ROS-dependent anticandidal activity of zinc oxide nanoparticles synthesized by using egg albumen as a biotemplate

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Abstract
Zinc oxide nanoparticles (ZnO NPs) have attracted great attention because of their superior optical properties and wide application in biomedical science. However, little is known about the anticandidal activity of ZnO NPs against \textit{Candida albicans} (\textit{C. albicans}). This study was designed to develop the green approach to synthesize ZnO NPs using egg white (denoted as EtZnO NPs) and investigated its possible mechanism of antimicrobial activity against \textit{C. albicans} \textit{077}. It was also notable that anticandidal activity of EtZnO NPs is correlated with reactive oxygen species (ROS) production in a dose dependent manner. Protection of histidine against ROS clearly suggests the implication of ROS in anticandidal activity of EtZnO NPs. This green approach based on egg white-mediated synthesis of ZnO NPs paves the way for developing cost effective, eco-friendly and promising antimicrobial nanomaterial for applications in medicine.

Keywords: nanoparticles, \textit{Candida albicans}, anticandidal, ROS

Classification numbers: 2.05, 4.02

1. Introduction
The brutality of antifungal drugs in pharmacotherapy has led to the development of widespread multidrug resistance (MDR) in \textit{Candida albicans} [1, 2]. Unfortunately, most of these drugs, being chemical in nature and having bulk form, are too reactive and unsuitable for human use [3]. With the rising toxicity and development of MDR in \textit{C. albicans} isolates, the search for new medical treatments beyond conventional antifungal drugs has become a key aim of public health research [4, 5]. The failure of drugs to control infection makes it crucial to find new alternatives to currently available drugs. Possible innovative strategies encompass the inhibition of \textit{C. albicans} growth through the use of nanomaterials.

Zinc oxide (ZnO) nanoparticles (NPs) are being widely used in health care commercial products due to their unique properties such as UV light absorption, and being catalytic, semi-conducting, magnetic and antimicrobial [6–8]. ZnO NPs exhibit unique characteristics that may completely differ from bulk-sized ZnO materials in terms of higher proportion of atoms on the surface of nano-sized materials, light absorption, electronic band gap, and being catalytic and antimicrobial compared to bulk-sized ones [7–11]. Therefore, owing to the large surface-to-volume ratio, ZnO NPs give rise to high reactivity towards biological responses [12, 13]. Numerous studies suggest that ZnO particles with different
sizes and shapes have different degrees of antimicrobial activities [12, 14–17]. ZnO NPs also have excellent stability, robustness, biocompatibility and longer shelf life compared to other antimicrobial agents [12, 18]. Although the toxic impact of ZnO NPs on living cells has been proved, relatively low concentrations of ZnO NPs are non-toxic to eukaryotic cells [18–22]. Consequently, ZnO NPs are believed to be non-toxic, biosafe and have been used as drug carriers in cosmetics and fillings in medical materials [13, 18, 23, 24].

Multiple lines of evidence propose that ZnO NPs increase oxidative stress through excess production of reactive oxygen species (ROS), namely hydroxyl radicals (•OH) and singlet oxygen (1^O_2), which dominantly contribute to the antimicrobial activity of ZnO NPs [12, 19, 25–28]. Other mechanisms such as cytoplasmic membrane disruption and electrostatic binding of ZnO NPs to the cell surface of the microbial pathogen have been reported [19, 27]. Although many controversies have been raised on the mechanistic aspects of the antimicrobial activity of ZnO NPs, recently the production of ROS governed by the electronic band gap property of metal-oxide NPs were considered to trigger the actual ‘mechanism’ [28]. The electronic band gap property of ZnO NPs is influenced by the structural parameters (size and pH) and carrier molecule concentrations [29, 30]. The debate in the scientific community continues in determining exact mechanisms that give rise to the antimicrobial properties of ZnO NPs. Therefore, a fundamental understanding on electronic band gap property of ZnO NPs becomes crucial to the tailoring of the new antimicrobial metal oxide NPs and effectively reduces experimental testing cost.

The properties of ZnO NPs are strongly dependent on their morphology and the size of the crystal [31]. Morphology and size can play a key role in many optoelectronic and antimicrobial applications. Therefore developing a shape-controlled ZnO NPs synthesis method is crucial for exploring the potential of ZnO NPs as a source of smart and functional materials. To date, numerous distinct molecules such as tri-n-octylphosphine oxide (TOPO), sodium dodecyl sulfate (SDS), polyoxyethylene stearly ether (Brij-76), bovine serum albumin (BSA) and citric acid have been used to control the size and shape of ZnO NPs during synthesis [11, 32]. In recent years, researchers have begun to use natural bioresource with excellent biocompatibility as a template for the synthesis of nanomaterials. However, information about mechanisms of antimicrobial potential of ZnO NPs synthesized via egg albumen acting as a template is not available. There is thus a need to identify a novel class of biocompatible anticandidal ZnO NPs, which could present us with new opportunities for the development of safe and effective antibiotic drugs for treating *C. albicans* [33, 34]. In present investigation we synthesized anticandidal ZnO NPs using egg albumen as a biotemplate.

### 2. Experimental details

#### 2.1. Materials

Zinc acetate, potassium bromide, ammonia and other chemicals were procured from SRL, India. Sabouraud’s dextrose (SD) nutrient media for the cultivation of *C. albicans* 077 were obtained from the HiMedia Laboratories, Mumbai, India. 2,7-dichlorofluorescin diacetate (DCFH-DA) and histidine were obtained from Sigma Aldrich (St Louis, Missouri, USA). All other chemicals used were of the highest purity available from commercial sources.

#### 2.2. Synthesis of ZnO NPs

The synthesis of ZnO NPs using egg albumen as a biotemplate (denoted as EtZnO NPs) was performed according to the
method of Nouroozi and Farzaneh [35]. In brief, freshly extracted 20 ml of egg albumens (5 mg ml−1) were prepared in mixed milli-Q (MQ) water and mixed drop-wise into 80 ml of aqueous zinc acetate [Zn(CH₃COOH)₂·2H₂O] solution. The mixture was stirred for 10 min at room temperature to form the colloidal solution. The colloidal solution was precipitated by the addition of ammonia (NH₃) at ~pH 7.0 and centrifuged at 5000 rpm for 10 min and twice washed with the MQ water. The washed material was collected and dried in the vacuum oven and grounded into a fine powder (figure 1(a)). The obtained dried powder was subjected to sintering at 500 °C for 3 h and synthesized EtZnO NPs were stored in a dry and dark place until further use (figure 1(b)).

2.3. Characterization of EtZnO NPs

The x-ray diffraction (XRD) patterns of the powdered sample were recorded on a MiniFlex™ II benchtop XRD system (Rigaku Corporation, Tokyo, Japan) operating at 40 kV [36]. For the morphological analysis, transmission electron microscopy (TEM) of aqueous EtZnO NPs was carried out on JEOL 100/120 kV TEM (JEOL, Tokyo, Japan) with an accelerating voltage of 80 kV. Briefly, a drop of aqueous EtZnO NPs was placed on the carbon coated copper grid and air dried under dark. The elemental analysis was determined using the Oxford Instruments INCAx-sight energy dispersive x-ray (EDAX) spectrometer equipped TEM. Thin film of the EtZnO NPs was prepared on the borosilicate glass slide for the analysis of surface morphology. The prepared thin film was analyzed on the atomic force microscopy (AFM) Innova SPM Veeco in tapping mode. Commercial etched silicon tips as scanning probes with an accelerating voltage of 80 kV .

2.4. Assessment of antifungal activity of EtZnO NPs

2.4.1. Growth condition. Clinical isolate of C. albicans 077 was obtained from Department of Microbiology, JN Medical College, Aligarh, UP, India. Stock culture was maintained on slants of SD agar (containing dextrose 40 g l⁻¹, mycoligal, peptone 10 g l⁻¹ and agar 15 g l⁻¹) at 4 °C. The primary culture of the C. albicans 077 was prepared from the stock slant into the SD broth medium and incubated at 37 °C for 48 h (stationary phase, 10⁶ cfu ml⁻¹). The primary culture (~1 ml) was re-inoculated into the 50 ml fresh SD broth and grown for ~12 h to mid-log phase (~10⁷ cfu ml⁻¹) at 37 °C. All experiments were performed from the mid-log phase (~10⁷ cfu ml⁻¹) freshly grown C. albicans 077 culture in triplicates.

2.4.2. Agar disc diffusion assay. For agar disc diffusion assay, 5 ml mid-log phase grown of C. albicans 077 was centrifuged at 4000 rpm for 5 min at 4 °C. Then, the pellet was washed with 1× phosphate buffer saline (PBS) and resuspended in 500 μl normal saline solution (NSS). A 100 ml of the suspended cells were spread uniformly on SD agar plates and the plates were incubated at 37 °C for 30 min. The seeded petri plates were used for loading various concentrations of EtZnO NPs (0, 5, 10 and 15 μg ml⁻¹) onto the pre-sterilized filter paper discs. The petri plates were incubated at 37 °C for 40 h, after that zone of inhibition was recorded.

2.4.3. In vitro killing assay. Cell suspension of C. albicans 077 was obtained similarly as described in the agar disc diffusion assay and suspension (100 μl) was dispensed into the 96-well microtiter plate in triplicates. Various concentrations of EtZnO NPs (0, 5, 10 and 15 μg ml⁻¹) diluted in sterile SD broth medium were added and incubated at 37 °C for 2 h. The whole suspension of the plate wells was spread on the SD agar plate and incubated at 37 °C for 40 h. Anticandidal activity was detected by the dose dependent reduction in cfu ml⁻¹.

2.4.4. Growth kinetics assay. To see the effect of EtZnO NPs on the growth kinetics of C. albicans, 50 ml of SD broths in individual flask were inoculated with 100 μl of the NSS suspended cells. Different concentrations of EtZnO NPs (0, 5, 10 and 15 μg ml⁻¹) to be tested were applied in the individual flask. The flasks were incubated at 37 °C for 40 h and time dependent growth kinetics were recorded turbidometrically at A₅₉₅ nm. The turbidity backgrounds induced by EtZnO NPs were subtracted from the final reading.

2.5. Measurement of intracellular ROS generation

The produced intracellular ROS was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA) [37]. The DCFH-DA passively enters the cell where it reacts with ROS to form highly fluorescent dichlorofluorescein (DCF). Briefly, DCFH-DA (10 mM) stock solution in methanol (HPLC grade) was diluted in culture medium to yield a working solution (100 μM). At the end of exposure, C. albicans 077
cells were washed twice with ice-cold 1× PBS and then incubated in 1 ml of working solution of DCFH-DA at 37 °C for 30 min. The C. albicans 077 cells were treated with different concentrations of EtZnO NPs for 40 h, lysed in alkaline solution and centrifuged at 5000 rpm for 10 min. Then, a 200 µl of supernatant was transferred to the other fresh well of microtiter plate and fluorescence was measured at excitation of $\lambda_{485\text{nm}}$ and emission of $\lambda_{520\text{nm}}$ using a microplate reader (Bio-Rad laboratories Inc., Hercules, CA, USA).

Figure 2. EtZnO NPs structural characterizations. (a) XRD patterns of EtZnO NPs were recorded in the range of 20–80° of 2θ angles. XRD pattern of EtZnO NPs depicted the well-resolved diffraction peaks of the crystalline wurtzite particles structure. (b) SEM of EtZnO depicts the microstructure EtZnO NPs. (c) The EDAX spectrum proves the elemental composition of as-synthesized EtZnO NPs. (d) EDAX map indicates the presence of Zn and O. (e) TEM of EtZnO depicts the NPs structure. (f) 2D and (g) 3D atomic force micrographs illustrate the nanostructure of as-synthesized EtZnO NPs, respectively.
3. Results and discussion

3.1. Characterization of EtZnO NPs

3.1.1. Structural characterization. The crystal structure of EtZnO NPs was characterized by XRD with CuKα radiation (λ = 0.15418 nm). The data revealed that the well resolved 11 XRD peaks were obtained at 2θ = 31.01°, 34.21°, 35.64°, 47.10°, 56.02°, 62.15°, 65.68°, 67.51°, 69.01°, 72.08° and 76.24° which correspond to the crystal planes [100], [002], [101], [102], [110], [103], [200], [112], [201], [004] and [202] of polycrystalline wurtzite structure (Zincite, JCPDS [5...]...
The FTIR spectrum of EtZnO NPs shows absorption band at 511 cm\(^{-1}\), which corresponds to E\(_2\) mode of hexagonal ZnO wurtzite structure [40]. The band at the position 511 cm\(^{-1}\) reflects EtZnO NPs stretching frequency of Zn–O bonds. The intermediate product hydrozincite (without sintering) shows the absorption band at 677 cm\(^{-1}\) due to the presence of the hydroxide phase of the EtZnO NPs (figure 3). The sintering process also indicated the disappearance of the egg albumen signature peaks which confirmed its decomposition from the EtZnO NPs. The comparative FTIR spectra confirmed that the transformation of the hydroxide to the oxide phase occurred during the sintering process of EtZnO NPs. The bands at \(\sim\)1557 and 3452 cm\(^{-1}\) were due to the stretching frequency of hydroxyl groups of absorbed water from ambient atmosphere [41, 42].

3.1.2. Optical and thermal characterization. The electronic structure of ZnO NPs is characterized by the band gap \((E_g)\), which is essentially the energy interval between the valence band \((E_v)\) and the conduction band \((E_c)\), each of which has a high density of states [28]. The generation of a specific type of ROS such as \(\cdot\text{OH}, \cdot\text{O}_2,\) or \(\cdot\text{O}_2^-\) is governed by the metal oxide NPs related to the electronic structures as well as the redox potentials (EH) of different ROS generation reactions [28, 43, 44]. The oxidative stress induced by ZnO NPs is thought to be the main mechanism of their antimicrobial activity [1, 9, 28, 43–45]. Therefore, we calculated the electronic band gap energy \((E_g)\) of EtZnO NPs because of their broad application in antimicrobial properties. The ZnO-NPs (10 \(\mu\text{g ml}^{-1}\)) were dispersed in ethanol by using ultra sonication and then the solution was used to perform the UV–Vis measurement (figure 4(a)). The spectrum reveals a characteristic absorption peak of EtZnO NPs at wavelength of \(\sim\)360 nm which can be assigned to the intrinsic band-gap absorption of EtZnO NPs due to the electron transitions from the valence band to the conduction band \((\text{O}_2\rightarrow \text{Zn}_{3d})\) (figure 4(a)) [46, 47]. The sharp absorption peak of EtZnO NPs also indicated the narrow nanosize particle distribution. Moreover, the egg albumen showed the absorbance at wavelength of \(\sim\)360 nm due to the presence of the proteins in their composition (figure 4(a)). Thus,
Figure 6. Anticandidal activity of EtZnO NPs. (a) Zone inhibition and (b) in vitro killing of assays show the anticandidal activity of EtZnO NPs against *C. albicans* 077. (c) The graph shows the dose-dependent size of the zone of inhibition formed by EtZnO NPs. (d) SEM based observation of change in cell morphology of *C. albicans* 077, when treated with 15 µg ml⁻¹ of EtZnO NP. (e) Growth curve analysis depicts the growth inhibition of *C. albicans* 077 in the presence of different concentrations of EtZnO NPs.

Results indicate that the egg albumen does not influence the absorption of EtZnO NPs, suggesting that EtZnO NPs were fully functional. The electronic band gap ($E_g$) of the EtZnO NPs was determined by employing Tauc relationship as follows:

$$a h \nu = A (h \nu - E_g)^{n},$$

where $a$ is the absorption coefficient (2.303A/t), $h$ is Planck’s constant, $\nu$ is the photon frequency, and $E_g$ is the electronic band gap. The value of $n = 1/2$, 3/2, 2 or 3 depending on the nature of the electronic transition responsible for absorption and $n = 1/2$ for direct band gap semiconductor. An extrapolation of the linear region of a plot of $(ah\nu)^2$ on the $y$ axis versus photon energy ($h\nu$) on the $x$-axis gives
of EtZnO NPs alone, suggesting the involvement of ROS in anticandidal activity of EtZnO NPs. albicans 077 cells, resulting in abrogate the antimicrobial property of EtZnO NPs, however, growth inhibition was recorded in the presence of EtZnO NPs alone, suggesting the involvement of ROS in anticandidal activity of EtZnO NPs.

The optical properties of ZnO are more interesting since confinement of charge carriers in the restricted volume of the small particles can lead to effects such as widening of $E_g$ [51]. Since the EtZnO NPs have a wide $E_g = 3.55$ eV good electron transporting properties [52], they can be utilized as an anticandidal agent, which can efficiently kill the C. albicans via ROS production. The data suggest that the egg albumen facilitates the electronic band gap widening effect via controlling the nucleation and surface capping of the intermediate products (hydrogencite) [51]. The amino acid moiety in the egg albumen is sufficient to form proper capping, resulting in the formation of smaller sized EtZnO NPs, so as to keep the wide $E_g$ [53].

The photoluminescence behavior of EtZnO NPs could give information on energies and dynamics of photogenerated charge carriers as well as on the nature of the emitting states [54]. Figure 4(c) shows photoluminescence emission spectra in the visible range of EtZnO NPs and monitoring by measuring the dose-dependent changes in the intensity of EtZnO NPs. The emission spectra have a broad band with a maximum $\sim$532 nm which can be ascribed to the singly ionized oxygen vacancy with exited EtZnO NPs at $\sim$A$_{370}$nm. The green emission in the visible region arises when a photogenerated hole (O$^-$) trapped at a deep level above the valence band recombines with an electron trapped at a shallow level below the conduction band [55]. Usually, the emission intensity and band width are related to the size and nature of the carrier trapped states located at the surface of the nanocrystals [56]. From figure 4(d) we can see that about $\sim$5% of the total weight loss of EtZnO NPs might be due to the evaporation of water adsorbed on the surface of NPs [57, 58]. The differential thermal analysis (DTA) of EtZnO NPs shows the endothermic reaction peak at 135°C possibly due to change of phases [59].

3.2. Anticandidal activity of EtZnO NPs

Before starting the experiments we determined the stability of EtZnO NPs (45 $\mu$g ml$^{-1}$) in SD broth culture medium up to 40 h at 37°C through the change in UV–visible absorbance characteristics. Significant change in agglomeration and absorbance of EtZnO NPs was not observed (figure 5(a)), suggesting that the SD broth culture medium does not significantly affect EtZnO NPs’ stability, size and integrity. It was also observed that the colloidal solution of EtZnO NPs remained stable for 90 days and the significant change in the absorbance does not decrease (figure 5b). Similarly, no significant changes were found when surface modified NPs incubated in culture medium [37, 60].

3.2.1. Anticandidal activity of EtZnO NPs

In light of the evidence, the rapid global spread of resistant in clinical isolates of C. albicans and new families of antimicrobial agents have a short life assurance, thus, the need to find new anticandidal agents is of supreme importance [61]. Researchers are increasingly turning their attention to nanomaterials, looking for new leads to develop better nano-antimicrobial drugs against MDR strains of C. albicans. In the present study we assessed the anticandidal activity of EtZnO NPs against MDR strain 077 of C. albicans. Anticandidal assays revealed that the EtZnO NPs efficiently suppressed the growth of C. albicans 077 in a dose dependent manner (figures 6(a)–(c)). The cells treated with the EtZnO NPs (15 $\mu$g ml$^{-1}$) also exhibited cavity formation, examined by SEM analysis (figure 6(d)). These cavities possibly reflected the formation of apoptosome in the C. albicans 077 cells, indicating promising anticandidal activity. The untreated sample cells showed a normal pattern of growth with a lag phase of $\sim$4 h, active exponential phase of 8 to $\sim$21 h
before attaining stationary phase. However, EtZnO NPs led to the suppression of growth and delay exponential phases of C. albicans 077 with minimum inhibitory concentration (MIC) \(\sim29.7\ \text{µg ml}^{-1}\) (figure 6e), again proving strong antimicrobial activity of EtZnO NPs against C. albicans 077. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth. The almost complete cessation of growth was observed at 34.1 \(\text{µg m}^{-1}\) concentration. The obtained anticandidal activity of EtZnO NPs is corroborated with previous published reports on anticandidal nanomaterials [62, 63].

3.2.2. Role of ROS in anticandidal activity of EtZnO NPs.

Recently, our group synthesized biosurfactant stabilized anticancer CdS QDs. This effect was associated with the production of ROS [37]. In light of these results, we concluded that the anticandidal effect of EtZnO NPs may be ROS dependent. As shown in figure 7(a), exposure of C. albicans cells to EtZnO NPs increased the intracellular ROS production in a time- and dose-dependent manner, when compared to untreated control. ROS-mediated oxidative stress is a well known inducer of cytotoxicity and apoptotic cell death in C. albicans [4]. Therefore, we sought to examine the possible role of ROS in anticandidal activity of EtZnO NPs. C. albicans were pretreated with 5 mM of histidine, a known scavenger of hydroxyl radicals (\(\cdot\text{OH}\)) and singlet oxygen (\(1^\text{O}_2\)). The data revealed that the histidine completely abrogates the antimicrobial property of EtZnO NPs (figure 7(b)). This clearly indicates that the anticandidal activity of EtZnO NPs is due to ROS production. The electronic band gap (\(E_g\)) structures of the metal oxides NPs with the redox potentials (EH) of the different ROS generation reactions have been proposed [23]. The metal oxide NPs when excited with energy higher than the \(E_g\), the electrons (\(e^-\)) of metal NPs were promoted across the band gap to the conduction band (\(E_c\)), which creates a hole (\(h^+\)) in the valence band (\(E_v\)). The electrons in the \(E_c\) and holes in the \(E_v\) exhibit high reducing and oxidizing power, respectively [28, 64].
The $e^{-}$ reacted with molecular oxygen to produce superoxide anion ($O^{2-}_2$) through reductive reactions. The $h^{+}$ can extract electrons from water and/or hydroxyl ions to generate $^{\cdot}OH$ (figure 8(a)). Taken together, results have confirmed that the ROS principally contributes the antifungal activity of the EtZnO NPs (figure 8(b)). However, various research articles in the last five years have been published on generation of ROS by various metal-oxide NPs. The literature survey revealed that very limited research has been done on the role of the electronic band gap property of metal-oxide NPs in ROS generation [28, 65].

4. Conclusion

We examined that EtZnO NPs exhibit strong antifungal activity against C. albicans 077 by alleviating ROS-mediated oxidative stress. An in depth understanding on energy band gap would definitely allow us to tailor new antimicrobial metal-oxide nanomaterials and effectively reduce experimental testing cost. In future, we suggest in-depth in vitro and in vivo studies that will help to identify antifungal potential of EtZnO NPs which can be utilized in the management of diseases caused by MDR strains of C. albicans.

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References

[1] Lipovský A, Nitzan Y, Gedanken A and Lubart R 2011 Nanotechnology 22 105101
[2] Ying S and Chunyang L 2012 Mycoses 55 50
[3] Brambilla G, Mattioli F, Robbiano L and Martelli A 2012 Mutagenesis 27 387
[4] Liang R, Cao Y, Zhou Y, Xu Y, Gao P, Dai B, Yang F, Tang H and Jiang Y 2010 Acta Pharmacol. Sin. 31 616
[5] Tani N, Rahnasto-Rilla M, Wittkeindt C, Salminen K A, Ritvanen A, Ollakkara R, Koskiartana J, Raunio H and Juvonen R O 2012 Eur. J. Med. Chem. 47 270
[6] Porter F 1991 Zinc Handbook: Properties, Processing, and Use in Design (Boca Raton, FL: CRC Press)
[7] Li M, Pokhrel S, Jin X, Mässler D, Damaoiseaux R and Hoek EM 2011 Environ. Sci. Technol. 45 755
[8] Hub A J and Kwon Y J 2011 J. Control. Release. 156 128
[9] Baek M, Kim M K, Cho H J, Lee J A, Yu J, Chung H E and Choi S J 2011 J. Phys.: Conf. Ser. 304 012044
[10] Yousef J M and Danial E N 2012 J. Health Sci. 2 38
[11] Brayner R 2008 Nanotechnology 3 48
[12] Raghupathi K R, Koodali R T and Manna A C 2011 Langmuir 27 4020
[13] Palanikumar L, Ramasamy S and Harirhan G 2012 Appl. Nanosci. 5 1
[14] Jones N, Ray B, Koodali RT and Manna AC 2008 FEMS Microbiol. Lett. 279 71
[15] Padmavathy N and Vijayaraghavan R 2008 Sci. Technol. Adv. Mater. 9 035004
[16] Seving B A and Hanley L 2010 J. Biomed. Mater. Res. B 94 22
[17] Akhavan O, Mehrabian M, Mirabasazadeh K and Azimiral R 2009 J. Phys. D: Appl. Phys. 42 225305
[18] Rosi N L and Mirkin C A 2005 Chem. Rev. 105 1547
[19] Brayner R, Ferarri-Iliou R, Brivois N, Djediat S, Benedetti MF and Fiévét F 2006 Nano Lett. 6 866
[20] Zhou J, Xu N and Wang Z L 2006 Adv. Mater. 18 2432
[21] Reddy K M, Feris K, Bell J, Wingett D G, Hanley C and Punnoose A 2007 Appl. Phys. Lett. 90 2139021
[22] Adiseshaiah P P, Hall J B and McNeil S E 2010 Nanomed. Nanobiotechnol. 2 99
[23] Xiong H, Xu Y, Ren Q and Xia Y 2008 J. Am. Chem. Soc. 130 7522
[24] Tang X, Choo E S G, Li L, Ding J and Xue J 2009 Langmuir 25 5271
[25] Xia T, Kovochich M, Lione M, Adler L M, Gilbert B, Shi H, Yeh J I, Zink J I and Nel A E 2008 ACS Nano 2 2121
[26] Yang H, Liu C, Yang D, Zhang H and Xi Z 2009 J. Appl. Toxicol. 29 69
[27] Zhang L, Jiang Y, Ding Y, Daskalakis N, Jeuken L, Povey M, O’Neill A J and York D W J 2010 Nanopart. Res. 12 12
[28] Li Y, Zhang W, Niu J and Chen Y 2012 ACS Nano 26 5164
[29] Zhang H Y et al. 2012 ACS Nano 6 4349
[30] Smijs T G and Pavel S 2011 Nanotechnol. Sci. Appl. 4 95
[31] Cho S, Jang J W, Jung S H, Lee B R, Oh E and Lee K H 2009 Langmuir 25 3825
[32] Moazzen M A M, Borghesi S M and Taleshi F 2012 J. N 295
[33] Bacci A, Montagnoli C, Perruccio K, Bozza S, Gazziano R, Pitzurra L, Velardi A, d’Ostiani C F, Cutler J E and Romani L 2002 J. Immunol. 168 2904
[34] Gullo A 2009 Drugs (Suppl. 1) 65
[35] Nouroozi F and Farzaneh F 2011 J.Braz. Chem. Soc. 22 484
[36] Musarrat J, Dwivedi S, Singh B R, Al-Khedhairy A A, Azam A and Naqvi A Q 2010 Bioresourc Technol. 101 8772
[37] Singh B R, Singh B N, Khan W H and Naqvi A H 2012 Biomaterials 33 5753
[38] Ansari S A, Nisar A, Fatma B, Khan W and Naqvi A H 2012 Mater. Sci. Eng. B 177 428
[39] Singh B R, Dwivedi S, Al-Khedhairy A A and Musarrat J 2011 Colloid Surf. B 85 207
[40] Thangaraj P, Rajan J, Durai S, Kumar S, Phani A R and Neri G 2011 Vacuum 86 140
[41] Chu X and Zhang H 2009 Mod. Appl. Sci. 3 177
[42] Faisal M S, Khan B, Rahman M M, Jamal A, Akhtar K and Abdullah M M 2011 J. Mater. Sci. Technol. 27 594
[43] Grätzl M 2001 Nature 414 338
[44] Vecitis C D, Zodrow K R, Kang S and Elimelech M 2010 ACS Nano 4 5471
[45] Zeynow O, Thill A, Chauvat F, Menguy N, Cassier-Chauvat , Oréar C, Daraspe C, Auffan J, Rose M and Spalla JO 2009 Nanotoxicology 3 284
[46] Zak A K, Abrishami M E, Majid W H, Yousefi R and Hossein S M 2011 Ceram. Inter. 37 393
[47] Zak A K, Razali R, Majid W H and Darroudi M 2011 Int. J. Nanomed. 6 1399
[48] Carotta M et al. 2009 Sensors Actuators B 137 164
[49] Takagahara T and Takeda K 1992 Phys. Rev. B 46 15578
[50] Ahmed A S, Shafeeq M M, Singla M L, Tabassum S, Naqvi A H and Azam A 2011 J. Lumin. 131 1
[51] Singla M L, Shafeeq M M and Kumar M 2009 J. Lumin. 129 434
[52] Lee H, Park I, Kwak J, Yoon D Y and Lee C 2010 Appl. Phys. Lett. 96 153506
[53] Kathiravan A, Paramaguru G and Renganathan R 2009 J. Mol. Struct. 934 129
[54] Sengupta A, Jiang B, Mandal K C and Zhang J Z 1999 J. Phys. Chem. B 103 3128
[55] Kahn M L, Cardinal T, Bousquet B, Monge M, Jubera V and Chaudret B 2006 *Chem. Phys. Chem.* **7** 2392
[56] Smith A M and Nie S 2010 *Acc. Chem. Res.* **16** 190
[57] Hong R Y, Li J H, Chen L L, Liu D Q, Li H Z, Zheng Y and Ding J 2009 *Powder Technol.* **189** 426
[58] Jayanhi S K and Chawla S 2010 *Appl. Surf. Sci.* **256** 2650
[59] Omondi C A, Sakwa T W, Ayodo Y K and Khanna K M 2012 *Int. J. Phys. Math. Sci.* **2** 159
[60] Wang L, Nagesha D K, Selvarasah S, Dokmeci M R and Carrier R L 2008 *J. Nanobiotechnol.* **6** 11
[61] Macherla C, Sanchez D A, Ahmadi M S, Vellozzi E M, Friedman A J, Nosanchuk J D and Martinez L R 2012 *Front Microbiol.* **3** 193
[62] Hwang I-S, Lee J, Hwang J H, Kim K-J and Lee D G 2012 *FEBS J.* **279** 1327
[63] Panáček A, Kolar M, Vecerova R, Prucek R, Soukupova J, Krystof V, Hamal P, Zboril R and Kvitek L 2009 *Biomaterials* **30** 6333
[64] Lin H F, Liao S C and Hung S W 2005 *J. Photochem. Photobiol. A* **174** 82
[65] Burello E and Worth A P 2011 *Nanotoxicology* **5** 228