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THE EFFECT OF Ag-DOPING ON THE CYTOTOXICITY OF ZnO NANOSTRUCTURES GROWN ON Ag/Si SUBSTRATES BY APMOCVD

The search and development of new nanostructures and nanomaterials are very important for the progress of nanotechnology and modern microbiology. Due to the unique properties of silver and zinc oxide, these nanoparticles are the optimal basis for creating nanostructures with potential antiviral activity. An important issue in these studies is the establishment of cytotoxicity of these nanoparticles and their composites. ***Aim.*** *To define the influence of substrate temperature and Ag concentration in ZnO lattice on the microstructure and cytotoxicity of zinc oxide nanostructures.* ***Methods.*** *Pure and Ag-doped ZnO nanostructures were grown on Ag/Si substrates by atmospheric pressure metalorganic chemical vapor deposition method using a mixture of zinc acetylacetonate and silver acetylacetonate powders as a precursor. Argentum thin films were deposited on Si substrates by a thermal evaporation method. MTT-assay was used for the analysis of MDBK and MDCK cell viability in the definition of zinc oxide nanostructure cytotoxicity.* ***Results.*** *Ag-doped zinc oxide nanostructures were grown and characterized by X-ray diffraction, scanning electron microscopy, and energy dispersive X-ray spectroscopy. It was found that Si substrate and pure zinc oxide do not inhibit the cell viability of both epithelial cultures whereas Ag-doped ZnO nanostructures inhibit the cell viability because of all-time exposure in a sample without dilution. The cytotoxic effect was not observed at higher dilutions for Ag-doped zinc oxide nanostructures.* ***Conclusions.*** *The investigation of the effect of Ag-doping on the morphology and cytotoxicity of zinc oxide nanostructures is very important for implementing zinc oxide nanostructures into the current optoelectronics and photocatalysis.*

Keywords: *ZnO nanostructures, Ag doping, APMOCVD, scanning electron microscopy, cytotoxicity.*

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According to the International Committee on Taxonomy of Viruses, viruses are one of the most diverse groups of microorganisms [1]. A lot of them can lead to the significant cause of morbidity, mortality worldwide, and socio-economic consequences. This is once again confirmed by the COVID-19 pandemic, which spread to the whole world [2].

In recent years, much attention has been paid to the study of nanostructures with antimicrobial and antiviral activity [3]. Surface contamination has been recognized as an essential contributor to the spread of diseases. Infected individuals promote constant recontamination of surfaces, which then are touched by non-infected people leading to the rapid dissemination of the disease. This effect has a significant contribution to viral spread [4]. Given this, it is important to create a nanostructure and surfaces with antiviral and viricidal activity.

With the rapidly growing interest in this area, the toxicity of nanoparticles (NP) and nanostructures (NS) is becoming a more and more important issue in nanotechnology. A lot of new types of nanoparticles and nanostructures are synthesized and studyed every year. Also, the application of nanoparticles in biomedicine, e.g. in drug and gene delivery, biosensors, cancer treatment, and diagnostic tools, has been extensively studied over the past decade [5]. For those purposes, the definition of toxicity of nanoparticles and nanostructures is a critical factor to be taken into account when evaluating their application potential [6].

According to the literature, zinc oxide (ZnO) nanoparticles have been shown to exert antimicrobial and antiviral activities against various human pathogens [7]. ZnO is a well-known wide-gap semiconductor material intensively studied over last decades due to its unique multifunctional properties which are perspective for device designing in such scientific fields as material science, physics, chemistry, biochemistry, etc. [8, 9]. The studies of ZnO in the form of nanostructures are a promising and interesting trend in view of potential applications for the development of nano-scale optoelectronic devices and effective photocatalyst materials. Recently, pure and doped ZnO NS have been studied as a fast cost-effective eco-friendly photocatalytic material for the clean-up of the environment from persistent organic pollutants as well for air disinfection from bacteria and viruses [10,11,12].

In order to increase the performance of ZnO NS and to shift photocatalytic activity to visible light, many researchers apply doping with various metal and non-metal elements including rare earth elements. The basics of photocatalytic mechanism and an overview of other major research results and application of ZnO nanostructures can be found in Ref. [13]. Cytotoxicity of ZnO nanoparticles was investigated in [14] over MCF-7 cells. It was demonstrated that the cytotoxicity and oxidative stress response of ZnO nanoparticles were enhanced by Ag-doping. among various elements used for ZnO doping (ZnO:Ag) and creating ZnO NS composites (ZnO-Ag), silver is considered the most promising metal due to the possibility to absorb and scatter more light thanks to the surface plasmon resonance appeared in metal nanoparticles surrounded by a dielectric matrix [15, 16, 17]. Thus, determining the cytotoxicity of ZnO and its combination with Ag is the first step in working with the cell culture system.

We used the commercially attractive atmospheric pressure metal-organic chemical vapor deposition (APMOCVD) method for the growth of pure and Ag-doped ZnO NS on Ag-coated Si (Ag/Si) substrates. This work presents the investigation of the effect of the level of Ag-doping on the structure and the cytotoxicity of ZnO nanostructures, which is very important for implementing ZnO NS into realistic optoelectronic and photocatalytic applications.

Material and methods. Cell cultures. The culture of MDCK (Madin-Darby canine kidney cell line) cells obtained from L.V. Hromashevs-

koho Institute of Epidemiology and Infection Diseases of NAS of Ukraine and MDBK (Madin-Darby canine bovine cell line) cells obtained from the collection of the museum of tissue cultures of the Institute of Virology of the Bulgarian Academy of Sciences (Sofia, Bulgaria) were used in this study. Cells were grown in plastic vials in a medium consisting of 46% DMEM (Biowest, France), 46% RPMI 1640 (Biowest, France), 8% fetal bovine serum inactivated by heating (Biowest, France), and antibiotic penicilin (100 μg/mL, Biowest, France) in an atmosphere of 5% CO_2 at 37 °C.

Sample growth. Pure and Ag-doped ZnO NSs were deposited on Ag/Si substrates by the APMOCVD technique [18, 19]. We grew a thin 120 nm layer of Ag as a nucleation layer for growing ZnO NS with developed geometry on Si substrates by thermal evaporation method [19]. Before deposition of pure and Ag-doped ZnO NS, Ag/Si substrates were cleaned in acetone, ethanol, and deionized water for 10 min at each stage. After that, the Ag/Si substrates were dried in a nitrogen flow. To grow ZnO, zinc acetylacetonate hydrate (Zn(AcAc)) from Sigma Aldrich was used as a precursor. The mixture of zinc and silver acetylacetonates with 1 and 10 wt.% of silver acetylacetonate (Ag(AcAc)) was used for growth of Ag-doped ZnO samples, resulting in the proportional incorporation of the Ag dopant into the ZnO lattice. Four sample sets (pure ZnO, ZnO:0.5at.%Ag nanorods (NR), ZnO:7.0at.%Ag NR, and ZnO:7.0at.%Ag nanowires (NW) were grown at different substrate temperatures ranging from 250 °C to 500 °C for 30 min. Information about the growth conditions and the corresponding material properties of the samples is collected in Table.

Characterization. The crystal structure was investigated by X-ray diffraction (XRD) using a DRON-3 diffractometer with Cu-Kα radiation (λ = 0.1542 nm). The microstructure of pure and Ag-doped ZnO samples were studied by scanning electron microscopy (SEM) (Leo 1550 Gemini SEM) at the standard aperture 30 μm and operating voltage 5 kV. An elemental analysis of pure and Ag-doped ZnO samples was performed by ZEISS EVO 50 XVP SEM using energy dispersive X-ray spectroscopy (EDX) furnished with INCA 450 (OXFORD Instruments). The operating voltage for EDX analysis was to 20 kV. Thus, the atomic content of Ag was determined for three ZnO samples grown with different contents of Ag precursor in the precursor mixture.

Determination of the cytotoxic effect of ZnO NS doped with Ag of different concentrations and pure ZnO film for comparison was performed by using the MTT test, which is based on determining the functioning of the dehydrogenase activity of mitochondria. Cell viability was assessed by the ability of living cells to reduce the yellow MTT dye to a blue formazan crystal. The procedure included 24 hr of exposure of the plates in a growth medium for cell cultures at 37 °C. The growth medium was then added to the monolayer of cell cultures without dilution and with dilutions of 1:10 and 1:100. The

The growth conditions and properties of pure and Ag-doped ZnO samples deposited on Ag/Si substrates

Samples	Precursors		Substrate temperature, °C	Ag content in ZnO, at.%	NS diameter, nm
	$Zn(AcAc)_2$, wt.%	Ag(AcAc), t.%			
Pure ZnO films	100	0	250	0	—
ZnO:0.5 at.%Ag nanorods (NR)	99	1	400	0.5	100
ZnO:7.0 at.%Ag nanorods (NR)	90	10	400	7.0	250
ZnO:7.0 at.%Ag nanowires (NW)	90	10	500	7.0	60

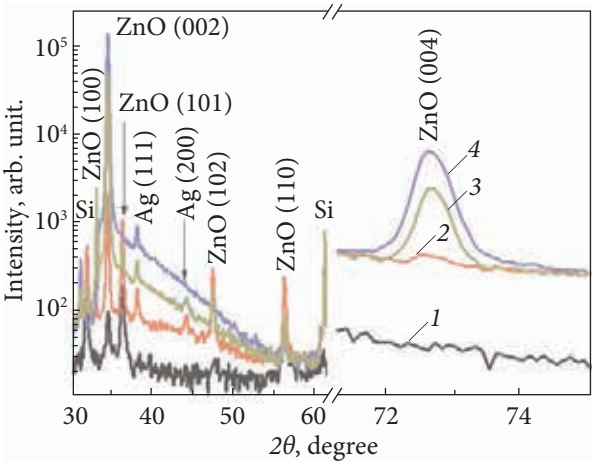

Fig. 1. Color online. XRD patterns of ZnO samples: pure ZnO (*1*), ZnO:0.5 at.%Ag NR (*2*), ZnO:7.0 at.%Ag NR (*3*), and ZnO:7.0at.%Ag NW (*4*)

dilutions were prepared on the growth medium. Each dilution was analyzed in 3 replicates. The cells were analyzed at 24, 48, and 72 hr by adding 20 μL of MTT solution (5 mg/mL). After a four-hour incubation in the dark at 37 ^{0}C (5% CO_2), the formazan crystal formed by MTT reduction was dissolved in DMSO, and absorbance was measured at 538 nm using a Multiskan FC microtiter plate ELISA reader (Thermo Scientific, USA). The obtained optical density of the test sample was compared with that of the cell control sample without addition of NS and the formula for calculation of the percentage of cell viability different dilutions:

$$\% \text{ of cell viability} = (A \times 100 / B),$$

where A is the average optical density of test samples and B is the average optical density of cell control samples.

Results. We investigated the elemental composition of the grown ZnO samples by the energy dispersive X-ray spectroscopy (EDX). It was shown that all deposited samples contained zinc, oxygen, and carbon. The NS deposited from the mixtures ($Zn(AcAc)_2$) and (Ag(AcAc)) additionally contained silver with amount presented in Table 1. For all pure and Ag-doped ZnO samples, oxygen deficiency was found. Obviously, the presence of a low amount of carbon is the result of the decomposition of acetylacetonate precursors or surface organic contaminations.

XRD measurements were used to determine the crystal structures of the deposited samples. Fig. 1 presentsXRD patterns of pure (1) and Ag-doped (2—4) ZnO samples. Accordingly, JCPDS card number 36–1451 all grown ZnO samples have the hexagonal wurtzite structure of ZnO with the crystallographic planes (100), (002), (101), (102), (110), and additionally ZnO (004) XRD planes for Ag-doped samples testifying their good crystalline quality. The observed XRD peaks of Ag (111) and Ag (200) (JCPDS card number 04–0783) and Si belong to the Ag/Si substrate. No other diffraction peaks are shown in Fig. 1.

SEM measurements were carried out to study the change in the surface morphology for pure and Ag-doped ZnO NS depending on the substrate temperatures and Ag concentration in the mixture of precursors. Fig. 2 demonstrates the top SEM view and 30° tilted view for pure and Ag-doped ZnO samples. At a substrate temperature of 250 °C and by using only ($Zn(AcAc)_2$) precursor, the formation of ZnO films is observed (Fig. 2, *1*). The cross-sectional view of these NS films demonstrates that the film thickness is about 200 nm. In conclusion, the decomposition of pure ZnO precursor leads to the formation of ZnO NS with a film-like surface morphology. A more significant change in the surface morphology of Ag-doped ZnO samples was found. When we added 1 wt.% of the $Zn(AcAc)_2$ precursor to the mixture of precursors and increased the substrate temperature to 400 °C, the growth of well-defined ZnO NR with characteristic hexagonal facets having a diameter of about 100 nm (Table) and a height of about 1 μm takes place (Fig. 2, *2*). When we added 10 wt.% of the $Zn(AcAc)_2$ precursor and used the substrate temperature of 400 °C, the formation of well-defined ZnO NR with characteristic hexagonal facets having a diameter of about 250 nm and height of about

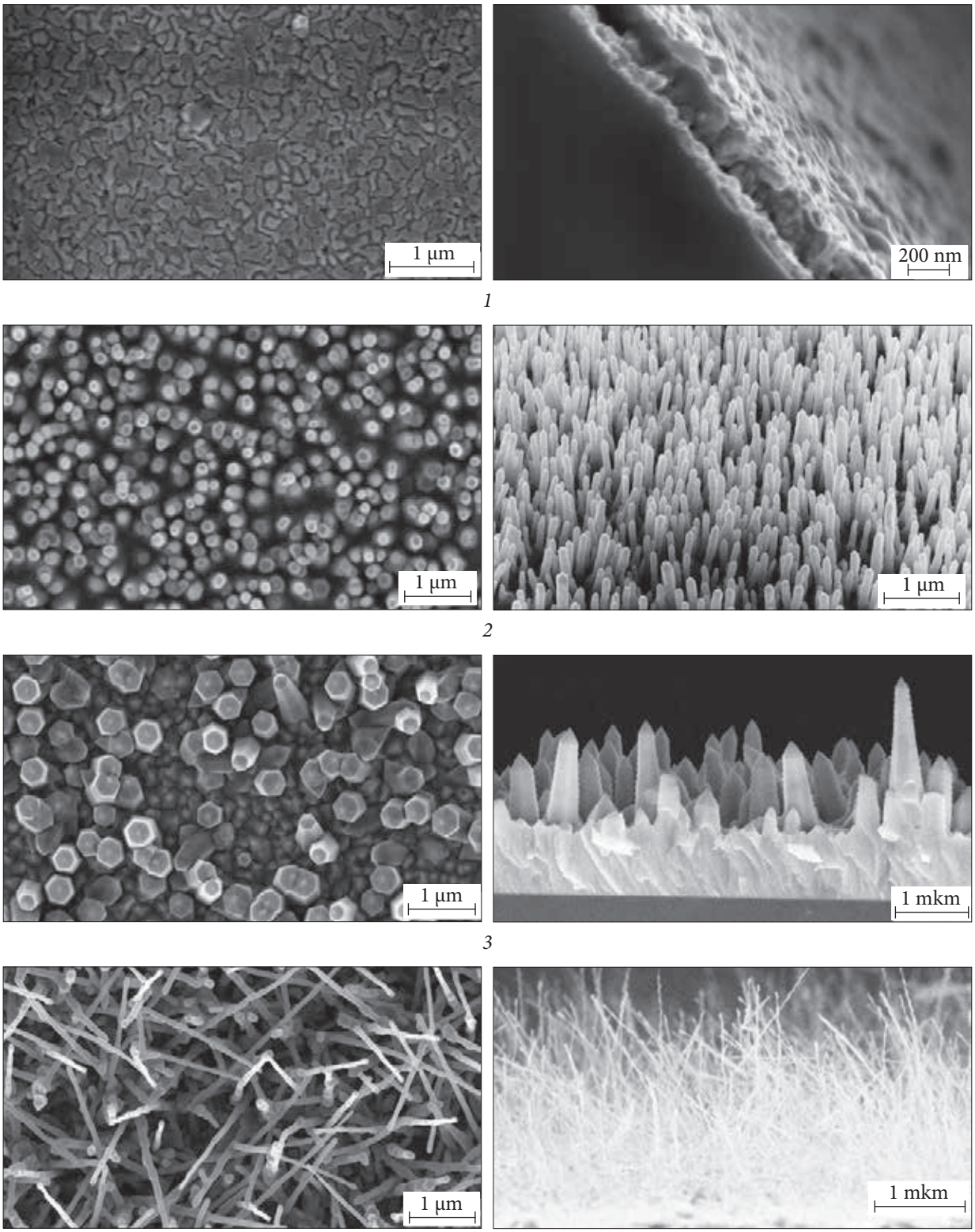

Fig. 2. SEM surface images of ZnO samples: pure ZnO (*1*), ZnO:0.5 at.%Ag NR (*2*), ZnO:7.0 at.%Ag NR (*3*), and ZnO:7.0at.%Ag NW (*4*). The left side is a top SEM view, while a 30° tilted view is shown on the right

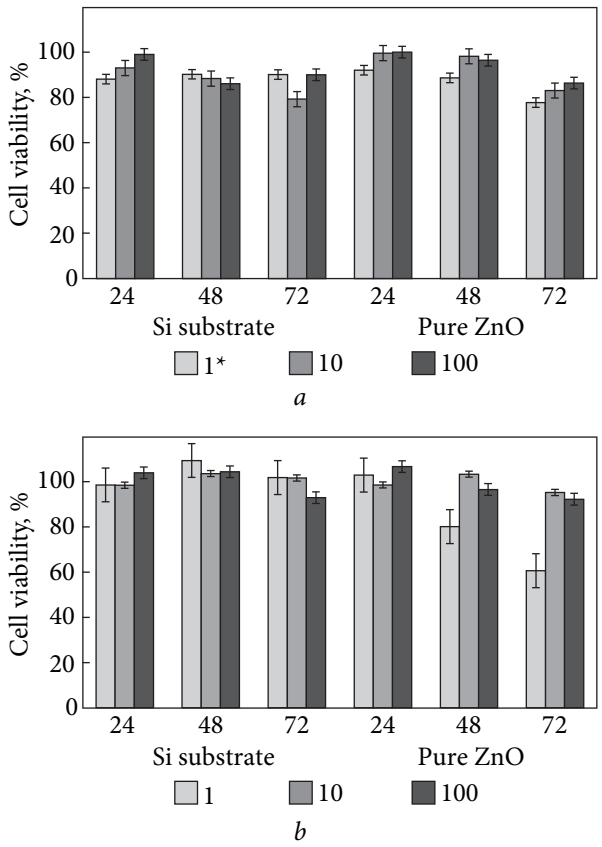

Fig. 3. Cell viability of MDCK (*a*) and MDBK (*b*) cell cultures after exposition with an eluent of nanostructures; * — dilution of the eluent after incubation with nanostructures

1.5 μm took place (Fig. 2, *3*). These nanocrystals are also well aligned with the *c*-axis perpendicular to the substrate. With increasing substrate temperature to 500 °C, the growth of disordered ZnO NW with a diameter of about 60 nm is observed (Fig. 2, *4*). The NWs uniformly cover the Ag/Si substrate and have a height of about 2.5 μm (Fig. 2, *4*, right column). Consequently, adding 10 wt.% of Ag precursor to the pure ZnO precursor leads to the formation of well-defined ZnO NR and NW at different substrate temperatures. Hence, Ag doping affects the ZnO microstructure via changing the nucleation mode into heterogeneous and thus transforming the polycrystalline films into a matrix of highly *c*-axis-textured hexagonally-faceted NR.

At first, the cytotoxicity of Si substrate and pure ZnO was studied. We used pure ZnO films to compare with Ag-doped ZnO NS and defined their toxic effects. As shown in Fig. 3, the substrate does not lead to inhibition of the cell viability in both cultures. Even with prolonged exposure (72 h), cell viability ranged from 80 to 100% for MDCK cells and 93–104% for MDBK cells. After exposure of pure ZnO films, the cell viability was from 77 to 100% for the MDCK cell line (Fig. 3, *a*—*b*). However, the level of MDBK cell viability was lower and exceeded 60% for prolonged exposure (72 h). At the same time, 24 h and 48 h exposures do not inhibit the cell viability (80–100%).

As shown in Fig. 4, the influence of Ag-doped ZnO NS on the living of MDBK and MDCK cells was more toxic than on the substrate. A pronounced cytotoxic effect was determined for the Ag-doped ZnO NS samples deposited with a high silver content (ZnO:0.7at.%Ag NW) in MDCK cells (Fig. 4, *a*); cell viability was in the range of 61–64% in samples without dilution. No cytotoxic effect of Ag-containing ZnO nanostructure with less silver (0.5 and 0.7at%Ag NR) on the living of MDCK cells was observed (Fig. 4, *a*). On the other hand, it was established that the height cytotoxic effect occurs in MDBK cells (Fig. 4, *b*). All samples studied for MDBK cells without dilution inhibit cell viability (11–31%) at all exposure times. It should be noted that an increase in Ag content in the ZnO NS samples leads to the enhancement of their toxic effect. In addition, the cytotoxic studies of diluted Ag-doped ZnO NS samples as 1:10 and 1:100 showed a non-toxic effect of Ag-doped ZnO samples on epithelial cell lines.

Discussion. Due to their unique properties, nanoparticles are on the top of interest for scientists. ZnO is a perfect candidate for creating new nanostructures and materials as an effective photocatalyst and an antimicrobial and antiviral agent [20]. Thus, the studies of the cytotoxicity of pure and Ag-doped ZnO nanostructures are

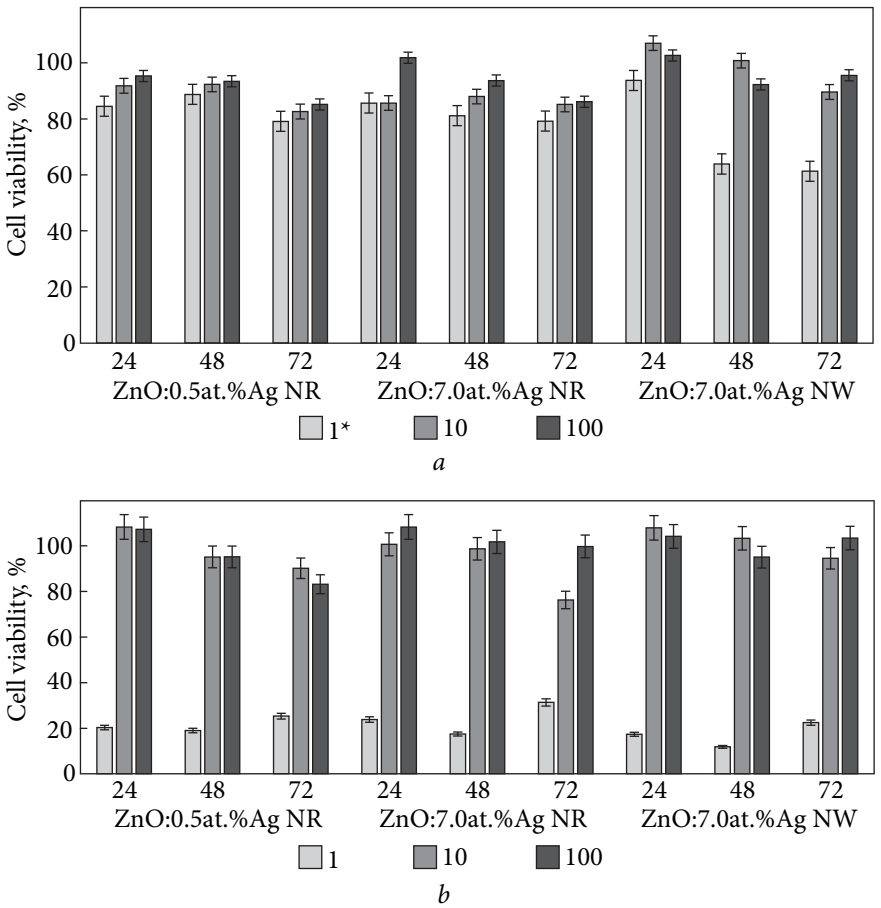

Fig. 4. Cytotoxic effect of ZnO samples in MDCK (*a*) and MDBK (*b*) cells culture: ZnO:0.5at.%Ag NR, ZnO:0.7at.%Ag NR, and ZnO:0.7wt.%Ag NW; * — dilution of the eluent after incubation with nanostructures

an essential step in developing new composites as promising materials for practical applications.

Our study showed the difference in the toxicity of ZnO nanostructure with different concentrations of Ag at epithelial cell cultures. Thus, ZnO:7.0at.%Ag NW and ZnO:7.0at.%Ag NR are more toxic than ZnO:0.5at.%Ag NR in both cell cultures. This can be explained by the contrast of the shape and size of nanostructures to the specific surface areas shown by SEM (Fig. 2) as well as of different Ag concentrations in ZnO samples. Generally, smaller nanostructures, i.e. having a larger surface-to-volume ratio, are chemically more active (e.g., in catalysis, photo corrosion, etc.). Also, according to the literature, smaller Ag nanoparticles show a higher cytotoxic effect on cell lines and change biological activity [21, 22]. Besides, ZnO nanoparticles have a size-depended biological effect, especially cytotoxic [23]. It should be noted that the grown by us Ag-doped ZnO samples condensed on Ag/Si substrates in different shapes of NR and NW with different diameters (Table 1). Thus, the size and shape of NS also might influence their toxicity and, in general, the biological activity. *E.g.,* , Kononenko et al. have described that when ZnO NPs dissolve, Zn ions are released, which can disrupt cellular homeostasis, resulting in lysosomal and mitochondria damage and ultimately cell death [24]. Moreover, zinc oxide did not inhibit cell viability in both cultures. According to the literature, the most clearly studied aspect of NPs is inhibitory activity against bacteria. [25]. Since we studied Ag-ZnO composites, let us consider possible effects associated with silver. Silver's mode of action is presumed to depend on Ag+ ions, which

strongly inhibit bacterial growth by suppressing respiratory enzymes and electron transport components along with interfering with DNA functions [26]. It can be assumed that silver ions have a similar effect on mammalian cell cultures. Zhang et al. have shown that the penetration of Ag NPs induces a series of effects including oxidative stress, cell cycle rest, inflammatory responses, DNA damage, apoptosis, etc. [27].

With increasing dilution, , the toxic effect disappeared. This indicates the effect of ion concentration on the cytotoxicity of nanostructures. Obtained results have shown that studied ZnO:Ag samples affect the cell viability only in the case of non-diluted samples. Thus, Ag-doped ZnO nanostructures should be studied as an antiviral agent.

Conclusions. To summarize, pure and Ag-doped ZnO samples were successfully grown on Ag/Si substrates using the APMOCVD method. It is shown that the changing of substrate temperature and in situ Ag doping influence the ZnO microstructure and level of the ZnO lattice doping with Ag. Investigation of the cytotoxicity of the nanostructures showed a different effect on mammalian cell lines. According to the results obtained, Ag-doped ZnO samples' toxic impact depends on the Ag concentration and exposure time. Obtained results have shown that Ag-doped ZnO nanostructures do not demonstrate a highly toxic effect.

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REFERENCES

1. Walker P, Siddell S, Lefkowitz E, Mushegian A, Dempsey D, Dutilh B, et al. Changes to virus taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. Archives Virol. 2019; 164:2417–2429.
2. Hodek J, Zajícová V, Lovětinská-Šlamborová I, Stibor I, Müllerová J, Weber J. Protective hybrid coating containing silver, copper and zinc cations effective against human immunodeficiency virus and other enveloped viruses. BMC Microbiol. 2016; Suppl 1(56).
3. Schio L, Michels F, Fongaro G, Figueroa A. Trends in the antiviral chemical activity of material surfaces associated with the SARS-CoV-2 outbreak. Front Chem. Eng. 2021; 3:636075.
4. Toledo G, Toledo V,. Lanfredi A, Escote M, Champi A, Carlan da Silva C, et al. Promising Nanostructured Materials against Enveloped Virus. Health Sciences. 2020; 92(4):e20200718.
5. Yang S, Liu J, Wang J, Yuan Y, Cao A, Wang H, et al. Cytotoxicity of zinc oxide nanoparticles: the importance of microenvironment. J Nanosci Nanotechnol. 2010; 10(12):8638–8645.
6. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. Small. 2008; 4(1):26–49.
7. Ghaffari H, Tavakoli A, Moradi A, Tabarraei A, Bokharaei-Salim F, Zahmatkeshan M, et al. Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: another emerging application of nanomedicine. J Biomed Sci. 2019; 26(1):70–80.
8. Lashkarev G, Shtepliuk I, Ievtushenko A, Khyzhun O, Kartuzov V, Ovsiannikova L, et al. Properties of solid solutions, doped film, and nanocomposite structures based on zinc oxide. Low temperature physics. 2015; 41(2):129–140.
9. Golovynskyi S, Ievtushenko A, Mamykin S, Dusheiko M, Golovynska I, Bykov O, et al. High transparent and conductive undoped ZnO thin films deposited by reactive ion-beam sputtering. Vacuum. 2018; 153:204–210.
10. Khan S, Pathak B. ZnO based Photocatalytic Degradation of Persistent Pesticides: A Comprehensive Review. Environmental Nanotechnology, Monitoring and Management. 2020; 13:100290.
11. Ong C, Ng L, Mohammad A. A review of ZnO nanoparticles as solar photocatalysts: Synthesis, mechanisms and applications. Renewable and Sustainable Energy Reviews. 2018; 81:536–551.
12. Lee K, Lai C, Ngai K, Juan J. Recent developments of zinc oxide based photocatalyst in water treatment technology: A review. Water Research. 2016; 88:428–448.

13. Samadi M, Zirak M, Naseri A, Khorashadizade E, Moshfegh A. Recent progress on doped ZnO nanostructures forvisible-light photocatalysis. Thin Solid Films. 2016; 605:2–19.
14. Ahamed M, Khan M, Akhtar M, Alhadlaq H, Alshamsaan A. Aluminum doping tunes band gap energy level as well as oxidative stress-mediated cytotoxicity of ZnO nanoparticles in MCF-7 cells. Scientific Reports. 2017; 7:17662.
15. Saravanan R, Mansoob Khan M, Gupta V, Mosquera E, Gracia F, Narayanan V, Stephen A. ZnO/Ag/CdO nanocomposite for visible light-induced photocatalytic degradation of industrial textile effluents. Journal of Colloid and Interface Science. 2015; 452:126–133.
16. Raji R, Sibi K, Gopchandran G. ZnO:Ag nanorods as efficient photocatalysts: Sunlight driven photocatalytic degradation of sulforhodamine B. Applied Surface Science. 2018; 427(B):863–875.
17. Zhizhong H, Lili R, Zhihui C, Chongqi C, Haibo P, Jianzhong C. Ag/ZnO flower heterostructures as a visible-light driven photocatalyst via surface plasmon resonance. Applied Catalysis B: Environmental. 2012; 126:298–305.
18. Ievtushenko A, Karpyna V, Eriksson J, Tsiaoussis I, Shtepliuk I, Lashkarev G, et al. Effect of Ag doping on the structural, electrical and optical properties of ZnO grown by MOCVD at different substrate temperatures. Superlattices and Microstructures. 2018; 117:121–131.
19. Ievtushenko A, Tkach V, Strelchuk V, Petrosian L, Kolomys O, Kutsay O, et al. Solar Explosive Evaporation Growth of ZnO Nanostructures. Applied Science. 2017; *7*(4):383–392.
20. Ghaffari H, Tavakoli A, Moradi A, Tabarraei A, Bokharaei-Salim F, Zahmatkeshan M, et al. Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: another emerging application of nanomedicine. J Biomed Sci. 2019; 10; 26(1):70–80.
21. Raza A, Kanwal Z, Rauf A, Sabri N, Riaz S, Naseem S. Size- and Shape-Dependent Antibacterial Studies of Silver Nanoparticles Synthesized by Wet Chemical Routes. Nanomaterials. 2016; 6(4):74–89.
22. Akter M, Sikder T, Rahman M, Ullah A, Hossain B, Banik S, Hosokawa T, et al. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. J Adv Res. 2017; 2(9):1–16.
23. Costa C, Rodrigues E, Tokuhara K, Oliveira C, Lisboa-Filho N, Rocha A. ZnO Nanoparticles with Different Sizes and Morphologies for Medical Implant Coatings: Synthesis and Cytotoxicity. BioNano Sci. 2018; 8(2):587–595.
24. Kononenko V, Repair N, Marušič N, Drašler B, Romih T, Hočevar S, Drobne D. Comparative *in vitro* genotoxicity study of ZnO nanoparticles, ZnO macroparticles and $ZnCl_2$ to MDCK kidney cells: Size matters. Toxicol In Vitro. 2017; 40:256–263.
25. Pal S, Tak K, Song M. Does the antibacterial activity of silver nanoparticles depend on the nanoparticle's shape? A study of the gram-negative bacterium Escherichia coli. Appl Environ Microbiol. 2007; 73:1712–1720.
26. Galdiero S, Falanga A, Vitiello M, Cantisani M, Marra V, Galdiero M. Silver nanoparticles as potential antiviral agents. Molecules. 2011; 16(10):8894–8918.
27. Zhang T, Wang L, Chen Q, Chen C. Cytotoxic potential of silver nanoparticles. Yonsei medical journal. 2014; 55(2):283–291.

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ВПЛИВ Ag-ЛЕГУВАННЯ НА ЦИТОТОКСИЧНІСТЬ НАНОСТРУКТУР ZnO, ВИРОЩЕНИХ НА СУБСТРАТАХ Ag/Si МЕТОДОМ APMOCVD

Пошук і розробка нових наноструктур та наноматеріалів дуже важливі для прогресу нанотехнологій та сучасної вірусології, мікробіології та медицини. Завдяки унікальним властивостям срібла та оксиду цинку, ці наночастинки є оптимальною основою для створення наноструктур з потенційною противірусною активністю. Важливим питанням у цих дослідженнях є встановлення цитотоксичності наночастинок та їх композитів. **Метою** дослідження було визначити вплив температури підкладки та концентрації срібла у гратці оксиду цинку на мікроструктуру та цитотоксичність наноструктур оксиду цинку. **Методи**. Чисті та леговані сріблом наноструктури ZnO вирощували на кремнієвих підкладках, вкритих плівкою срібла, методом осадження з хімічних парів металоорганічних сполук при атмосферному тиску з використанням суміші порошків ацетилацетонату цинку та порошку ацетилацетонату срібла як прекурсора. Тонкі плівки срібла наносилися на кремнієві підкладки методом термічного випаровування. Визначення цитотоксичності проводили стандартним колориметричним методом з використанням МТТ (за порівнянням метаболічної активності в присутності дослідного зразку та контролю клітин). Вивчали цитотоксичний вплив чистих та легованих наноструктур ZnO з різною концентрацією срібла на клітини MDBK та MDCK. **Результати.** У цій роботі вирощено наноструктури оксиду цинку, леговані сріблом та охарактеризовано їх рентгенівською дифракцією, скануючою електронною мікроскопією та енергодисперсійною рентгенівською спектроскопією. Встановлено, що кремнієва підкладка та чистий оксид цинку не є токсичними в культурах MDBK та MDCK, оскільки за час експерименту (24, 48 та 72 год) не виявлено пригнічення життєздатності клітин обох епітеліальних культур. Наноструктури оксиду цинку, леговані сріблом, пригнічують життєздатність клітин у зразку без розведення на 24, 48 та 72 годину досліджень в культурі MDBK. Зменшення життєздатності клітин MDCK відмічали у зразку з найбільшим вмістом срібла та поверхнею у вигляді нанопроводів. Цитотоксичний ефект не спостерігається при більш високих розведеннях для всіх наноструктур оксиду цинку, легованих сріблом, в обох культурах клітин. **Висновки.** Вміст срібла та морфологія наноструктур ZnO впливає на їхній рівень цитотоксичності. Дослідження цього впливу є дуже важливим для впровадження ZnO наноструктур в сучасні оптоелектронні та фотокаталітичні застосування.

Ключові слова: *наноструктури оксиду цинку, легування сріблом, APMOCVD, скануюча електронна мікроскопія, цитотоксичність, культура клітин.*