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Investigation on the effect of nano-TiO_2 synthesized by the hydrothermal method on LNCaP cancer cells

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The nanosized TiO_2 synthesis was performed through the hydrothermal method by using titanium alkoxides. The structural, physical and chemical characteristics of the nanosized TiO_2 were determined via XRD, SEM and particle size analyzer. The synthesized nano-TiO_2 particles were obtained in a completely anatase form. Different concentrations of nano-TiO_2's turned into sol were applied into the LNCaP cells in the cell culture media, and their anti-tumour/proliferation activities were tried to be determined photocatalytically with and without the UV-ray. For this, the numbers of living and dead cells were detected by means of MTT method, and thus, the anticancer characteristic was determined.

Keywords: Nano-TiO_2, hydrothermal method, MTT assay method, LNCaP

INTRODUCTION

TiO_2 nanoparticles are used in a number of fields, such as photocatalyst [1-3], solar cell [4, 5] and gas sensors [6]. Separately, TiO_2 is also used efficiently in the degradation of organic compounds and coloring agents as well as neutralizing/warding off microorganisms. TiO_2 is a semi-conductor with a band-gap energy of 3.2 eV, and it has a far more quantum effect compared to normal substances [7]. In an aqueous medium, reactive oxygen species (ROS) like hydroxyl radicals, superoxide anions and hydrogen peroxide occur thanks to e^-/h^+ gap pairs that occur on TiO_2 nanoparticles under the UV ray [8-10]. These active oxygen species play an efficient role in several chemical reactions as well as annihilating microorganisms due to their high redox activities. It has been reported in the studies conducted recently that TiO_2 could also be applied in the treatment of cancer [11-15]. Within the cancer cells and in the cell membrane [15] have the active oxygen species (ROS) been effective, as well. In such oxidation reactions, the cell toxicity is dependent on the cell stability and the chemical combination (order) on the surface structure of the cell [16].

In this study, the nano-sized TiO_2 was synthesized through the hydrothermal method, and its effect on the LNCaP cells was examined under the effect of the UV-ray, without the presence of the UV-ray.

EXPERIMENTAL

Chemicals and Reagents

Titanyum (IV) isopropoksit (TTIP) (97%, Alfa-Aesar), isopropyl alcohol (99.5%, LabKim), Hydrochloric acid (%37, Merck), Acetic acid (99.5%, J.T.Baker), Newborncalf serum (FCS) and pensilin-streptomycin (Biological Industries), Sodium chloride, Sodium hydroxide, Dimetyl sulfoxide (DMSO) (Merck) were purchased. The water used in the experiment was doubly distilled and deionized.

Berghoff BTR-2000A Model hydrothermal unit interfaced with a temperature controller and timer unit was used for synthesizing nano-sized TiO_2. Malvern Nanoseries Zetasizer was used for particle size analyzer. The crystalline phase of the nano-TiO_2 particles was analyzed by X-ray powder diffraction (XRD) pattern obtained from Rigaku Geigerflex D Max/B diffractometer with Cu Kα radiation (λ = 0.15418 nm) in the region 2θ = 10–90° with a step size of 0.04°. The crystallite size of the anatase particle was calculated from the X-ray diffraction peak, according to the Scherrer's equation. SEM (LEO EVO 40) was used to examine the surface morphology.

Preparation of nano-TiO_2

Titanium (IV) isopropoxide, [$Ti(OPr^i)_4$], was added into i-propanol in the way that it would reach 10% in mass, and thus, a homogeneous mixture was obtained. As the catalyst, the mixture of HCl/HAc was added into the reaction medium in the way that the Acid/$Ti(OPr^i)_4$ rate would reach

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0.05 and 0.07, respectively. In order for the hydrolysis-condensation reaction to start, the prepared water was instilled in the way that the $H_2O/Ti(OPr^i)_4$ rate would be (mol/mol) 3, and then, a homogenous and transparent/ crystalline solution was obtained after the reaction had lasted for approximately 10 minutes more. The sol-solution was first placed into a Teflon cup of 250 mL and then onto the hydrothermal unit. After having been kept at 200 °C for 4 hours, it was taken out of the hydrothermal unit and was cooled at room temperature. The obtained solid TiO_2 particles, after having been separated from the solution medium through a centrifuge, were dried up/dehydrated at 40 °C in the vacuum incubator. Hence, the nano-sized TiO_2 particles were obtained.

Preparation of the sols in the RPMI-1640 medium

The sol of the pure nano-TiO_2 particles which was synthesized through the hydrothermal method was prepared within RPMI-1640 medium. In this process, RPMI-1640 medium was added onto 1 gram of TiO_2 particles in the way that it would make up 1% sol by weight and then was placed into the ultrasonic bath. In order to prepare a solution in 4 different concentrations, a 1% main stock sol was used. The nano-TiO_2 to be tested was dissolved in the medium, and their 1-100 mM concentrations were prepared to be used during the experiment. The stock solutions were kept at the +4 °C throughout the experiment.

Cell Cultures

LNCaP cells were obtained from The Middle East Technical University (METU), the Department of Biology (Ankara). All the cells were fed by f (that which is prepared by adding into it 10% FCS, 100 U/mL penicillin and 0.1mg/mL streptomycin) within the culture flasks of 25 cm^2. The mediums of the cells kept in the carbon dioxide (5% CO_2) incubator, within a humid environment at 37 °C were changed twice a week. When the cells were confluent, they were removed from the flasks by using a trypsin-EDTA solution and were then poured into the plaques with 96 wells to be used in 3-(4,5-dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide (MTT) analyses.

Statistical Analyze

Statistical analysis of the data was performed with SPSS program (16.0). Non-parametric method of analysis was used because of the number of repetitions in the groups are n<20. Due to the number of groups are more than 2, Kruskal-Wallis analysis of variance was used for multiple comparisons. Statistically significant $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Characterization of the nanocomposite

Whether or not the nano-sized pure particles synthesized through the hydrothermal method were of a crystalline structure was determined by using X-ray dust diffractometry. Throughout the analysis, CuK_α ray was applied, and 2θ values were selected as 0-80°, and the scanning rate as 0.04 cm/s. It was determined that the peaks seen in the XRD spectrums (Fig. 1) of the particles had corresponded to the crystalline reflections determined for TiO_2 in the form of anatase crystal (1 0 1), (0 0 4), (2 0 0) and (2 1 1). No reflections of rutile and brookite, which are the other crystalline modifications of TiO_2, were found.

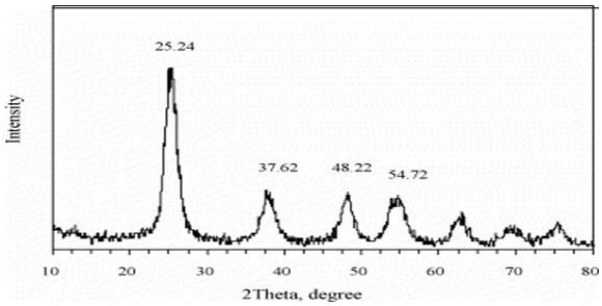

Fig. 1. XRD results for the nano-TiO_2

SEM analysis of anatase TiO_2 particles with nano-size was performed on the surface of the particles, which was covered by carbon. It can be seen from SEM analysis results (Fig. 2) that the particles resemble one another quite a great deal in terms of their structure and that they had a spheric form in general; yet, a total spheric structure failed to take shape among the particles in some areas. The reason for this is that the particles are likely to be exposed to agglomeration due to a thermal treatment, with the result that the sizes of some of the particles may grow, whereas those of some others could become smaller.

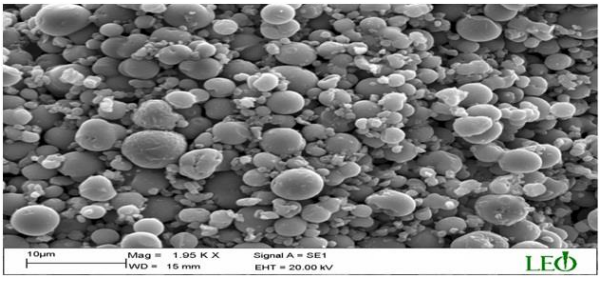

Fig. 2. SEM images for the nano-TiO_2

The volumetric particle-size distributions of the nano-sized TiO_2 particles were determined via Zeta-

sizer. The sol of the synthesized nano-TiO_2 particles, which were prepared in the water, was used for measurements. As the result of the measurements performed for determining the particle-size distribution, the TiO_2 particles were found to be 4.78 nm at a rate of 95.9% (Fig. 3).

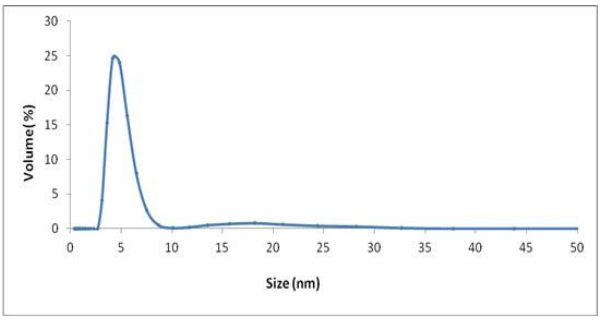

Fig. 3. Particle size distribition results for the nano-TiO_2

Cytotoxicity tests

The synthesized nano-particles were screened for their cytotoxicity against prostate cancers (LNCaP) by using MTT assay method. The pale-yellow tetrazolium salt [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] (MTT), was cleaved by active mitochondria to form a dark blue formazan product, that can be completely solubilized in acidified isopropanol and detected by a microtiter plate reader [17]. The assay provides a simple way to detect living and growing cells without using radioactivity. Briefly, $15x10^3$ prostate cancer cells were plated in triplicate in 96-well flat bottom tissue culture plates, and treated with different concentrations (0.01 mM, 0.1 mM, 1 mM and 10 mM) of agents. The culture plates were exposed to UV light at four different time points (0, 5, 15 and 30 minutes) and then cells were incubated for 24 h at 37°C in 5% CO_2 humidified incubator. MTT (0.005 g/mL in phosphate buffer saline) was added to the cell culture and incubated for 4 h. The formazan crystals formed during the reaction of active mitochondria with MTT, were dissolved in 0.04 N (100 mL) in isopropanol and readings were taken by a microtiter plate reader (Biotek Synergy) using a 570 nm filter. Each data represented an average of 10 measurements.

It was seen as the result of the measurements that there was no mortality in LNCaP cells without the UV-ray; on the contrary, it was observed that this situation brought about proliferation. On the other hand, mortality in LNCaP cells was observed when the UV-ray was applied. As the period of exposure to the UV-ray extended, so did the cell death increase. The best results were observed in the 0.01 mM nano-TiO_2 concentration exposed to the UV-ray for 30 minutes, and almost 65% mortality occurred in the cells (Fig. 4).

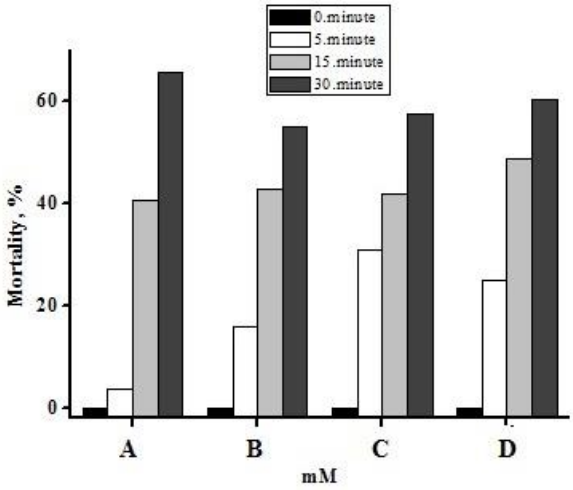

Fig. 4. Results of the mortality (%) for LNCaP cells. (A, B, C and D symbols was represented concentrations respectively 0.01, 0.1, 1 and 10 mM.) Statistically significant $p<0.05$ was considered significant.

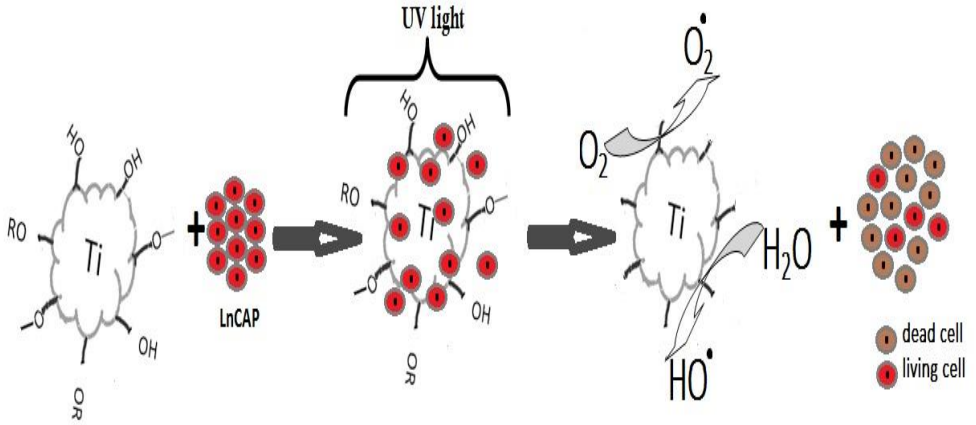

Fig. 5. The formation of active oxygen species at the surface of TiO_2.

The fact that there is a proliferation without the UV-ray, but a decrease in the vitality of the cancer cells with the effect of the UV-ray indicates the fact that active regions are generated at the surface of TiO_2 with the effect of the UV-ray, allowing for the emergence of active oxygen species (ROS) in the medium [18]. The active oxygen species form non-duplicated electron pairs. Hydroxyl radical ($OH^{\cdot}$), along with superoxide radical ($^{\cdot}O_2$) and non-radical H_2O_2 and singlet oxygen ($_1O_2$) constitute an oxidation-reduction reaction [19]. Active oxygen species are also used in cancer drugs, such as procarbazine, doxorubicin, buthionine sulfoximine, motexafin gadolinium and rituximab [20-23]. Active oxygen species (Fig. 5) are also formed at the surface of TiO_2, which lead to the death of cancer cells.

CONCLUSION

In conclusion, in this study, the nano-sized TiO_2 particles were successfully synthesized, and their effect on the LNCaP cell was analyzed. The nano-sized TiO_2 particles synthesized through the hydrothermal method showed a photocatalytic and anti-cancer characteristic on the LNCaP cell. The death of cancer cells was accomplished by means of the active oxygen species that occurred on the surface of TiO_2 via the applied UV-ray. Approximately 65% of mortality took place in 30 minutes. The use of the synthesized nano-TiO_2 could be possible in the treatment of cancer. If the nano -TiO_2's are applied to the cancerous area exposed to the UV-ray, they may cause the cancer cells to diminish or to get destroyed. The X-rays can even be used for starting photocatalytic reactions on the nano-TiO_2 [24]. In order to be able to pull the applied wavelength to the visible area, different studies can be performed by attaching transition metal dopants into the nano-TiO_2 [25].

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REFERENCES

1. M. Asiltürk, F. Sayılkan, S. Erdemoğlu, M. Akarsu, H. Sayılkan, M. Erdemoğlu, E. Arpaç, *J. Hazard. Mater.*, **129**, 164 (2006).
2. K. Nakata, A. Fujishima, *Photochem. Photobiol. C: Photochem. Rev.*, **13(3)**, 16 (2012).
3. T. Fotiou, T. M. Triantis, T. Kaloudis, A. Hiskia, *Chem. Eng. J.*, **261,** 17 (2015).
4. [4] S. J. Kwon, H. B. Im, J. E. Nam, J. K. Kang, T. S. Hwang, K. B. Yi, *Appl.Surf. Sci.*, **320**, 487 (2014).
5. S. J. Lue, Y.L. Wu, Y.L. Tung, C.M. Shih, Y. C. Wang, J. R. Li, *J. Power Sources*, **274**, 1283 (2015).
6. B. Lyson-Sypien, A. Czapla, M. Lubecka, P. Gwizdz, K. Schneider, K. Zakrzewska, K. Michalow, T. Graule, A. Reszka, M. Rekas, A. Lacz, M. Radecka, *Sens. Actuators, B: Chemical*, **175**, 163 (2012).
7. X. Chen, S. S. Mao, *Chem. Rev.*, **107**, 2891 (2007).
8. Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, *Br. J. Cancer*, **70**, 1107 (1994).
9. A. Mills, S. L. Hunte, *J. Photochem. Photobiol. A*, **108,** 1 (1997).
10. G.F. Fu, P.S. Vary, C.T. Lin, *J. Phys. Chem. B*, **109,** 8889 (2005).
11. N.P. Huang, M.H. Xu, C.W. Yuan, R.R. Yu, *J Photochem Photobiol A: Chem.*, **108**, 229 (1997).
12. A.P. Zhang, Y.P. Sun, *World J Gastroenterol.*, **10,** 3191 (2004).
13. J. W. Seo, H. Chung, M.Y. Kim, J. Lee, I.H. Choi, J. Cheon, *Small*, **3**, 850 (2007).
14. P. Thevenot, J. Cho, D. Wavhal, R. B. Timmons, L. Tang, *Nanomed Nanotech Biol Med.*, **4**, 226 (2008).
15. L. Liu, P. Miao, Y. Xu, Z. Tian, Z. Zou, G. Li, *J. Photochem. Photobiol. B: Biol.*, **98,** 207 (2010).
16. D. M. Blake, P. C. Maness, Z. Huang, E. J. Wolfrum, J. Huang, *Sep Purif Methods*, **28**, 1 (1999).
17. Z.K. Genc, S. Tekin, S. Sandal, M. Sekerci, M. Genc, *Res. Chem. Intermed* **41**, 4477 (2015).
18. R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, A. Fujishima, *Cancer Res.*, **52**, 2346 (1992).
19. K.A. Conklin, *Integr. Cancer Ther.*, **3**, 294 (2004).
20. J.M. MateÂs, M. Francisca, J.Â SaÂnchez, *Int J Biochem Cell B*, **32**, 157 (2000).
21. M.F. Renschler, *Eur. J. Cancer*, **40**, 1934 (2004).
22. T. Ozben, *J. Pharm. Sci.*, **96**, 2181 (2007).
23. J. Wang, J. Yi, *Cancer Biol. Ther.*, **7**, 1875 (2008).
24. T. Kazuhisa, O. Yoshihisa, K. Hiroyuki, Y. Hideki, T. Tetsu, F. Akira, M. Jun'ichiro, *Electrochim Acta*, **52**, 6938 (2007).
25. N. Siva, I. Reddy, B. Thirupathi, S. Makram, G. S. Panagiotis, *Appl Catal B-Environ.*, **144**, 333 (2014).

ИЗСЛЕДВАНЕ НА ЕФЕКТА НА НАНОЧАСТИЦИ ОТ TiO_2, СИНТЕЗИРАНИ ПО ХИДРОТЕРМАЛНИЯ МЕТОД ВЪРХУ LnCap-РАКОВИ КЛЕТКИ

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(Резюме)

Извършена е синтеза на наноразмерен TiO_2 по хидротермалния метод от титанови алкоксиди. Структурните, физичните и химичните свойства на наночастиците са определени чрез рентгеноструктурен анализ, сканираща електронна микроскопия и анализ на размера на частиците. Синтезираните TiO_2 – наночастици са изцяло в анатаз – полиморфна модификация. Наночастиците в различни концентрации и приведени в зол-състояние са приложени спрямо LNCaP-ракови клетки в културална среда, а техната анти-туморна и забавяща пролиферацията активност е изпитана фотокаталитично с и без ултравиолетово лъчение. Броят на живите и мъртвите клетки беше установен по MTT-метода, като с това са определени противо-раковите свойства на наночастиците.