

# Synthesis, characterization, size and shape-dependent antibacterial activity and cytotoxicity profile of ZnO nano- and microparticles

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## Research Article

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# Abstract

The aim of our work was the synthesis of ZnO nano- and microparticles and to study the effect of shapes and sizes on cytotoxicity towards normal and cancer cells and antibacterial activity toward two kinds of bacteria. We fabricated ZnO nano- and microparticles through facile chemical and physical routes.

The crystal structure, morphology, textural properties, photoluminescent properties were characterized by powder X-ray diffraction, electron microscopies, nitrogen adsorption/desorption measurements, and photoluminescence spectroscopy.

The obtained ZnO structures were highly crystalline and monodispersed with intensive green emission. ZnO nanoparticles and nanorods showed the strongest antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* compared to microparticles due to their high surface area. However, the ZnO hierarchical particles at higher concentrations also strongly inhibited bacterial growth. *S. aureus* strain was more sensitive to ZnO particles than the *E. coli*. Nanoparticles and nanorods were more harmful to cancer cell lines than to normal ones at the same concentration.

## 1. Introduction

Successful fabrication of materials with desired properties using simple and low-cost approaches attracts a great deal of attention. Materials properties are strongly dependent on their characteristics, mainly phase composition, crystallinity, shape and size, dispersion, etc. Materials morphology plays an important role in optics, photonics, sensors, solar cells to obtain high-quality devices<sup>1-3</sup>. For example, it is well-known that the photocatalytic activity of semiconductor materials (e.g. NiO, TiO<sub>2</sub>) depends strongly on their particle sizes, basically due to enhancing specific surface area<sup>4,5</sup>.

Materials for bioapplication, such as biomarkers, biosensors, or drug carriers with improved optical, sensing, and surface properties can be obtained due to changing their shape, size, and dimension<sup>6,7</sup>. Nanoparticles due to their small size have exciting physicochemical properties, such as a high surface-to-volume ratio, a size similar to the size of biomolecules allowing for their greater mobility and passing through the cell biological membranes<sup>8</sup>. Morphology and surface area affect the antibacterial activity, therefore, it is necessary to synthesize a material with a novel morphology having a higher amount of adsorption sites and surface area<sup>9-11</sup>. Thus, verifying the morphology of the materials and hence their surface area can show great potential for bioapplication because the properties can be tuned and improved.

Decreasing the particle size (nanosized materials, such as 0D materials (e.g. quantum dots, QDs)) is one of the effective ways to increase the surface area. Despite many advantages of the nanoparticles, their main disadvantage is particle agglomeration. Modern approaches allow obtaining high-quality nanoparticles without any stabilizers and surfactants<sup>12,13</sup>. Anisotropic multidimensional and multicompartamental particles with intriguing morphologies are the subjects of extensive research. One

dimension materials (1D) offer high surface area because of their unique morphologies. 3D materials are seen to be more suitable for many applications than other morphologies/structures as they may be assembled from lower dimensional materials and provide a large surface area and fast electron transport<sup>14</sup>.

Metal oxide nanoparticles have great attention in materials science and nanotechnology for a large variety of applications. From semiconductor materials, zinc oxide (ZnO) is an important multifunctional material with broad applications in many scientific fields. As a promising semiconductor oxide (large exciton binding energy (60 meV and wide bandgap  $E_g = 3.37$  eV) ZnO possess excellent physicochemical properties, such as high thermal and chemical stabilities, thermal conductivity, UV protection or electronic, photonic, unique optical, and luminescent properties. At the same time, ZnO is a low-cost material, its synthesis is simple and it can be obtained with a big variety of morphology using physical and chemical approaches. ZnO with different morphology and dimension (*e.g.* bulk ZnO, nanoparticles, films, 3D hierarchical structures) has broad applications as sensors, catalysts, solar cells, optoelectronic devices<sup>15-17</sup>. Furthermore, zinc is a necessary element to our health and has high biocompatibility to human cells, low toxicity, and good antimicrobial activity<sup>10,18,19</sup>. All of these properties allow using ZnO in medicine as biomarkers, drug carriers, and therapeutic<sup>20,21</sup>. Thus, Warheit D.B. *et al.* studied the effect of ZnO morphology on the toxicity of lung cells<sup>22</sup>. They showed a low cytotoxic effect on rat L2 lung epithelial cells and primary rat lung alveolar macrophage treated with ZnO nanoparticles (NPs). Z. Li. *et al.* studied the cell viability of ZnO nanowires using two cell lines<sup>23</sup>. Results showed that ZnO nanowires were biocompatible to HeLa cells up to the highest concentration used (up to 100  $\mu\text{g}/\text{mL}$ ), whereas for L929 cells were deleterious even at lower concentrations.

ZnO possesses unique antibacterial, antimicrobial and antifungal properties, ZnO-based materials can initiate antibacterial activity even without the presence of light<sup>24</sup>. In presented studies<sup>19,25,26</sup> authors obtained ZnO with different morphology and indicated that ZnO and its composites can be used as good antibacterial agents toward Gram-positive, as well as Gram-negative bacteria. Thus, despite intensive research in this field, the evaluation of ZnO antibacterial activity and biocompatibility due to changing the particle morphology is still important and can significantly expand the scope of this material in biomedical applications.

The main aim of our work was a synthesis of ZnO nano- and microparticles, the study of their crystal structures, and the effect of morphology and surface area on luminescent properties, cytotoxicity, and antibacterial activity toward Gram-positive and Gram-negative bacteria.

## 2. Results

### 2.1. Fabrication of ZnO nano- and microparticles

ZnO nano- and microparticles with different morphology were fabricated using facile chemical and physical methods. We performed the study of the shape and size of the obtained ZnO nano- and

microparticles using the transmission (HRTEM) and scanning electron microscopes (SEM). Indeed, in nanoparticles synthesis, we obtained the highly crystalline and monodispersed ZnO nanoparticles (ZnO NPs) and nanorods (ZnO NRs) using the sol-gel approach without any additional surfactants or ligands. ZnO NPs had a perfect spherical shape and uniform size distribution with a mean nanoparticle's size of approximately 7 nm (Fig. 1a, S1a). The FFT digital diffractogram pattern presented in Fig. S1b from nanoparticles with [010] zone axis orientation, demonstrated individual point reflections that indicate that particles had a single crystalline structure. The interplanar distance between adjacent lattice fringes is 2.57 Å corresponding to d(002).

Changing the synthesis parameters in ZnO NP synthesis (reagent concentration and synthesis time, and temperature) we obtained ZnO NRs and ZnO particles (ZnO Ps), (Fig. 1b and 1c). The mean size of ZnO NRs was approximately 5×17 nm (width×length), Fig. S1c. After annealing of ZnO NPs at 900°C for 2h, we obtained ZnO Ps with sizes of ~ 150 nm (Fig. 1c).

To obtain ZnO with the hierarchical structure (ZnO HSs) we used a solvothermal reaction. The SEM image in Fig. 1d confirmed the formation of the flower-like (peony) structure of ZnO. All individual particles had a spherical shape, were highly crystalline, and monodispersed with sizes of approximately 4 μm and the thickness of the individual plates of about 100 nm (Fig. S1d).

We also obtained ZnO in the shape of tetrapods using thermal evaporation in the air. Tetrapods were well faceted with four uniform legs connected to a central nucleus (Fig. 1e, S1e). The legs had hexagonal morphology with a length of about 5.2 μm and approximately 170 nm in thickness.

## **2.2. Structural characterization of ZnO nano- and microparticles**

Next to determine the crystal structure and phase purity of the obtained ZnO materials we performed XRD measurements. Figure 2 shows the XRD pattern of ZnO particles with different morphology. X-ray powder diffraction confirmed the formation of a single-phase for all ZnO samples.

Table 1  
Crystallographic parameters of the obtained ZnO nano- and microstructures.

Sample	XRD		
	Cell parameters		
	a, nm	c, nm	V, nm <sup>3</sup>
ZnO NPs	0.324	0.526	4.811
ZnO NRs	0.326	0.528	4.853
ZnO Ps	0.324	0.520	4.753
ZnO HSs	0.324	0.521	4.766
ZnO TPs	0.325	0.521	4.764

All diffraction peaks are indexed according to the hexagonal phase of ZnO wurtzite crystal structure with main (100), (002), (101), (102), (110), (103), (112), and small (201) and (202) crystal planes. No additional peaks indicating the presence of impurity phases were found in all studied samples. The broadening of the peaks for ZnO NPs and ZnO NRs samples (Fig. 2, black and red lines) can be attributed to the small particle size of ZnO. Calculated crystallographic parameters of zinc oxide particles are presented in Table 1.

The results indicate higher lattice constant values for ZnO NPs and ZnO NRs samples compared with the others. The lowest lattice constant *a* and *c* are observed for ZnO Ps and are the results of their preparation condition (high-temperature annealing). Similar results were presented by Lupan O. *et al.*<sup>27</sup> indicating a decrease of the lattice constant of ZnO with the increase of the temperature of its treatment.

## 2.3. Textural properties of ZnO nano- and microparticles

The next stage was the study of the textural properties of the investigated samples and we performed their N<sub>2</sub> adsorption-desorption analysis on powdered ZnO samples at -196°C. The specific surface area, pore volume, and pore size distribution of samples were specified. The results are summarized in Table 2 and presented in Fig. 3. The adsorption/desorption isotherms of investigated ZnO materials correspond to type IV typical for mesoporous materials. The estimated BET specific surface area of the obtained ZnO nano- and microparticles were 83.5 m<sup>2</sup>/g (NPs), 83.8 m<sup>2</sup>/g (NRs), 3.0 m<sup>2</sup>/g (Ps), 4.5 m<sup>2</sup>/g (HSs), and 29.4 m<sup>2</sup>/g (TPs). The lowest surface area, as well as pore volume, showed ZnO Ps and ZnO HSs.

Table 2  
Surface area and pore volume of ZnO samples with different morphology.

Samples	S <sub>BET</sub> , m <sup>2</sup> /g	Pore volume, cm <sup>3</sup> /g
ZnO NPs	83.5	0.18
ZnO NRs	83.8	0.17
ZnO Ps	3.0	0.08
ZnO HSs	4.5	0.04
ZnO TPs	29.4	0.04

## 2.4. Photoluminescence properties of ZnO nano- and microparticles

The PL spectra of ZnO nanostructures are shown in Fig. 4a. All investigated ZnO nanostructures demonstrate the intense and broad peak in the visible range. The intense emission for ZnO TPs and Ps is mostly associated with oxygen vacancies (Fig. 4b), whereas the PL for ZnO NPs, NRs, and HSs is attributed to oxygen interstitials (Fig. 4c).

## 2.5. Antibacterial properties of ZnO nano- and microparticles

As a next important step, to use our ZnO-based materials as potential antibacterial agents, the antimicrobial activity of ZnO nano- and microstructures was evaluated against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* bacterial strains using optical density measurements after 24-hour incubation, as summarized in Fig. 5. By measuring the optical density at 570 nm (OD<sub>570</sub>), the growth of bacteria can be quantified, based on turbidity resulting from light scattering<sup>28</sup>. For all samples, the bacteria number decreased in a concentration-dependent manner. The most effective towards *E. coli* bacterial strains were ZnO nanoparticles and nanorods, as well as ZnO hierarchical structures at the highest concentration, and the least antibacterial effect was observed for ZnO Ps and TPs. The Gram-positive *S. aureus* bacteria strains were more sensitive to the antimicrobial action of ZnO nano- and microparticles than *E. coli*. However, also here, the greatest lowering of the bacteria number was visible after incubation with ZnO NPs, NRs, and HSs with the highest surface areas.

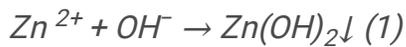
## 2.6. Cytotoxicity studies of ZnO nano- and microparticles

The impact of distinct ZnO nano- and microparticles on human cells was evaluated by cytotoxicity analysis by WST-1 assay, performed on cervical cancer cell line (HeLa) and normal human fibroblasts (MSU1.1) (Fig. 6). Five different ZnO nano- and microparticles concentrations of 0.1, 1.0, 10, 100, and 1000 µg/mL were employed, and the cell viability was determined after 24h of treatment. The cell viability

data revealed that for both cell types at the concentration up to 10 µg/mL all kinds of ZnO particles were biocompatible, maintaining a high level of viability (up to 80%). However, at a higher concentration range (100–1000 µg/mL) the cell viability declines to 40%, indicating a high level of toxicity. Interestingly, at a concentration of 100 µg/mL, a similar level of viability was observed for all samples on MSU1.1 cells, while for HeLa cells significant differences were visible.

### 3. Discussion

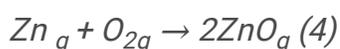
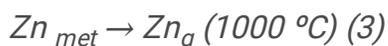
It is known that materials with different morphology can be obtained due to changing the synthesis parameters. ZnO NPs and NRs were obtained by facile sol-gel approach, which is based on the hydrolysis of zinc acetate with the addition of NaOH in a methanol solution. The mixing of the main precursors led to fast sol formation and then to the white homogeneous colloid solution (gel) of ZnO according to reactions (Eq. 1,2).

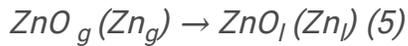


The obtaining of larger ZnO nanoparticles (ZnO Ps with sizes approximately 150 nm) was performed due to Ostwald ripening, where small crystals dissolve and redeposit onto larger crystals using high temperatures<sup>29</sup>.

ZnO HSs were formed from ZnO seeds by their aggregation to rods, assembly to plates, and finally to the spherical framework. The formation of the hierarchical structures is due to the use of “structural director” (generally organic polar molecules). In our case, trisodium citrate dihydrate was used for this purpose.

Obtaining the ZnO TPs was due to the fast formation of the ZnO seeds and then the formation of legs with preferred growth direction along the *c*-axis of the hexagonal unit cell. It is generally accepted that the nucleation and growth of ZnO tetrapods are understood to occur in the vapor phase during synthesis<sup>30</sup>. The growth mechanism of the tetrahedral ZnO particles can be explained by the growth model proposed by Alsultany F.H. *et al.* and Markushev V.M. *et al.*<sup>31,32</sup>. In our case, where no metal catalyst is used, the growth of ZnO TPs can be divided into several steps - nucleation and growth. Extremely high temperature leads to the reaction of the metal Zn ( $Zn_{met}$ ) and then Zn gas ( $Zn_g$ ) with  $O_2$  gas ( $O_{2g}$ ) to form ZnO gas seeds ( $ZnO_g$ ), (Eq. 3,4). Then during constant heating, gaseous ZnO or unoxidized Zn will condense into a liquid state of  $Zn_l$  or  $ZnO_l$  (Eq. 5), and finally following  $O_2$  absorption leads to the crystallization of ZnO solid seeds ( $ZnO_s$ ).





According to the octa-twin nucleus model proposed by Iwanaga H. *et al.*<sup>33</sup>, the ZnO seeds have an octahedral shape. These octahedral seeds are substrates for the following growth of ZnO TPs.

Obtained all ZnO nano- and microparticles were highly crystalline. It is well known that particles in nanorange or materials with unique structures. Including hierarchical structure possess high specific surface area. Often the specific surface area is strongly related to the particle size and increased with decreasing particle size<sup>34</sup>. These results are consistent with the results of particle size estimated by SEM/TEM analysis. The samples with the highest surface areas (ZnO NPs and ZnO NRs) showed narrow pore size distribution with most of the pores in the size range from 3 to 10 nm, whereas the samples with lower surface areas possess additionally pores with a diameter above 10 nm.

Analysis of PL spectra for different semiconductor nanostructures is a powerful tool to investigate their morphology features, defects, and even chemical composition<sup>35</sup>. Typical ZnO nanostructures, such as nanoparticles, nanorods, etc., exhibit two luminescence bands located at the UV region (the near band emission - NBE) and a broad long-wavelength band at the Visible spectrum (the deep-level emission - DLE)<sup>35</sup>. The unique optical properties are very important for medical and biological visualization applications<sup>36</sup>. Depending on the PL intensity, PL peaks position, and the ratio  $I_{\text{NBE}}/I_{\text{DLE}}$ , one can conclude about the structural features of produced ZnO nanostructures. It is well known that the DLE is associated with different ZnO defects, such as zinc vacancies ( $\text{Zn}_i^{++}$ ), single ( $\text{V}_o^+$ ), and double ( $\text{V}_o^{++}$ ) ionized oxygen vacancies, neutral oxygen vacancies ( $\text{V}_o$ ), and oxygen interstitials ( $\text{O}_i$ ). According to previous studies, there are three main defects involved in the DLE:  $\text{V}_o^+$  (2,45 eV),  $\text{V}_o^{++}$  (2,23 eV),  $\text{O}_i$  (2 eV)<sup>35</sup>. Besides, in our case, the ratio  $I_{\text{NBE}}/I_{\text{DLE}}$  indicates the good crystallinity of produced nanostructures. It was shown previously, that the increased concentration of oxygen sites in the ZnO leads to the variation of antibacterial properties of produced nanoparticles<sup>37,38</sup>. Therefore, it is expectable that the different ZnO nanostructures would demonstrate various antibacterial behaviors.

The OD measurement is mainly used as a quick and affordable method to monitor the growth of bacteria during their culture in liquid media but can also be applied for testing antibacterial properties of different nanostructures and nanomaterials<sup>39-41</sup>. The higher the number of bacteria in the solution, the greater the  $\text{OD}_{570}$  value, and thus the lower antibacterial activity of the added material, ZnO nano- and microparticles in this case.

The high antibacterial activity towards *E. coli* bacteria showed ZnO NPs, NRs, and HSs (Fig. 5). This could be related to the surface area, which is the largest for 3D heterostructures, and volume to surface ratio, the highest for nanoparticles and nanorods, respectively. The lowest surface area of ZnO Ps and TPs led

to a decrease in antibacterial activity. Even at the highest concentration (1 mg/mL), their viability reached about 62% and 76%, respectively.

As it was mentioned above, the OD<sub>570</sub> is proportional to the total number of bacteria, however, it does not provide any information regarding their viability. Thus, additionally, the LIVE/DEAD BacLight staining with the use of confocal laser scanning microscopy was carried out. To recognize live and dead bacteria two fluorescence dyes were used. SYTO 9 stains in green both live and dead cells, and propidium iodide (PI) stains in red dead cells, that have lost membrane integrity. Confocal images of *E. coli* and *S. aureus* were shown in Figs. 7 and 8, respectively. All of the untreated bacterial cells showed green fluorescence, due to the viable cells, indicating intact cell wall structure.

As can be seen in Fig. 7, the co-incubation of *E. coli* cells with ZnO nano- and microparticles for 2h was enough to influence the bacteria viability. In all cases, the number of bacterial cells decreased. Moreover, the red signals, indicating dead cells appeared. The counting of both signals, collected from nine randomly selected images for all samples, allowed us to determine the percentage of live and dead cells. Based on this, we can conclude that ZnO NRs and ZnO HSs were the most effective towards *E. coli*, with the number of dead cells above 50%. As it turned out after optical density measurements, also here, the least effective were ZnO particles and tetrapods.

For the *S. aureus* strain (Fig. 8) ZnO materials exhibited a stronger antibacterial effect than for *E. coli*. The number of bacterial cells was significantly reduced compared to the non-treated control. Moreover, after ZnO nanoparticles treatment the number of dead cells was about 50:50 to living cells, whereas, for nanorods and heterostructures, the percentage of viable cells decreased to about 20%. Also, the ZnO TPs decreased cells viability, however in this case the general number of cells seems to be higher. As in the case of *E. coli*, the ZnO particles show the weakest antibacterial activity. The obtained results for viability analysis were compatible and comparable with optical density measurements. As it was mentioned before the antibacterial effect could be related to the surface-to-volume ratio of ZnO materials, which is consistent with Azam A. *et al.*<sup>42</sup> whose indicated that the antimicrobial activity increased due to a decrease in particle size of zinc oxide nanoparticles, as well as with Yamamoto O.<sup>43</sup>, who indicated that smaller size of zinc oxide nanoparticles exhibits greater antibacterial activity than microscale particle.

Four mechanisms of action have been proposed as responsible for the antibacterial properties of zinc oxide particles, namely the production of reactive oxygen species (ROS)<sup>44,45</sup>, the loss of cellular integrity after contact of ZnO materials and the cell wall<sup>46</sup>, ZnO NPs internalization<sup>47</sup>, as well as the release of Zn<sup>2+</sup> ions<sup>48,49</sup>. The mechanism of nanomaterials toxicity is not specific, and thus bacteria are not able to get the resistance for nanoparticles treatment<sup>50</sup>. The differences in antimicrobial activity of ZnO materials used in this study depending on their size and shape could be related to the distinct mechanism of action. The smallest nanoparticles and nanorods probably internalize bacterial cells, whereas particles of micrometer size like tetrapods and heterostructures can interact with cell walls, through ion diffusion and free radicals generation, which further enter the cells, destroying cellular components such as DNA, proteins, and lipids.

Generally it is thought that Gram-negative bacteria are more susceptible than Gram-positive to attack by external factors, such as metal nanoparticles like it was observed for silver<sup>51</sup> and gold nanoparticles<sup>52</sup>. As the main reason for differences in bacterial susceptibility and resistance the bacterial cell walls composition is suggested. In the case of Gram-negative bacteria, bacterial cells are covered by a layer of lipopolysaccharides (1–3 µm thick) and thin peptidoglycans (~ 8 nm thick), whereas Gram-positive bacteria possess a peptidoglycan layer (~ 80 nm thick) with covalently attached teichoic and teichuronic acids<sup>53</sup>. However, here we observed that Gram-negative *Escherichia coli* were less susceptible to ZnO materials than Gram-positive *S. aureus*, which is consistent with Tayel and co-workers<sup>54</sup>, who showed that the inhibition of Gram-negative bacteria requires higher concentrations of ZnO NPs. This is likely because the peptidoglycan layer that surrounds Gram-positive bacteria can promote ZnO attack inside the cell, while the cell wall components of Gram-negative bacteria, such as lipopolysaccharides, can counter this attack. Similar results were found by d'Agua R.B. *et al.*<sup>55</sup>, who showed that Gram-positive bacteria were more sensitive to peroxide hydrogen than Gram-negative bacteria. It was also seen in our earlier studies with gelatin-ZnO nanofibers<sup>56</sup>.

At cytotoxicity studies (Fig. 6), results showed that all nano- and microparticles are biocompatible at low concentrations. The greatest decrease in viability above 100 µg/mL was seen with the administration of ZnO nanorods and nanoparticles, and then heterostructures. The differences could be related to the distinct mechanism of action, and different levels of nanoparticles internalization. The toxicity mechanism is comparable with antibacterial action, which means that the ROS generation, mechanical harm due to direct interaction of ZnO materials with the cells, cells internalization, as well as zinc ions releasing could be responsible for cytotoxic activity towards human cells. Moreover, Cho W-S. *et al.*<sup>57</sup> indicated that zinc oxide nanoparticles rapidly dissolve under acidic conditions (pH 4.5), which may occur after absorption of nanoparticles into lysosomes in the process of endocytosis, leading in turn to cell death.

## 4. Conclusion

The simple chemical and physical methods were used to obtain ZnO nano- and microparticles with different morphologies. The morphology effect on luminescent and surface properties, antibacterial activity toward Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, and cytotoxicity toward normal and cancer cells was studied. All obtained ZnO materials were highly crystalline with hexagonal wurtzite crystal structures and monodispersed. N<sub>2</sub> adsorption-desorption analysis showed that ZnO in nanorange (nanoparticles and nanorods) had a higher surface area. All samples performed high green emission caused by structural defects in ZnO. Due to high crystallinity ZnO tetrapods (TPs) showed the strongest photoluminescence. The antimicrobial activity measurements of ZnO nano- and microparticles indicated that for all samples, the bacteria number decreased in a concentration-dependent manner. Results showed that surface area has a significant impact on antibacterial performance. The high surface area of ZnO nanoparticles led to the most effective antibacterial activity towards *E. coli* bacterial strains. Among used bacteria, the Gram-positive *S. aureus*

strains were more sensitive to the antimicrobial action of ZnO nano- and microparticles than *E. coli*. All kinds of ZnO materials were biocompatible towards cervical cancer cell line (HeLa) and normal human fibroblasts (MSU1.1), maintaining a high level of viability (up to 100 µg/mL).

As we can see materials of different sizes and varieties of morphology can be used in biomedical fields. According to our present results, highly luminescent and biocompatible ZnO particles in nanorange (especially ZnO NPs and NRs) are planned to be used for cancer diagnostic and therapy. At the same time, the microparticles will be used for biosensors, drug adsorption, and release experiments.

## 5. Material And Methods

### 5.1. Materials

Zinc acetate dihydrate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \times 2\text{H}_2\text{O}$ ,  $\text{Zn}(\text{Ac})_2$ , Sigma Aldrich), sodium hydroxide (NaOH, Stanlab), methanol (MeOH, Sigma Aldrich), ethanol (EtOH, Sigma Aldrich), tri-sodium citrate dihydrate ( $\text{HOC}(\text{COONa})(\text{CH}_2\text{COONa})_2 \times 2\text{H}_2\text{O}$ , Sigma Aldrich), Zn powder (Sigma Aldrich) were all of the analytical grades and used as starting materials.

### 5.2. Synthesis of ZnO nano- and microparticles

#### 5.2.1 Synthesis of ZnO nanoparticles

ZnO nano- and microparticles (nanoparticles (ZnO NPs), nanorods (ZnO NRs), hierarchical flower-like structures (ZnO HSs), and tetrapods (ZnO TPs)) were synthesized using chemical and physical approaches presented in schematic illustration (Fig.S2).

The ZnO NPs and ZnO NRs were obtained by the modified low-temperature sol-gel route described in <sup>58</sup>. The proposed synthesis approach is facile and allows to obtaining of the crystalline monodisperse ZnO NPs (Fig.S2a) at a temperature below 100 °C. In ZnO NPs synthesis  $\text{Zn}(\text{Ac})_2$  was dissolved in methanol at a constant temperature of 60 °C. Then the solution of NaOH in methanol was added to  $\text{Zn}(\text{Ac})_2$  solution under vigorous stirring. The complete hydrolysis of zinc acetate with the addition of NaOH in a methanol solution results in the formation of ZnO colloid (gel). After about 3h of stirring, white ZnO nanoparticles were separated from the mother liquor, washed with methanol twice, and dried.

In the synthesis of ZnO NRs, the reaction mixture (prepared in the same manner as for ZnO NPs synthesis) was concentrated 10 times by solvent evaporation. After about 12h of stirring white precipitate was separated, washed, and dried as described above.

ZnO particles (ZnO Ps) were obtained using ZnO NPs (obtained earlier) by their annealing at 900 °C for 2h (Fig.S2b).

#### 5.2.2. Synthesis of ZnO microparticles

The ZnO 3D hierarchical structures (ZnO HSs) (Fig.S2c) were prepared by a template-free solvothermal approach according to Fang *et al.*<sup>59</sup>. First, Zn(Ac)<sub>2</sub> and sodium citrate were dissolved in H<sub>2</sub>O. Separately NaOH (1g) was dispersed in EtOH at 60 °C. After complete dissolution of both precursors, NaOH solution was added dropwise to Zn<sup>2+</sup> solution. The resulting mixture was agitated at room temperature for 1h. Then, for solvothermal reaction (150 °C for 24h) the resulting mixture was transferred into a Teflon-lined stainless steel autoclave. After cooling down final product was centrifuged, washed with H<sub>2</sub>O, and dried at 60 °C for 12h before characterization.

ZnO tetrapods (ZnO TPs), (Fig.S2d) were obtained by the simple catalyst-free oxidative-metal-vapor-transport method. The method was based on thermal evaporation of Zn powder at 1000 °C for 1h in the air<sup>29</sup>.

### 5.3. Characterization

Structural characteristics of the obtained ZnO nano- and microparticles were measured by powder X-ray diffraction (XRD). The studies were carried out on powdered samples using an Empyrean (PANalytical) diffractometer with Cu K $\alpha$  radiation ( $\lambda=0.154$  nm), reflection-transmission spinner (sample stage), and PIXcel 3D detector, operating in the Bragg-Brentano geometry. Scans were recorded at room temperature in angles ranging from 20° to 80° (2 $\theta$ ) with a step size of 0.006° and continuous scan mode. The morphology of the obtained samples was studied by high-resolution transmission electron microscopy (HRTEM; JEOL ARM 200F) and scanning electron microscopy (SEM, JEOL, JSM-7001F). For the determination of the specific surface area of the samples, the N<sub>2</sub> adsorption/desorption isotherms were measured at -196 °C on a Quantachrome Nova 1000 apparatus. The samples were outgassed at 150 °C for 15h in a vacuum before the measurements. The specific surface area was determined using the BET method. The total volume of pores was calculated using the single point mode (at p/p<sub>0</sub> = 0.98). The pore size distribution was determined by applying the Barrett-Joyner-Halenda (BJH) method from the desorption branch of the isotherm.

The photoluminescence (PL) of the samples was measured at room temperature using Kimmon HeCd laser. The excitation wavelength was 325 nm, and the power was around 2 mW. The PL spectra were recorded in the range from 360 to 1000 nm by Ocean Optics Spectrometer QE65 pro.

### 5.4. Biological characterization

#### 5.4.1 Cell line and cell culture conditions

Human cervical cancer cell line HeLa was obtained from American Type Culture Collection (ATCC). Human fibroblast cell line MSU1.1 was obtained from Prof. C. Kieda (CBM, CNRS, Orléans, France). Cells were cultured in a complete medium Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 units/ml penicillin, 100  $\mu$ g/mL streptomycin, and grown at 37 °C in a humidified atmosphere containing 5 % CO<sub>2</sub>.

### 5.4.2 Cytotoxicity analysis

Cervical cancer cells (HeLa) and normal fibroblasts (MSU1.1) were used for *in vitro* cellular toxicity studies of ZnO nano- and microparticles. Cells ( $1 \times 10^3$  cells/well) were seeded onto 96-well plates and incubated overnight at 37 °C under a 5 % CO<sub>2</sub> atmosphere. The medium in the wells was then replaced with a fresh medium containing increasing concentrations of ZnO particles (from 0.1 µg/mL to 1 mg/mL) and incubation was continued for 24h. The medium without ZnO particles was used as a negative control. The effect of the ZnO nano- and microparticles on cell proliferation and viability was determined by WST-1 assay according to the manufacturer's instructions. Briefly, 10 µL of WST-1 solution was added to each well and the plates were further incubated. After 2h the absorbance was measured with a microplate reader (AnthosZenyth 340rt) at 450 nm and 650 nm as reference. The mitochondrial function and, by extension, the relative cell viability (%) related to the negative control was calculated by test sample/negative control  $\times 100$  %. Data are reported as the average  $\pm$  standard deviation (SD) of wells performed in quadruplicate.

### 5.4.3 Bacterial growth inhibition study

Stock cultures of bacterial strains *E. coli* ATCC 35218 and *S. aureus* ATCC 29213 were stored in 30 % glycerol. The strains were cultured in LB Broth Lennox at 37 °C with constant agitation at 230 rpm for 24h. The bacterial cultures were then diluted between  $2.5 \times 10^5$  and  $5 \times 10^5$  cells/mL in LB broth medium. 100 µL of the cell suspension was then added to each well of a 96-well plate. Appropriate concentrations of freshly prepared ZnO particles solutions (10, 100, 250, 500, 1000 µg/mL) were added and placed at 37 °C in an incubator. Turbidity of the suspension, as a measure of bacteria growth, was recorded spectrophotometrically at 570 nm (OD<sub>570</sub>) with a microplate reader (Anthos Zenyth 340rt) after 24h. To avoid potential interference during optical measurements caused by the light scattering properties of the solutions, the same liquid medium without microorganisms, but containing the same concentration of studied samples, were used as blank controls. The positive control was bacterial cultures without ZnO particles treatment. All experiments were performed in triplicates.

### 5.4.4 Bacterial cells viability analysis

To visualize the effect of *S. aureus* and *E. coli* cells' interaction with ZnO particles with different morphology, the fluorescence assay LIVE/DEAD BacLight Bacterial Viability Kit (Life Technologies) was applied and observed under a confocal laser scanning microscope (Olympus, FV1000). In brief, the *S. aureus* and *E. coli* overnight cultures were used to inoculate of fresh LB medium. At the mid-log phase of bacterial growth, the ZnO nano- and microparticles solutions were added to the final concentration of 500 µg/mL and allowed to grow for 3h. From these cultures, 1 mL of each bacterial solution was centrifuged at 5000 rpm for 10 minutes. The pellets were resuspended in HEPES buffer, centrifuged, and washed with HEPES buffer three times more. Finally, the pellets were resuspended in 500 µL of HEPES buffer, and the combination of fluorescent dyes SYTO9 and PI were mixed in identical volumes. 1.5 µL of their mixture was added to each bacterial suspension and incubated in dark for 15 minutes. Fluorescence images were

taken by trapping 5  $\mu\text{L}$  of stained bacterial samples mounted on glass slides with mounting medium and cover with coverslips. For each sample, nine randomly selected images were captured by the microscope, and live/dead cells were counted to ascertain percentage viability. Data presented are live (green) and dead (red) cells as a percentage of the total cell number (live + dead).

## Declarations

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### Authors' contributions

N.B. wrote the first version of this manuscript and prepared all samples.

N.B., L.P., I.I, G.N., M.J., E.J., and SJ reviewed and prepared the final version of the manuscript.

L.P. performed bacterial culture and all bacteria-based experiments and human cell cultures. M.J. and N.B. performed XRD analysis of the obtained samples.

G.N. and I.I. performed scanning and transmittance electron microscopy.

E.J. provided and characterized the textural properties of the obtained samples.

I.I. studied the photoluminescence properties.

All authors commented and have approved the final version of the manuscript.

### Conflict of interests

There is no conflict of interests.

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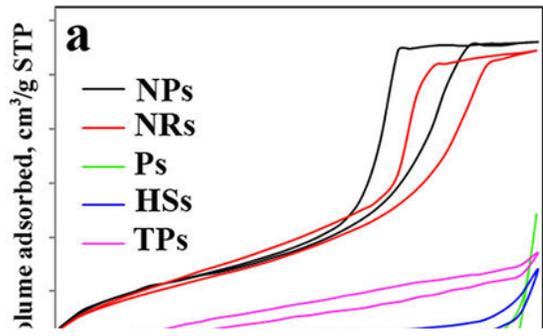
## Figures

### Figure 1

TEM and SEM images of ZnO nano- and microparticles: nanoparticles (a), nanorods (b), particles (b), hierarchical structures (d), tetrapods (e).

### Figure 2

Typical XRD pattern of ZnO nano- and microparticles.



**Figure 3**

Nitrogen physisorption isotherms and pore size distribution for indicated samples.

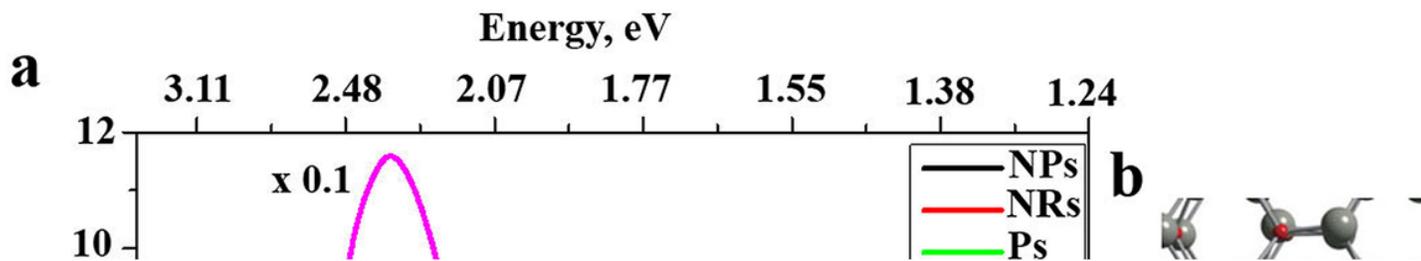
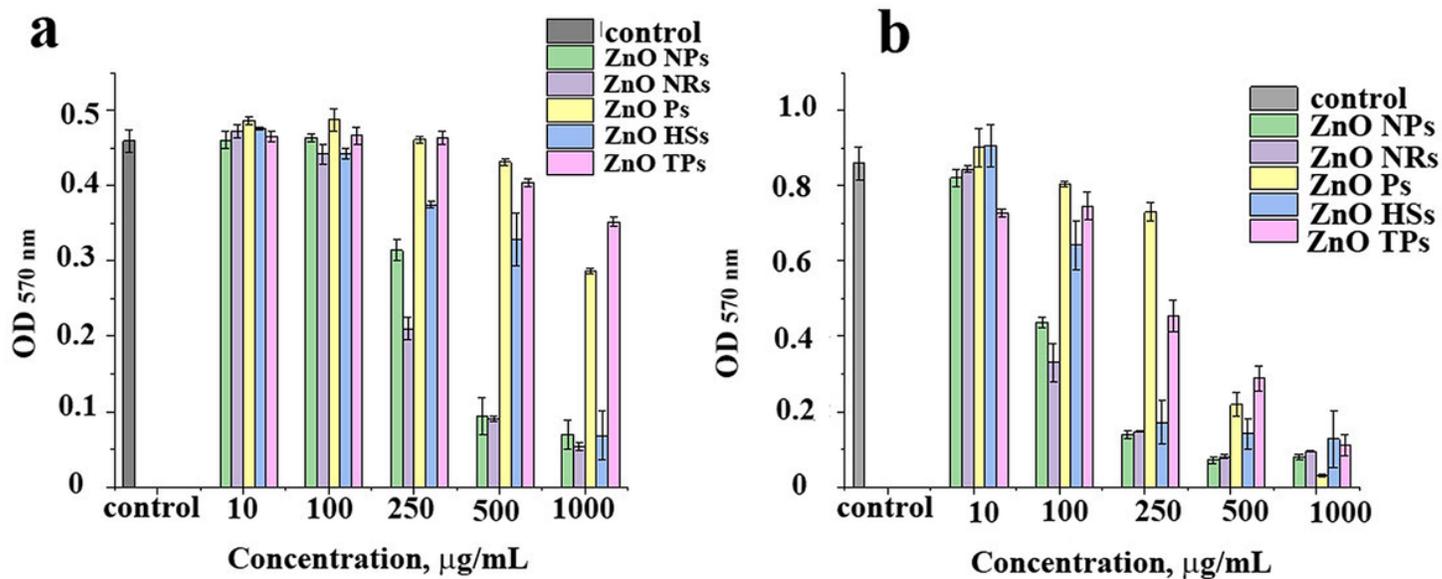


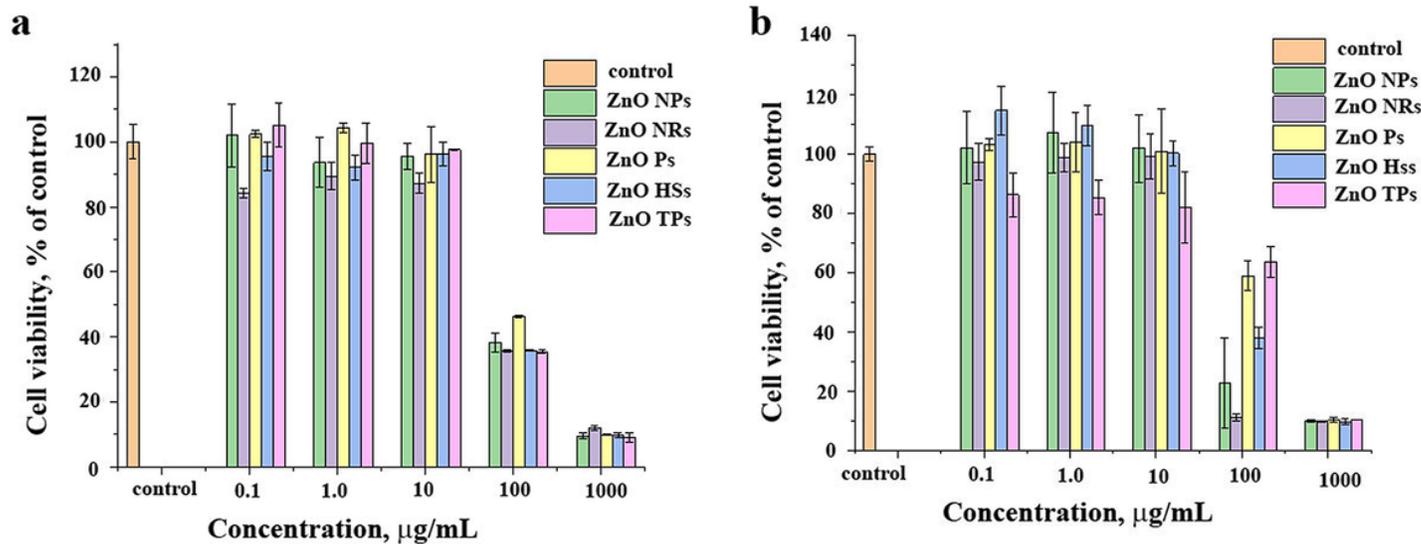
Figure 4

PL spectra of ZnO nano- and microparticles.



**Figure 5**

Antimicrobial activity of ZnO nano- and microparticles against *E. coli* (a) and *S. aureus* (b) evaluated by measuring optical density at 570 nm (OD<sub>570 nm</sub>) after incubation at 37 °C, 220 rpm for 24h. Data presented as mean ± SD of three independent experiments, each performed in triplicates.



**Figure 6**

WST-1 assay performed on normal human fibroblasts MSU1.1 (a) and cervical cancer HeLa cells (b) after 24h of incubation with indicated ZnO materials.

**Figure 7**

BAC Live/dead assay. *E. coli* bacteria (AATC: 35218 *E. coli* strain) after 2h incubation with ZnO nano- and microparticles on distinct morphology at concentration 500 µg/mL. Scale bar: 20 µm.

**Figure 8**

BAC Live/dead assay. *S. aureus* bacteria (AATC: 29213 *S. aureus* strain) after 2h incubation with ZnO nano- and microparticles on distinct morphology at concentration 500 µg/mL. Scale bar: 10 µm.

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