Supplementary Information

Zinc oxide nanocrystals as nano-antibiotic and osteogenic/osteinductive agents

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Figure S1. Size distribution curves deriving from Dynamic Light Scattering (DLS) measurements for ZnO and ZnO-NH₂ NCs in Ethanol (top) and water (bottom)
The FT-IR spectra (Figure S2) of ZnO and ZnO–NH$_2$ nanocrystals show both an intense mode at 440 cm$^{-1}$ typical of Zn–O vibration. In addition, the two spectra show, with different intensities, the symmetric and antisymmetric stretching of –CH$_2$ and –CH$_3$ groups at 2860 and 2925 cm$^{-1}$, respectively. These vibrations are attributed to residual acetate groups on the ZnO surface due to the precursors used in the synthetic procedure. However, in the case of ZnO–NH$_2$ NCs, the 2860 and 2925 cm$^{-1}$ stretching modes are more intense because of the presence of the propyl chain of the amine-functional group, thus confirming the successful functionalization of the ZnO surface. Moreover, the broad band from 3600 to 3400 cm$^{-1}$, due to the stretching vibrations of hydroxyl groups on the ZnO surface, are less pronounced in the ZnO–NH$_2$ sample with respect to the pristine ZnO. Since the APTMS moiety links through hydroxyl groups to the oxide surface (Zn–OH), leading to Zn–O–SiR bonds, the above-mentioned-observation further confirms the successful amine functionalization of our ZnO NCs.
Figure S3. Fluorescent microscope images of pre-osteoblasts cultured up to 70% of confluence after incubation for 4 days with ZnO NCs at different concentrations.
Figure S4. Fluorescent microscope images of pre-osteoblasts cultured up to 70 % of confluence after incubation for 4 days with ZnO-NH$_2$ NCs at different concentrations.
Figure S5. Differentiation assays in term of total protein content after 10 days of incubation with ZnO (top) and ZnO-NH$_2$ (bottom) NCs at different concentrations. (p < 0.05, significant differences compared to control denoted by an asterisk.)
Figure S6. Cell morphology evaluation by optical microscopy of MC3T3-E1 pre-osteoblast cells after 10 days of incubation with ZnO NCs.
Figure S7. Cell morphology evaluation by optical microscopy of MC3T3-E1 pre-osteoblast cells after 10 days of incubation with ZnO-NH$_2$ NCs.
Table S1. Reduction in bacteria viability after 24 h of incubation with ZnO and ZnO-NH$_2$ NCs, respectively. The values shown in bold indicate a CFU concentration less than $10^2$ CFU/ml, which is considered as positive effectiveness.

| Concentration | E. coli vs ZnO | E. coli vs ZnO-NH$_2$ | S. aureus vs ZnO | S. aureus vs ZnO-NH$_2$ |
|---------------|----------------|----------------------|------------------|------------------------|
| 5 μg/mL       | 99.999%        | 100%                 | 99.923%          | 99.935%                |
| 10 μg/mL      | 100%           | 100%                 | 99.965%          | 99.963%                |
| 25 μg/mL      | 100%           | 100%                 | 99.985%          | 99.990%                |
| 50 μg/mL      | 100%           | 100%                 | 99.940%          | 99.853%                |
| 100 μg/mL     | 100%           | 100%                 | 99.963%          | 99.883%                |