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Reducing ZnO nanoparticles toxicity through silica coating

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Abstract

ZnO NPs have good antimicrobial activity that can be utilized as agents to prevent harmful microorganism growth in food. However, the use of ZnO NPs as food additive is limited by the perceived high toxicity of ZnO NPs in many earlier toxicity studies. In this study, surface modification by silica coating was used to reduce the toxicity of ZnO NPs by significantly reducing the dissolution of the core ZnO NPs. To more accurately recapitulate the scenario of ingested ZnO NPs, we tested our as synthesized ZnO NPs in ingestion fluids (synthetic saliva and synthetic gastric juice) to determine the possible forms of ZnO NPs in digestive system before exposing the products to colorectal cell lines. The results showed that silica coating is highly effective in reducing toxicity of ZnO NPs through prevention of the dissociation of ZnO NPs to zinc ions in both neutral and acidic condition. The silica coating however did not alter the desired antimicrobial activity of ZnO NPs to *E. coli* and *S. aureus*. Thus, silica coating offered a potential solution to improve the biocompatibility of ZnO NPs for applications such as antimicrobial agent in foods or food related products like food packaging. Nevertheless, caution remains that high concentration of silica coated ZnO NPs can still induce undesirable cytotoxicity to mammalian gut cells. This study indicated that upstream safer-by-design philosophy in nanotechnology can be very helpful in a product development.

Keywords: Physical chemistry, Inorganic chemistry, Materials science

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1. Introduction

Foods contamination by pathogenic microorganisms, which may endanger consumer's health, could occur during slaughtering, processing, packaging, and shipping. Every year, there are estimated 48 million cases and 3000 deaths from foodborne illness in the United States [1]. These numbers are actually preventable. One of the solutions is introducing antimicrobial agents to reduce these deterioration of food products in order to prolong the shelf life of foods [2].

Nanotechnology shows much promise in biomedical applications but its influence in food technology have only just begun [3, 4, 5, 6, 7, 8]. However, there is a word of caution since nanoparticles (NPs) themselves may elicit toxic effects and toxicity should be taken into account the contextual circumstances [9, 10].

Zinc oxide (ZnO) NPs are the third highly produced metal oxide NPs [11]. The unique properties and low production cost of ZnO NPs has been attracting wide range of applications in different industry such as chemicals, semiconductors, electronics, and healthcare industries. Besides that, studies have showed that ZnO NPs also exhibit good antimicrobial properties that can be utilized in food packaging and food preservation [12, 13, 14]. This is to augment the area where use of antibiotics are not feasible like in food. However, the perceived high toxicity of ZnO NPs to mammalian cells has significantly curtailed the potential large scale use of ZnO NPs in food [15, 16, 17, 18, 19].

ZnO NPs were found to be able to dissolve in aqueous solution to release Zn^{2+} ion. The influx of Zn^{2+} ion could affect the zinc homeostasis in the cell [20, 21]. The sheer small size eases the cellular uptake of these nanoparticles. When in the cell, further dissolution of ZnO NPs further increases intracellular Zn^{2+} to supraphysiological levels where it becomes toxic through for example, release of Zn^{2+} ion sequestration in mitochondria, increased oxidative stress, inflammation, apoptosis, and other undesired events [22, 23, 24].

Due to the well reported toxic nature of ZnO NPs, there have been the test beds of several strategies to mitigate NPs toxic effects. Surface modification on NPs is a commonly used technique to tailor the physical, chemical, and biological properties of NPs in order to improve the desired properties and eliminate shortfalls [25, 26, 27]. Most of the study on surface modification of ZnO NPs have been done to alter the intrinsic properties of ZnO NPs [28, 29, 30], rather than to reduce its toxicity. Only a few attempts on reducing toxicity of ZnO NPs by doping and capping were found [31, 32]. For biological applications such as antimicrobial agents incorporated in foods or food related products, this surface modification of ZnO NPs need to be able to effectively reduce the toxicity of ZnO NP. As the mechanism of toxicity lies with its dissolution to Zn^{2+} especially in the acidic

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gastric fluids, any given surface modification should protect the ZnO from acidic dissolution [33].

Although surface modification of ZnO NPs might significantly reduce the toxicity of ZnO NPs, one needs to be mindful that the initial rationale of using ZnO NPs (in this case is the antimicrobial activity of ZnO NPs) and its specific property is not negated while trying to reduce the toxicity. Therefore, the selected surface modification must not compromise the antimicrobial activity of ZnO NPs [34]. In order to ride the tension arising from the requirements, we hypothesized that a thin silica coating could reduce the dissolution through partial protection of the ZnO NPs surfaces due to the innate acidic resistance and relatively benign nature of silica.

Although most of the *in vitro* studies take account of the exposure route of ZnO NPs in the selection of targeted cell lines for toxicity assessments, we rarely pay attention to the possible form of ZnO NPs before it reaches the targeted cells. Direct introduction of ZnO NPs into the as used *in vitro* culture medium might not accurately reflect the actual state of ingested ZnO NPs. The ingested ZnO NPs will encounter digestive juices secreted by different organs in digestive system. Each of these juices has different chemical compositions, enzymes and pH that could affect the form and properties of ZnO NPs [35]. To more accurately replicate the actual environment that would be encountered by any ingested ZnO NPs, we utilized synthetic gastrointestinal fluids to investigate the dissolution of uncoated and silica coated ZnO NPs in order to determine the effectiveness of silica coating in preventing dissolution of ZnO NPs.

2. Materials and methods

2.1. Materials

Commercial grade ZnO NPs were purchased from Sigma Aldrich, USA. The water used throughout this study is Ultrapure Millipore water (18.2 MΩ·cm)

2.2. Preparation of synthetic gastrointestinal juices

Synthetic saliva was prepared as follows; potassium phosphate dibasic (2.5 mM), sodium diphosphate (2.4 mM), potassium bicarbonate (157 mM), sodium chloride (10 mM), magnesium chloride (0.15 mM), citric acid (0.15 mM), and calcium chloride dehydrate (1.5 mM) were mixed in water. The pH of the solution was adjusted to pH 6.7 before autoclaved to sterilize. α-amylase (1 mg ml^{-1}) and lysozyme from chicken egg white (0.1 mg ml^{-1}) were added into the cooled solution [36].

Synthetic gastric juice was prepared as follows; glucose (0.4 mg ml^{-1}), yeast extract (3.0 mg ml^{-1}), peptone (1.0 mg ml^{-1}), porcine mucin (4.0 mg ml^{-1}),

http://dx.doi.org/10.1016/j.heliyon.2016.e00177

cysteine (0.5 mg ml^{-1}), sodium chloride (0.08 mg ml^{-1}), sodium hydrogen carbonate (0.4 mg ml^{-1}), potassium phosphate dibasic (0.04 mg ml^{-1}), potassium diphosphate (0.04 mg ml^{-1}), calcium chloride dihydrate (0.008 mg ml^{-1}), magnesium sulfate heptahydrate (0.008 mg ml^{-1}), xylan (1.0 mg ml^{-1}), soluble starch (3.0 mg ml^{-1}), pectin (0.002 USP ml^{-1}), and Tween 80 (0.1%) was dissolved in water. The pH of the solution was adjusted to pH 2.0. After sterilization by autoclave, pepsin (3.0 mg ml^{-1}) was added into the cooled solution [36].

2.3. Cell lines

Human colon adenocarcinoma cell (SW480), human colorectal adenocarcinoma epithelial cell (DLD-1), *Escherichia coli* bacteria (*E. coli*, ATCC 700926) and *Staphylococcus aureus* bacteria (*S. aureus*, ATCC 25923) were purchased from American Type Cell Culture, USA.

2.4. Mammalian cell culture

SW480 and DLD-1 were grown in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, USA) supplemented with Fetal Bovine Serum (10%; Thermo Fisher Scientific, USA) and Penicillin/streptomycin (1%; PAA Laboratories Inc., USA). The cells were maintained in a standard cell culture condition of 37 °C with CO_2 (5%) supply.

2.5. Synthesis of silica coated ZnO NPs

The silica coating was prepared as previously reported [37]. In brief, ZnO nanopowders (26 mg) were dispersed in ethanol and isopropanol (2:1 v/v) mixture. Water and 28% aqueous NH_3 (Sigma Aldrich, Singapore) were added into the mixture. The solution was sonicated in a bath sonicator for 30 min at room temperature. Tetraethyl orthosilicate (TEOS; Sigma Aldrich, Singapore) was added dropwise into the stirred mixture. The reaction was allowed to proceed at room temperature for 8 h with continuous stirring. The silica coated ZnO NPs were collected by centrifugation at 6000 rpm and washed with copious amount of water to remove any unreacted reagents.

2.6. Characterization of NPs

The physical characterization of silica coated and uncoated ZnO NPs followed an earlier paper [38].

The images of uncoated and coated ZnO NPs were captured by Transmission Electron Microscopy (TEM; JEM 2010; JEOL, Japan). ZnO NPs (50 μg ml^{-1}) dispersed in ethanol (Fisher Scientific, Singapore) was dropped on carbon coated

http://dx.doi.org/10.1016/j.heliyon.2016.e00177

copper grid and dried at room temperature for 3 h. The thickness of silica coating was determined from TEM images with the aid of ImageJ software.

The surface elements of NPs were determined by energy-dispersive X-ray spectroscopy (EDX). Before the EDX analysis, the dried NPs were coated with carbon. X-ray photoelectron spectroscopy (XPS) was used to confirm the surface elements of NPs. Uncoated and silica coated ZnO NPs were attached on glass surface by double sided carbon tape for XPS analysis.

The functional groups of the NPs were characterized by Fourier Transform Infrared Spectroscopy (FTIR; FTIR 8400S; Shimadzu, Japan). The preparation of the sample was as follows; NPs (2 mg) were throughout mixed with potassium bromide (198 mg) before pressed to form uniform sample pellet. The transmittance of sample from wavenumber of 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} was recorded.

The hydrodynamic size and zeta potential of NPs were characterized by using Zetasizer (Zetasizer Nano ZS; Malvern co., UK). Filtered water was used to prepare the sample (500 μM). All of the measurement was conducted at room temperature.

2.7. Antimicrobial testing

E. coli and *S. aureus* were maintained in Luria-Bertani (BD Biosciences, Singapore) broth at 37 °C with shaking (250 rpm). The sonicated NPs solution (1 ml) was added into the bacteria solution (4 ml) to achieve the OD_{600} value of 0.01. The bacteria were cultured at 37 °C for 3 and 21 h respectively. The bacteria culture after 21 h culture was diluted with the fresh LB solution before the OD_{600} measurement. The OD_{600} value of the bacteria solution at the beginning and the end of the experiment were determined by microplate reader (Synergy 2 Multi-mode reader; BioTek, USA). Ampicillin (50 μg ml^{-1}; Sigma Aldrich, Singapore) was used as the positive control to inhibit the growth of bacteria.

2.8. Dissolution of ZnO NPs in synthetic gastrointestinal fluid

Uncoated and silica coated ZnO NPs (1250 mM) were incubated in synthetic saliva at 37 °C water bath shaker for 30 min. The synthetic saliva treated pellet was obtained by centrifugation at 6000 rpm for 10 min. Synthetic gastric juice (40 ml) was added into the pellet for an incubation of 2 h at 37 °C water bath shaker. The supernatant and synthetic gastric juice treated pellet was separated by centrifugation at 6000 rpm for 10 min. The synthetic saliva and synthetic gastric juice treated pellets were re-dispersed in water. The composition of zinc was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP 6000 Series, Thermo Scientific, UK) with nitric acid (65%) digestion. The acid digested

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samples were further diluted (final acid concentration is 2%) and filtered with syringe filter (0.4 μm pore size) before analysis [39].

2.9. Annexin V/propidium iodide assay

The procedures of annexin V/propidium iodide assay were conducted as previous study [40]. In brief, SW480 and DLD-1 cells (cell seeding of 1.2×10^5 cells per well, cultured until confluent) were treated with NPs and Zn^{2+} (prepared from $ZnCl_2$ solution) mixture (the concentration of $ZnCl_2$ and NPs were based on the dissolution data determined from ICP-OES analysis) on 24-well plate for 12 h. All the dead and alive cells were collected and incubated with the dyes as manufacturer's instruction. The apoptotic and necrotic cells were assayed by Tali® Image cytometer.

2.10. Statistical analysis

Data reported are means ± standard deviation (SD) of a triplicate analysis. Statistical analysis was ascertained with paired sample Student's *t*-test comparison. Comparison was considered as statistically significant with p-value of <0.05.

3. Results and discussion

3.1. Confirmation of silica coating on ZnO NPs

Fig. 1 illustrated the preparation of silica coated ZnO NPs. The chemical reaction of ZnO NPs and TEOS precursor in reaction mixture can be described as follows;

$$ZnO(s) + Si(OC_2H_5)_4(aq) + 2H_2O(aq) \rightarrow ZnO - SiO_2(s) + 4C_2H_5OH(aq)$$

The hydrolyzed TEOS condensed on the surface of ZnO NPs to form Zn-O-Si linkage with the presence of ammonia catalyst. After that, siloxane bonds polymerized to form an intact silica shell on the ZnO NPs core [41].

The final product was not heat treated as non-calcined silica is safer as compared to calcined silica. Calcined silica is not food grade and therefore not suitable for food related application. The non-heat treated silica (non-calcined) remains amorphous and is inert [42].

The characterization of raw material (ZnO NPs) and the product of the reaction in Fig. 2 confirmed the formation of a silica coating on the ZnO NPs. Compared to the rod-shaped ZnO NPs in Fig. 2A, there is an additional layer on the silica coated ZnO NPs in Fig. 2B. The thickness of silica layer on ZnO NPs is 28.5 ± 4.0 nm with the TEOS to ZnO ratio of 11: 2 (based on n = 50 images per group as analyzed with ImageJ). Besides the amount of TEOS added, the coating thickness can also be affected by factors such as the incubation time for the cross linking reaction and size of the ZnO NPs. FTIR spectra of the layer showed the asymmetric

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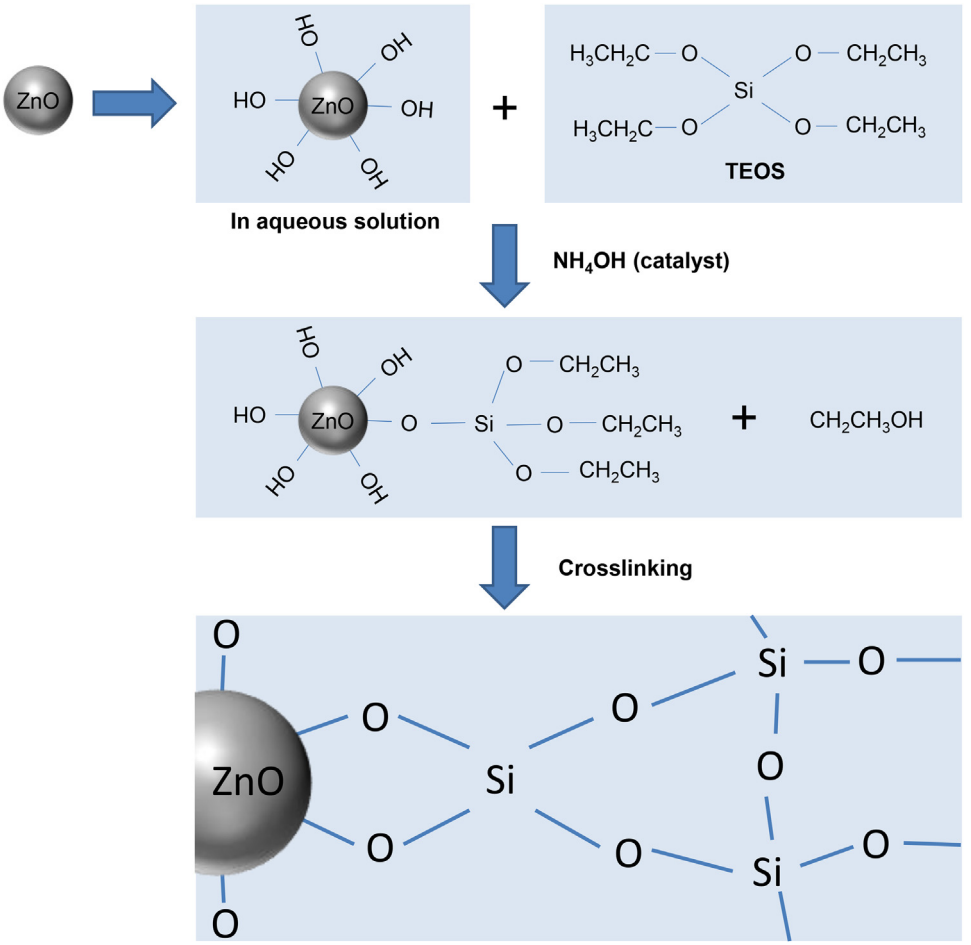

Fig. 1. Schematic diagram of the formation of silica coated ZnO NPs. Catalyst (ammonia) catalyzed the condensation of TEOS on the hydrolysed ZnO NPs. Polymerization of TEOS resulted in the formation of intact silica shell on the ZnO NPs (drawing not to scale).

vibration of Si-O-Si (siloxane bond by silica polymerization) at 950–1200 cm^{-1} broad band [43, 44]. The surface elemental composition analysis by XPS in Fig. 2D further agreed with the FTIR spectra that silica was the coating on the surface of ZnO NPs. Si 2s and Si 2p peak were found in the silica coated ZnO NPs, the missing of Zn 2p peaks in XPS spectra of silica coated ZnO NPs suggested that thick silica shell is presented on the surface of ZnO NPs. Therefore, inelastic scattering of electrons due to the long traveling distance significantly reduces the detection of zinc characteristic peak. However, the mechanical stirring might not effectively disperse the ZnO NPs for complete and even silica coating. This is to facilitate the functioning of ZnO NPs when exposed to food for antimicrobial property while still slowing down any dissolution in the gut.

The elemental composition analysis by EDX (Table 1) showed that the atomic percentage of silicon in the silica coated ZnO NPs is significantly larger than zinc

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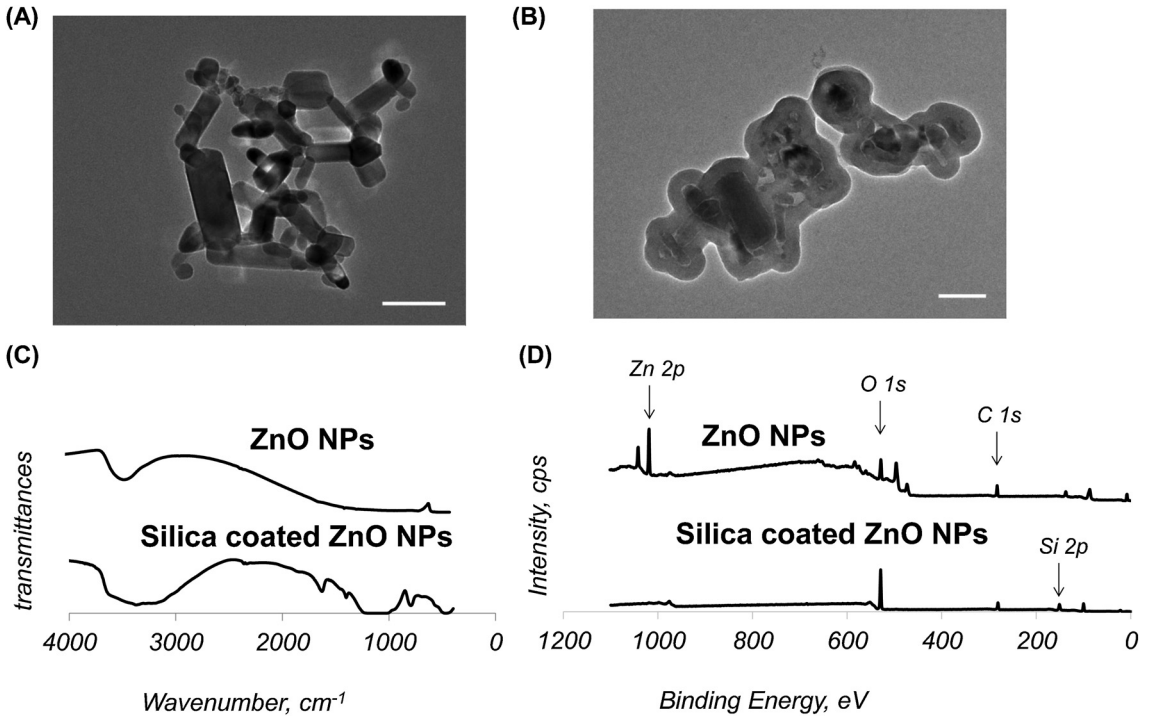

Fig. 2. Characterization of uncoated and silica coated ZnO NPs. TEM images of (A) uncoated ZnO NPs and (B) silica coated ZnO NPs revealed the shape and morphology of the NPs. (C) FTIR spectra showed the functional groups of the uncoated and silica coated ZnO NPs. (D) Surface elements on uncoated and silica coated ZnO NPs were determined by XPS spectra. Scale bars used in (A) and (B) are 100 nm.

(12-folds). These characterizations verified that the ZnO NPs were successfully coated with silica.

The hydrodynamic size and the surface charge of the uncoated and silica coated ZnO NPs were summarized in Table 2. With the presence of silica coating, the size of the ZnO NPs was increased 2.4-folds as compared to the uncoated ZnO NPs. Based on Fig. 2, the silica coating was found to coat the aggregated NPs into a cluster rather than specific individual coating. This might explain the large hydrodynamic size and polydispersed characteristics of silica coated ZnO NPs. The coating of silica change the surface charge of ZnO NPs from positive to negative, the increase of the magnitude in zeta potential suggested the improved stability of ZnO NPs in water by high hydrophilicity silica coating.

3.2. Silica coating retains the antimicrobial properties of ZnO NPs

After the confirmation of the silica coating on the ZnO NPs, we analyzed the effect of silica coating on the antimicrobial properties of ZnO NPs. The results in Fig. 3 showed that silica coating is able to retain or even slightly improve the

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Table 1. The atomic percentage of elements on the surface of uncoated and silica coated ZnO NPs.

Nanoparticles	Atomic percentage of O	Atomic percentage of Zn	Atomic percentage of Si	Atomic percentage of Br
Uncoated ZnO NPs	53.83	43.78	0.00	2.39
Silica coated ZnO NPs	72.84	2.02	24.92	0.23

antimicrobial properties of ZnO NPs to both the gram negative *E. coli* and gram positive *S. aureus* upon short (3 h) and long (21 h) exposure. In general, ZnO NPs are found to have better antimicrobial property than SiO_2 NPs (Fig. 3A, B). The exact antimicrobial mechanism of ZnO NPs is still remain unknown. Several mechanisms were postulated such as releasing of Zn^{2+} ion, formation of reactive oxygen species, and affecting the integrity of bacteria cell membrane. Jiang et al. found that the attachment of ZnO NPs to bacterial cell is less uniform and weaker than the SiO_2 NPs [45]. Therefore, the silica coating might have improved the attachment of the NPs on the cell wall of bacteria.

Although study reported that UV radiation can improve the antimicrobial activity of ZnO NPs [46], several components in foods such as vitamins, dyes, and aromatic compounds are sensitive to UV. The radiation of UV can accelerate the degradation of these components; thus, the use of UV radiation to improve anti-microbial properties is limited to certain food products only. To cover the broad range of food products, we eliminated the effects of UV radiation on the NPs in our anti-microbial study. Even if there is a slight compromise of the anti-microbial effect due to the silica coating, it would already be a safer improvement. Moreover, in a real application of ZnO NPs as anti-microbial agent on food packaging, the level of bacterial load is not going to be as high as what we have subjected our silica-ZnO NPs to. This further emphasizes the practical advantages of a protective silica coating on ZnO NPs.

3.3. Silica coating is stable in both neutral and acidic environment

To achieve the main objective of this study, we analyzed the effectiveness of the silica coating to protect ZnO NPs from dissolution in both synthetic saliva and

Table 2. The summary of the physical properties of uncoated and silica coated ZnO NPs in water.

Nanoparticles	Hydrodynamic size, nm	PDI*	Zeta potential, mV
Uncoated ZnO NPs	258.2 ± 8.4	0.37 ± 0.02	+17.2 ± 0.8
Silica coated ZnO NPs	623.8 ± 23.7	0.53 ± 0.05	-21.2 ± 0.5

* PDI denotes polydispersity index. All data are means ± SD of three independent measurements.

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synthetic gastric juice. Fig. 4 showed that majority of the ZnO NPs remained as ZnO when dispersed in synthetic saliva, the detected zinc ion in the ZnO NPs with or without silica coating is about 1%. When the NPs samples were dispersed in acidic synthetic gastric juice, about 98% of the uncoated ZnO NPs dissolved into Zn^{2+} ions. As expected, the dissolution of ZnO NPs was low in neutral solution but high in acidic solution. On the other hand, the silica coating effectively reduced the dissolution of ZnO NPs in an acidic environment from 98% to less than 15%. Study found that silica is very stable in high acid conditions [47].

In most of the *in vitro* study, ZnO NPs were directly introduced into culture medium with other biomolecules. The surface energy of NPs are high and coupled with a high surface area to volume ratio caused charged proteins from the media to adsorb onto the surface of NPs. This protein corona may reduce the direct contact of NPs with their immediate environment and solution and in turn affect the dissolution. While this is true in many other biological context where nanoparticles are found [7], this is probably not relevant in the context of food borne nanoparticles. The gut environment has sequential compartmentalization of reactions of varying pH ranges. The presence of enzymes such as pepsin in

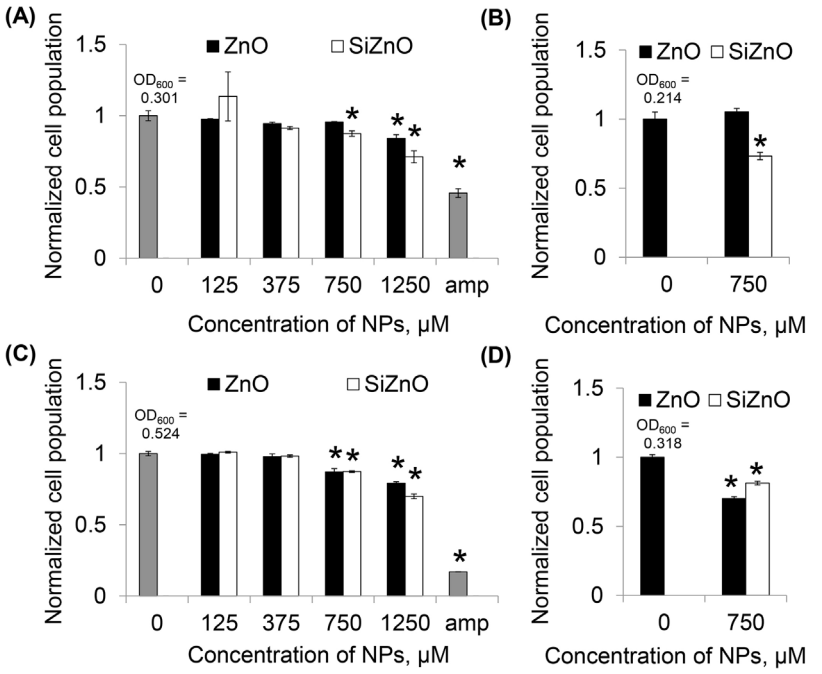

Fig. 3. Silica coated ZnO NPs have comparable antimicrobial activity as the uncoated ZnO NPs. The growth of *E. coli* bacteria was inhibited by uncoated and silica coated ZnO NPs upon (A) 3 h and (B) 21 h incubation. The growth of *S. aureus* bacteria was also significantly inhibited by uncoated and silica coated ZnO NPs upon (C) 3 h and (D) 21 h incubation. amp denoted 50 μg ml^{-1} of ampicillin, ZnO denoted ZnO NPs, SiZnO denoted silica coated ZnO NPs. Data represent means ± SD, $n = 3$. Student's *t*-test, $p < 0.05$, *significantly different between the group and untreated negative control (concentration = 0).

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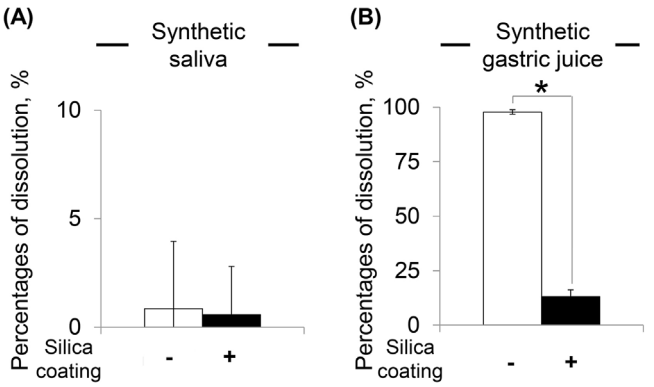

Fig. 4. Silica coating effectively reduce the dissolution of ZnO NPs in acidity synthetic gastric juice. (A) The dissolution of ZnO was low in synthetic saliva. (B) Silica coating significantly reduced the dissolution of ZnO NPs in synthetic gastric juice. Data represent means ± SD, $n = 3$. Student's t-test, $p < 0.05$, *significantly different between the two groups.

gastric juice could effectively degrade the protein corona into peptides and expose the NPs to the digestive fluids. This further argues the need to have a biologically inert coating and not rely on the conventional wisdom of a digestively labile protein corona to protect.

3.4. Silica protection effectively reduces ZnO NPs toxicity

The above observations have proved that silica coating is effective to reduce dissolution and yet maintain the antimicrobial properties of ZnO NPs. Here, the cytotoxicity of the NPs was determined. The colorectal epithelial cells, SW480 and DLD-1 were treated with NPs and Zn^{2+} ions equivalent to the concentration of ZnO NPs after gastric juice incubation to replicate the condition after stomach digestion to show that the reduced dissolved Zn^{2+} due to the silica coating helps with reducing cell cytotoxicity.

To ensure the toxicity assessment was done with a reasonable concentration, a dosage of 250 μM of zinc was introduced into the cell culture medium; at this concentration of zinc, it is about one eighth of the minimum recommended dietary intake for human [48, 49]. In comparison, this concentration of ZnO NPs was also found to be able to induce cytotoxicity [50]. Choosing this physiologically relevant concentration of Zn^{2+} ions can help us to compare the cytotoxicity of ZnO NPs and silica coated ZnO NPs. In Fig. 5, unprotected bare ZnO NPs, which dissociated into Zn^{2+} ions in gastric juice, induced high cytotoxicity to both colorectal epithelial cell lines (SW480 and DLD-1). After 12 h of exposure, more than 70% of the cells undergo necrosis. On the other hand, silica coating significantly reduced the cytotoxicity of ZnO NPs on both cell lines (11.4% and 15.3% died cells found in SW480 and DLD-1 respectively). Besides cytotoxicity, previous study also found that silica coating significantly reduce DNA damage induced by ZnO

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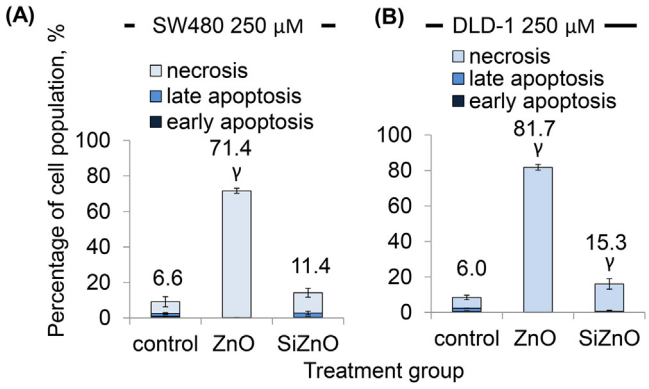

Fig. 5. Silica coating effectively reduced the cytotoxicity of ZnO NPs to colorectal cell lines. ZnO NPs and Zn^{2+} ions induced high cytotoxicity to both (A) SW480 and (B) DLD-1 cells upon 12 h of 250 μM treatment. The presence of silica coating significantly reduced this toxicity. ZnO denoted ZnO NPs, SiZnO denoted silica coated ZnO NPs. Data represent means ± SD, $n = 3$. Student's t-test, $p < 0.05$, $^{\gamma}$significantly different with the percentage of necrotic cell in control group (concentration = 0).

nanorods [51]. Comparison between ZnO NPs and SiO_2 NPs also found that ZnO NPs are more toxic [52, 53, 54, 55].

Although the silica coated ZnO NPs showed promising reduction in cytotoxicity at 250 μM, they are not completely benign. High cytotoxicity was observed in silica coated ZnO NPs treated groups when we increased the dosage to 1250 μM (Fig. 6). These findings proved that the appropriate and right dose of silica coated ZnO NPs must be used in appropriate amounts in real contextual circumstances and further testing is necessary to establish those relevant concentrations, especially so for the often unregulated consumable products.

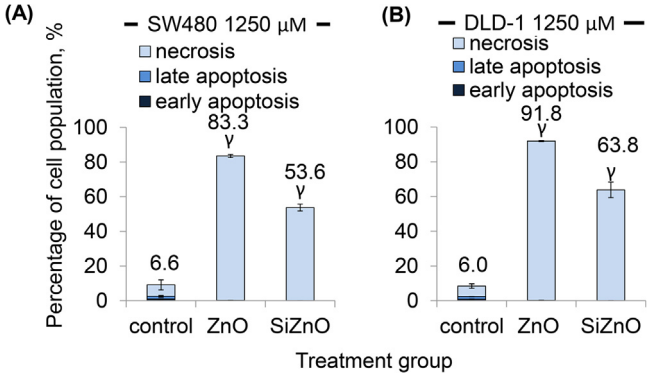

Fig. 6. High concentration of silica coated ZnO NPs induced high cytotoxicity. Silica coated ZnO NPs offered some protection to the toxicity of ZnO NPs to both (A) SW480 and (B) DLD-1 cells as compared to uncoated ZnO NPs upon 12 h of 1250 μM treatment. ZnO denoted ZnO NPs, SiZnO denoted silica coated ZnO NPs. Data represent means ± SD, $n = 3$. Student's t-test, $p < 0.05$, $^{\gamma}$significantly different with the percentage of necrotic cell in control group (concentration = 0).

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From a product development perspective, other mitigating strategies like incorporating the ZnO NPs on the outside surface of the food packaging, instead of on the food side of the packaging, might reduce the possibility of ZnO NPs diffusing into the food and being ingested.

4. Conclusion

The use of synthetic gastrointestinal fluids could more accurately determine the chemical form of ingested NPs for *in vitro* study. Silica coating, which formed by TEOS precursor, is stable in both neutral and acidic conditions. The presence of silica coating effectively reduces the toxicity of ZnO NPs and maintains the antimicrobial properties of ZnO NPs. This showed that surface modification by silica coating improves the safety of incorporating ZnO NPs in antimicrobial applications such as antimicrobial surfaces and additives in food products. Although silica coating largely reduces the cytotoxicity of ZnO NPs at different concentrations, silica coating is not ultimately benign. High concentration of silica coated ZnO NPs could also induce significant undesirable cytotoxicity to colorectal epithelial cells. Therefore, the use of the silica coated ZnO NPs for consumable products should be used at the moderate and right dose to prevent undesired toxic effects.

Declarations

Author contribution statement

Sing Ling Chia: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

David T. Leong: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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