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Enhanced antifungal activity of Ni-doped ZnO nanoparticles under dark conditions

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1. Antifungal test

1.1 Microbial culture conditions

Candida albicans (*C. albicans)* was obtained from the Korean Collection for Type Culture (KCTC 27242), and cultured in potato dextrose broth (PDB) under aerobic condition at 30 °C for 24 h in a shaking incubator at 180 rotations per minute (rpm). The *C. albicans* cells were centrifuged at 3500 rpm for 10 min at 4 °C in order to harvest the cells. The harvested cells were washed with phosphate buffered saline (PBS, pH ~ 7.4, Glibco, USA) followed by re-suspension in PBS (OD 600 of 0.1) to adjust the desired density (10^6 CFU/mL) after measurement with a hemocytometer. The number of colony-forming units (CFUs) was calculated by counting the colonies on each plate.

1.2 Minimum inhibitory concentration (MIC)

The growth inhibition of *C. albicans* was studied with the presence of undoped and Ni-doped ZnO NPs for different particle concentration (100, 200, 300, 400, 500 and 600 μg/mL). To determine the MIC, 200 μL of each inoculum suspensions was placed in triplicate in a 96-well microtiter plate and incubated on a reciprocal shaker (120 rpm) for 22 h. Then, 10 μL of resazurin indicator (0.18%) was added to each well and examined after incubation for another 2-3 h. The nystatin (10 μg/mL) was used as the standard antibacterial agents for positive inhibitory controls of *C. albicans*. After incubation under appropriate conditions, the lowest concentration of NPs that inhibited the growth (blue color) was recorded as the MIC for the test pathogen.

1.3 Cells viability test

The cell viability test was done according to the method described in Ref. 1. Briefly, about 1 x 10^7 cells per mL of bacterial strains were incubated in PBS suspensions of undoped and 6% Ni-doped ZnO NPs at different concentrations (0.5 x MIC, 1 x MIC and 2 x MIC) under shaking for 24 h at 30 °C. Aliquots of samples were withdrawn, diluted and then spread

onto PD agar plates. After incubation at 30 °C, the capability of the *C. albicans* to form colonies was measured by CFU counting method.

1.4 Morphological changes of the *C. albicans*

FESEM analyses were performed to investigate the morphological changes of the *C. albicans* after the MIC level treatment with the NPs. The *C. albicans* sample was prepared for FESEM analysis according to the method described by previously.[2] Briefly, treated cells were pelleted and washed using PBS and pre-fixed with 2.5% glutaraldehyde for 30 min. The pre-fixed cells were again washed by PBS and serially dehydrated using 30, 50, 70, 80, 90 and 100% ethanol. The fixed cells were dried and coated with platinum using ion sputter (E-1030, Hitachi, Japan). Treated samples were observed by FESEM at a voltage of 10 kV (S-4800, Hitachi, Japan).

1.5 Membrane permeability of *C. albicans*

Membrane permeability change of *C. albicans* with undoped and 6% Ni-doped ZnO NPs was investigated by determining the uptake of propidium iodide (PI, Sigma Aldrich, USA) according to the method described in Ref. 3. Briefly, the treated cells were incubated with PI (5 µg/mL) for 15 min. Subsequently, over staining were washed twice with PBS. Finally, the fluorescence images were observed using a Cari Zeiss LSM 5 Live confocal laser scanning microscope (CLSM) scan head integrated with the Axiovert 200 M inverted microscope (Carl Zeiss, Jena, Germany).

1.6 Cytotoxicity on human cells

To determine the cytotoxic level, HEp2 (ATCC CCL-23) and HEK293T (ATCC-11268) cells were treated with undoped and 6% Ni-doped ZnO NPs and determined the cell viability. Both types of cells were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) with 1% of antibiotics (Glibco, USA) and 10% of fetal bovine serum (FBS, Hyclone, USA). HEp2 and HEK293T cells were cultured in 6-well flat

bottom microtiter plates at a density of 3×10^5 cells/well and 5×10^5 cells/well, respectively, with 2 mL of medium (37 °C, 5% CO_2 atmosphere). After 12 h of culturing, the medium was aspirated out, and the cells were washed with PBS. Each well was treated with undoped and 6% Ni-doped ZnO NPs for different concentration (6.25, 12.5, 25, 50, 100 and 200 μg/mL) with the control as medium only. After 24 h post treatment, the cell viability was determined by MTT assay.

1.7 Metal ion release analysis

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) analysis was performed to detect quantitatively the metal ions released from the NPs. 6% Ni-doped ZnO NPs (at the MIC level concentration) were suspended in the PDB media and incubated overnight at 30 °C for 24 h. Then, the suspension was centrifuged at 15000 rpm for 1 h, and 1 mL portion of the supernatant was acidified with 1 mL of 7% ultrahigh purity nitric acid, and subjected to elemental analysis using ICP-AES (Perkin-Elmer Optima, USA) and ICP-MS (Perkin-Elmer SCIEX Elan DRCII).

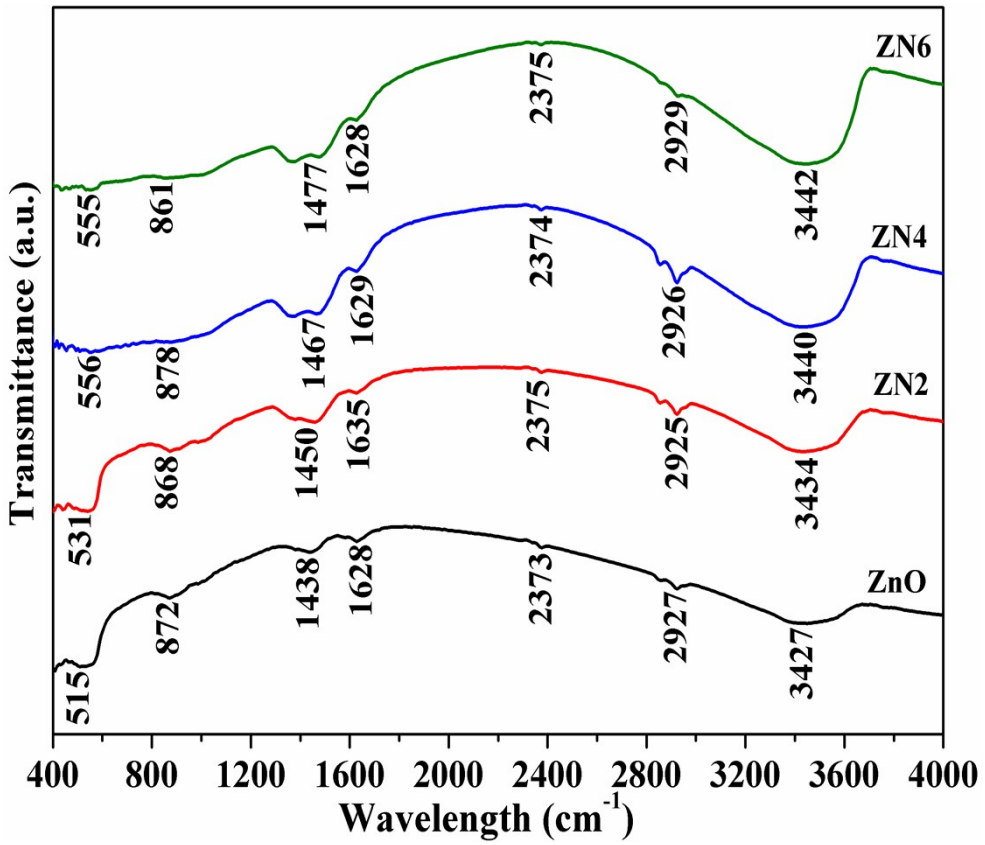

Fig. S1 FTIR spectra of undoped and Ni-doped ZnO NPs

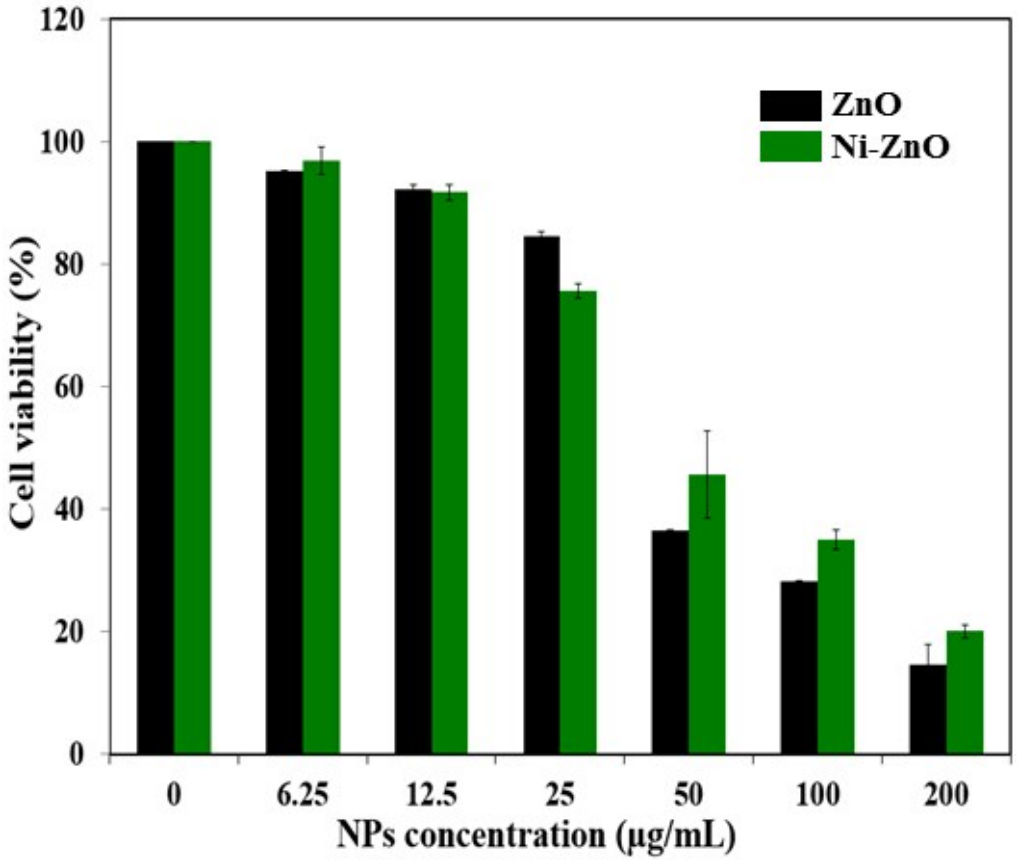

Fig. S2 Cytotoxic effects of undoped and 6% Ni-doped ZnO NPs with various concentrations on HEK293T cells. Cells without NPs were taken as control. Error bars represents the standard deviation (n=3).

References

[1] I. Rago, C. R. Chandraiahgari, M. P. Bracciale, G. De Bellis, E. Zanni, M. Cestelli Guidi, D. Sali, Alessandra Broggi, Claudio Palleschi, Maria S. Sarto and D. Uccelletti, *RSC Adv.*, 2014, **4**, 56031-56040.

[2] S. H. S. Dananjaya, G. I. Godahewa, R. G. P. T. Jayasooriya, J. Lee and M. D. Zoysa, *Aquacult.,* 2016, **450**, 422-430.

[3] S. H. S. Dananjaya, D. C. M. Kulatunga, G. I. Godahewa, J. Lee and M. De Zoysa, *RSC Adv.*, 2016, **6**, 33455-33461.