & Tofail, S. A. (2013). Biomedical applications of nanoalloys. In Nanoalloys (pp. 345-371).

[20.] McNamara, K., & Tofail, S. A. (2015). Nanosystems: the use of nanoalloys, metallic, bimetallic, and magnetic nanoparticles in biomedical applications. Physical Chemistry Chemical Physics, 17(42), 27981-27995.

[21.] Zeta Potential of Colloids in Water and Waste Water, ASTM Standard D 4187-82, American Society for Testing and Materials, 1985

[22.] Reinosa, J. J., Leret, P., Álvarez-Docio, C. M., del Campo, A., & Fernández, J. F. (2016). Enhancement of UV absorption behavior in ZnO-TiO2 composites. boletín de la sociedad española de cerámica y vidrio, 55(2), 55-62.

[23.] Rajiv, P., Rajeshwari, S., & Venckatesh, R. (2013). Bio-Fabrication of zinc oxide nanoparticles using leaf extract of Parthenium hysterophorus L. and its size-dependent antifungal activity against plant fungal pathogens. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 112, 384-387.

[24.] Park J, Wrzesinski SH, Stern E, Look M, Criscione J, Ragheb R, Jay SM. (2012). Demento SL, Agawu A, Licona Limon P, Ferrandino AF, Gonzalez D, Habermann A, et al. Combination delivery of TGF-β inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. Nat Mater. 11, 895-905.

[25.] Su Y, Wei X, Peng F, Zhong Y, Lu Y, Su S, Xu T, Lee ST, He Y. (2012).Gold nanoparticles-decorated silicon nanowires as highly efficient near-infrared hyperthermia agents for cancer cells destruction. Nano Lett.12,1845-50.

[26.] Guan M, Zhou Y, Zhu QL, Liu Y, Bei YY, Zhang XN, Zhang Q. (2012).N-trimethyl chitosan nanoparticle-encapsulated lactosyl-norcantharidin for liver cancer therapy with high targeting efficacy. Nanomedicine. 8, 1172-81.

[27.] Adiseshaiah PP, Crist RM, Hook SS, McNeil SE. (2016).Nanomedicine strategies to overcome the pathophysiological barriers of pancreatic cancer. Nat Rev Clin Oncol. 13,750-65.

[28.] Cheng HJ, Wu TH, Chien CT, Tu HW, Cha TS, Lin SY. (2016).Corrosion-activated chemotherapeutic function of nanoparticulate platinum as a cisplatin resistance-overcoming prodrug with limited autophagy induction. Small. 12, 6124-33.

[29.] Zhang X, Yang Y, Liang X, Zeng X, Liu Z, Tao W, Xiao X, Chen H, Huang L, Mei L. (2014). Enhancing therapeutic effects of docetaxel-loaded dendritic copolymer nanoparticles by co-treatment with autophagy inhibitor on breast cancer. Theranostics. 4,1085-95.

[30.] Dawidczyk CM, Kim C, Park JH, Russell LM, Lee KH, Pomper MG, et al. (2014).State-of-the-art in design rules for drug delivery platforms: lessons learned from FDA-approved nanomedicines. J Control Release. 187,133–44.

[31.] Ferrari, M. (2005). Cancer nanotechnology: opportunities and challenges. *Nature reviews cancer*, *5*(3), 161.

[32.] Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. (2007).Nanocarriers as an emerging platform for cancer therapy. Nature nanotechnology. 2(12):751-60.

[33.] Chen, J., Gu, W., Yang, L., Chen, C., Shao, R., Xu, K., & Xu, Z. P. (2015). Nanotechnology in the management of cervical cancer. Reviews in medical virology, 25, 72-83.

[34.] Guo, D., Bi, H., Liu, B., Wu, Q., Wang, D., & Cui, Y. (2013). Reactive oxygen species-induced cytotoxic effects of zinc oxide nanoparticles in rat retinal ganglion cells. Toxicology in Vitro, 27(2), 731-738.

[35.] Mano SS, Kanehira K, Sonezaki S, Taniguchi A. (2012).Effect of polyethylene glycol modification of TiO_2 nanoparticles on cytotoxicity and gene expressions in human cell lines. Int J Mol Sci.13, 3703–3717.

[36.] Devanand Venkatasubbu G, Ramasamy S, Ramakrishnan V, Kumar J. (2013).Folate targeted PEGylated titanium dioxide nanoparticles as a nanocarrier for targeted paclitaxel drug delivery. Adv Powder Technol 24, 947–954.