Supplementary information

Hybrid nanoparticles from chitosan and nickel for the enhanced biocidal activities

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SI 1: Characterization of the Cs/Ni/NiO HNPs

The Cs/Ni/NiO HNPs developed were characterized by X-ray diffractometer (XRD) (model: X'PERT PRO PANalytical) and it was recorded in the 2θ range of between 10-80^{o} for Cs/Ni/NiO HNPs, with a monochromatic Cu-K_{α} radiation of wavelength 1.5406 Å. The NanoPlus dynamic light scattering (DLS) nanoparticle sizer was, used for the particle size analysis of the Cs/Ni/NiO HNPs. The morphology and elemental analyses of HNPs were carried out by using the field emission scanning electron microscopy (FESEM) (Carl Zeiss Ultra 55 FESEM) with energy dispersive X-ray spectrometry (EDX) (model: Inca). The morphologies of the Cs/Ni/NiO HNPs were, examined by using the transmission electron microscope (TEM) (Tecnai F20 model) instrument, at an accelerating voltage of 200kV. The

attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectrum was, recorded in the wavenumber range of between 400-4000 cm^{-1} by using the Perkin-Elmer spectrometer. The photoluminescence (PL) spectra were, taken by using the spectrometer Perkin Elmer-LS 14.

SI 2: Antibacterial activity assay

Typical gram-positive and gram-negative bacteria, such as the *Staphylococcus aureus* and *Escherichia coli* were, used to assess the antibacterial activities of Cs/Ni/NiO HNPs (Tamilselvi, Yelilarasi, Hema, & Anbarasan, 2013). Pathogenic bacteria was, swabbed alone on the MHA Petri plates, having 25 mL of sterile Muller Hinton Agar (MHA, Himedia). The activity of bacteria was, examined at a concentration of 1 mg/mL of the Cs/Ni/NiO HNPs, dispersed in dimethyl sulfoxide (DMSO). After it was, incubated overnight at 37°C, the measurement of the zone of inhibition was, done after 24 hrs. As a positive control, a standard amoxicillin antibiotic was, used.

SI3: Bacterial microscopic examination*:*

Bacteria were, preserved in a 2% glutaraldehyde for 24 hrs, after exposure. Fixed HNPs were dehydrated in an acetone gradient (35, 50, 70, 80, 95 and 100 %) for 3 min after being post-fixed with 1 % osmic acid for 2 hrs. SEM was, employed to examine the air-dried materials (model: Carl Zeiss Ultra 55 FESEM).

SI4: MIC and MBC determination:

In this investigation, the Agar dilution method was, employed to measure the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for Cs/Ni/NiO HNPs. 2 mL of the test HNPs was added to a 19 mL of molten nutrient Agar (MHA). The HNPs were thoroughly, mixed into the medium. The HNPs concentrations in each plate were: 100, 300, 500, 800, 1000, 1300, 1500, 1800 and 2000 µg/mL. Each plate

was, injected with the test bacteria and a colony formation was, used to determine growth. The amount of Cs/Ni/NiO HNPs necessary for bacterial activity was, investigated by adding: 100, 300, 500, 800, 1000, 1300, 1500, 1800 and 2000 µg/mL concentrations of the HNPs-treated against *S. aureus* and *E. coli*. The MBC is the lowest concentration treated and the bacteria did not re-grow when removed from the test plate to a new medium. The MIC is the lowest concentration at which 99% bacterial growth was, inhibited. The experiment was, repeated three times, for the data collected to be statistically relevant.

SI4: a) Anti-proliferative assay

A 5% CO_2 atmosphere at 37 ^oC was, used for the inoculation of human breast cancer cells (MCF-7), procured from Pune NCCS on DMEM (Dulbecco's Modified Eagles Medium) and fortified with 10% of Fetal Bovine Serum (FBS), 100 µg/mL streptomycin and 100 µg/mL penicillin.

b) MTT Assay

The Cs/Ni/NiO HNPs were, tested for their antiproliferative potentials against MCF-7 and fibroblast viable cells (L929) by the MTT assay. Briefly, the cultured MCF-7 cells were, collected by trypsinization and introduced into a 15 mL tube. After that, the cells were, plated at a density of 1×10^5 cells/mL (200 µL), into a 96-well tissue culture plate in a DMEM medium, containing 10% FBS and 1% antibiotic solution between 24 and 48 hrs at 37°C. The wells were, washed with sterile PBS and treated with various concentrations (1-100 µg/mL) of Cs/Ni/NiO HNPs in a serum-free DMEM medium. They were thereafter, incubated at 37°C in a humidified atmosphere of a 5% CO_2 incubator for 24 hrs. After the incubation period, MTT (20 µL of 5 mg/mL) was added to each well. The cells were incubated for another period of between 2 and 4 hrs until purple precipitates were visible under an inverted polarizing microscope. Finally, the medium with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µL). Furthermore, to dissolve formazan crystals, DMSO (100

μL) was added, and the plate was shaken for 5 min. The absorbance for each well was, measured at 570 nm wavelength by using a microplate reader (Thermo Fisher Scientific, USA). The percentage cell viability and IC_{50} value were, calculated by using the GraphPad Prism 6.0 software (USA).

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\left(\bar{A}_c - \bar{A}_s\right)}{\bar{A}_c} \times 100 \quad (1)$$

where $\bar{A}_c$ is the mean absorbance of the control and $\bar{A}_s$ is the mean absorbance of the sample

SI5 Statistical analysis

The mean and standard deviation of the MIC and MBC determination tests on the samples are, used to express the data. Statistical comparisons were, made by using an SPSS version 12.0 MS Windows and a one-way analysis of variance (ANOVA), followed by a Duncan's Multiple Range Test (DMRT). If the *p*-value is less than 0.05, the results are, considered statistically significant.