ZnO and TiO_2 Nanoparticles Genotoxicity According to their Structural and Morphological Characteristics Used for Medical Purposes

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Defined by their small size, nanomaterials rapidly developed due to their physicochemical properties at nanoscale. Nanoparticles possess a high biological reactivity compared to their bulk size suggesting a high toxicity when the genetic material is involved. Nanogenotoxicity field refers to multidisciplinary sciences relevant for evaluation of genotoxic effect of various nanostructured materials. Due to their widespread use in medical area, zinc oxide and titanium dioxide nanoparticles are receiving researcher's attention. The major objective of this work was to find a relationship between the structure and morphology of ZnO and TiO_2 nanoparticles and their genotoxic potential.

Keywords: ZnO nanoparticles, TiO_2 nanoparticles, structural characteristics, morphology, genotoxicity

In last few years zinc oxide and titanium dioxide nanoparticles have gained an increased attention due to their various applications especially in pharmaceutical industry and medicine. These nanosized particles (less than 100 nm) at inappropriate concentrations can easily transcend biological cell membranes, similarly to other potentially toxic substances, into the bloodstream reaching some organs thereby causing their damage [1-3]. It is expected that physical and chemical properties of ZnO and TiO_2 nanoparticles expressed in their structure and morphology to be related to their toxicological effect [4, 5]. Several studies revealed that these nanosized particles may interact with some components of cellular system such as membranes, DNA, proteins, organelles and also with biological fluids. At the moment the mechanism of zinc oxide and titanium dioxide nanoparticles toxicity is not yet elucidated. Some authors have hypothesized that lipid peroxidation and oxidative stress induce DNA alteration influencing thus cell viability [6-8].

Recently, studies highlighted the possibility of large-scale use of ZnO and TiO_2 nanoparticles in medicine. Moreover, synthesis and applications of various nanosized zinc oxide and titanium dioxide nanoparticles had a significant increase. Researchers found that nanomaterials used as coatings for medical devices present a different toxicity features compared to the larger size because of their high interactions with proximate surface [9-31].

In our study we focused on creating a connection between physico-chemical characteristics of ZnO and TiO_2 nanoparticles and their potential genotoxic effects. The major objective of this study was to determine the toxic effects of ZnO and TiO_2 particles on human health in order to use them in safe doses in medical field. For this purpose XRD and SEM techniques were used.

Experimental part

Materials and methods

ZnO nanoparticles were prepared by using a simple method implying zinc acetate and citric acid in a molar ratio of 1:1 dissolved in deionized water. The solution was then vigorously stirred for 1 h at 100°C till the gel was formed. Next step consisted in thermally treatment of gel at 200°C followed by annealing at 450°C for 2 h in order to obtain crystalline zinc oxide nanoparticles [32-34].

TiO_2 nanoparticles were prepared by using titanium isopropoxide [Ti{OCH(CH_3)2}$_4$] hydrolyzed in a mixture solution of ethanol and water. The obtained gel was then dried at 80°C for 5 h. The obtained white powder containing titanium dioxide was calcined at 550°C [35].

Results and discussions

XRD pattern (not shown) presents highly intense peaks indicating crystalline structure of ZnO nanoparticles. The obtained peaks at 2θ degree of 31.72°, 34.4°, 36.2°, 47.49°, 56.5°, 62.8°, 66.26°, 67.85° confirm the purity of zinc oxide sample (table 1). Moreover, from XRD spectra was calculated ZnO crystal size with an average of 17.1 nm [30]. XRD pattern of TiO_2 nanoparticles (not shown) exhibit peaks in 2θ range at 25.32°, 36.96°, 37.79°, 38.59°, 48.8°, 53.89°, 62.15°, 62.72°, 68.77°, 76.11° confirming both anatase and rutile structure of titanium dioxide nanoparticles also indicating lack of impurities (table 1) [35, 36].

SEM image of ZnO nanoparticles (fig. 1) reveals an average size of 15-25 nm. Particle size and shape for TiO_2 sample determined by SEM (fig. 2) shows roughly spherical nanoparticles in the range of 10-20 nm.

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Genotoxicity profile

Cell culture

Neoplastic HeLa and normal Vero cells were grown in DMEM supplemented with 10% fetal bovine serum and antibiotics (100 μg/mL streptomycin, 100 IU/mL penicillin), being plated in 96-well at a density of 8/103 cells/well and allowed to attach and grow for 24 h at 37°C in a humidified air incubator containing 5% CO_2. Once the cells have

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Zinc Oxide				Titanium Oxide			
hkl	*d(Å)/d(nm)*	*2θ(°)*	*I(%)*	*hkl*	*d(Å)/d(nm)*	*2θ (°)*	*I(%)*
100	2.817/0.2817	31.72	56.7	101	3.512/0.3512	25.32	100
002	2.603/0.2603	34.40	46.1	103	2.428/0.2428	36.96	2.1
101	2.478/0.2478	36.20	100	004	2.377/0.2377	37.79	5.9
102	1.912/0.1912	47.49	21.3	112	2.33/0.233	38.59	11
110	1.626/0.1626	56.50	25.1	200	1.89/0.189	48.8	19.3
103	1.477/0.1477	62.80	26.5	105	1.699/0.1699	53.89	10.3
200	1.408/0.1408	66.26	3	211	1.664/0.1664	55.11	14.1
112	1.379/0.1379	67.85	1.6	213	1.491/0.1491	62.15	1.1
201	1.359/0.1359	68.98	7.8	204	1.479/0.1479	62.72	3.9
004	1.301/0.3010	72.52	1.8	116	1.363/0.1363	68.77	8.4
202	1.239/0.2390	76.84	2.4	220	1.336/0.1336	70.36	4
104	1.181/0.1181	81.31	1.6	107	1.278/0.1278	74.06	1.5
203	1.093/0.1093	89.49	4.9	215	1.263/0.1263	75.09	5.2
210	1.064/0.1064	92.61	1.2	301	1.249/0.1249	76.11	1.9
211	1.043/0.1043	95.13	3.5	008	1.188/0.1188	80.73	0.2
114	0.016/0.016	98.50	2.7	303	1.17/0.117	82.23	0.3
212	0.985/0.0985	102.75	1.3	224	1.165/0.1165	82.74	1.5
105	0.976/0.0976	104.05	4.4	312	1.159/0.1159	83.24	1.8
203	0.956/0.0956	107.28	0.5	217	1.059/0.1059	93.28	1.3
300	0.939/0.0939	110.15	1.2	305	1.05/0.105	94.28	1.2
213	0.907/0.0907	116.05	3.7	321	1.042/0.1042	95.27	1.8
302	0.883/0.0883	121.29	1.6	109	1.017/0.1017	98.34	0.2
006	0.867/0.0867	125.04	0.5	208	1.006/0.1006	99.84	0.4
205	0.837/0.0837	133.71	2.2	323	0.995/0.0995	101.34	0.3
106	0.830/0.0830	136.36	0.7	316	0.954/0.0954	107.56	3.8
214	0.824/0.0824	138.21	0.5	400	0.945/0.0945	109.13	1
220	0.813/0.0813	142.44	0.7	307	0.923/0.0923	112.92	0.5
a = b = 3,2533 Å, c = 5,2072 Å, α = β = 90°, γ= 120°				a = b = 3.78 Å, c = 9.51 Å, α = β = γ = 90°,			

Table 1
XRD PARAMETERS OF ZnO AND TiO_2 NANOPARTICLES

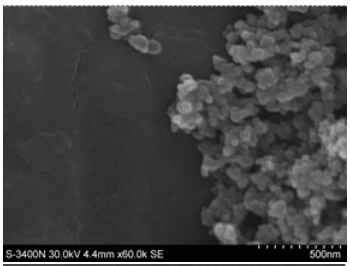

Fig. 1. SEM images of ZnO nanostructures

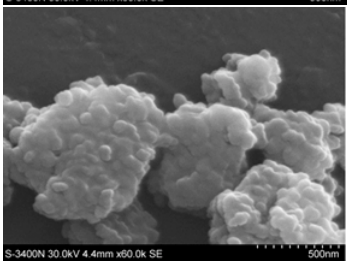

Fig. 2. SEM images of TiO_2 nanostructures

reached confluence in the monolayer stage, were treated with different doses from tested compounds. The incubation time of the cells in the presence of the tested compounds, was of 24 h. After the incubation time has expired, the cells viability was assessed by MTT assay.

Cell viability assay

After 24 h incubation with the tested compounds in different doses (μg/mL) the cell viability was assessed by MTT test [36], with minor modifications. Briefly, the cells were washed with PBS and the medium was replaced by fresh growth medium (100 μL/well) and MTT (10 μL/well) was added. After 3 h incubation at 37°C, the formazan dye, generated by the reduction of MTT in living cells, was dissolved in DMSO (100 μL/well) and further quantified at 540 nm. The cell viability (%) was calculated as 100(AS/AC), where AS and AC are the absorbance of the formazan dye in the cells incubated with essential oil dilutions and sham control, respectively (table 2).

Table 2
GENOTOXICITY ASSAY

Viability %	HeLa Cells	Vero Cells	Viability %	HeLa Cells	Vero Cells
	Average± standard error	Average± standard error		Average± standard error	Average± standard error
Standard	100±0.00	100.00±0.00	Standard	100±0.00	100.00±0.00
ZnO – 15 ug/mL	95.20±1.30	107.35±2.52	TiO2 – 15 ug/mL	86.13±0.52	98.56±5.45
ZnO – 30 ug/mL	94.56±1.15	105.69±3.60	TiO2 – 30 ug/mL	84.56±3.26	89.08±0.64
ZnO – 60 ug/mL	92.16±3.18	102.80±4.25	TiO2 – 60 ug/mL	83.28±4.41	87.35±4.15
ZnO – 125 ug/mL	93.22±1.84	97.94±5.06	TiO2 – 125 ug/mL	84.15±3.91	86.81±2.60
ZnO – 250 ug/mL	93.88±4.90	98.08±4.15	TiO2 – 250 ug/mL	83.13±2.30	88.22±4.58

The impact of the tested compounds on cells viability is minor, suggesting a reduced cytotoxicity on both cell lines. Some differences in the reactivity were registered between normal and tumoral cells, with a better tolerability in the case of the normal cells.

Conclusions

Nanotechnology has attracted public interest by various uses of nanomaterials in many fields such as pharmacy, medicine and public health. Zinc oxide and titanium dioxide nanoparticles are two compounds possessing very wide applications due to their physicochemical properties. So it is essential to reveal the harmful effect caused by these small in size materials on human health. Our study concluded that structural and morphological features of as synthesized ZnO and TiO_2 nanoparticles has a minor impact on tested cells suggesting a reduced genotoxicity on them.

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