Supplementary Information

Biosynthesis, characterization, and augmented anticancer activity of ZrO_2 doped ZnO/rGO nanocomposite.

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Endpoint chromogenic limulus amebocyte lysate (LAL) assay

The endpoint chromogenic limulus amebocyte lysate (LAL) assay kit (Lonza, Basel, Switzerland) was used to examine endotoxin contamination in ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs. This assay has a sensitivity range of 0.1 EU/ml - 1.0 EU/ml. Briefly, ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs at a concentration of 50 μg/ml was were mixed with the LAL supplied in the test kit and incubated at 37 °C for 10 min. A peptide substrate solution was then mixed with the LAL-sample mixture and incubated at 37 °C for next 6 min. The reaction was then stopped by addition of stop reagent supplied with the kit. In addition to the complete reaction mixture (i.e. NPs/NCs+LAL+substrate), two additional mixtures were prepared to check the possible interference of NPs/NCs in the assay, namely NPs/NCs+LAL and BT NPs+substrate. If endotoxin is present in the sample, a yellow color should develop only in the complete reaction mixture, not in other two mixtures. The absorbance of the enzymatically cleaved p-nitroaniline part of the substrate

peptide was measured at 405 nm in a microplate reader (Synergy-HT, BioTek, Vinnoski, VT, USA). Since this absorbance is in direct proportion to the amount of endotoxin present, the concentration of endotoxin can be calculated from a standard curve using LPS.

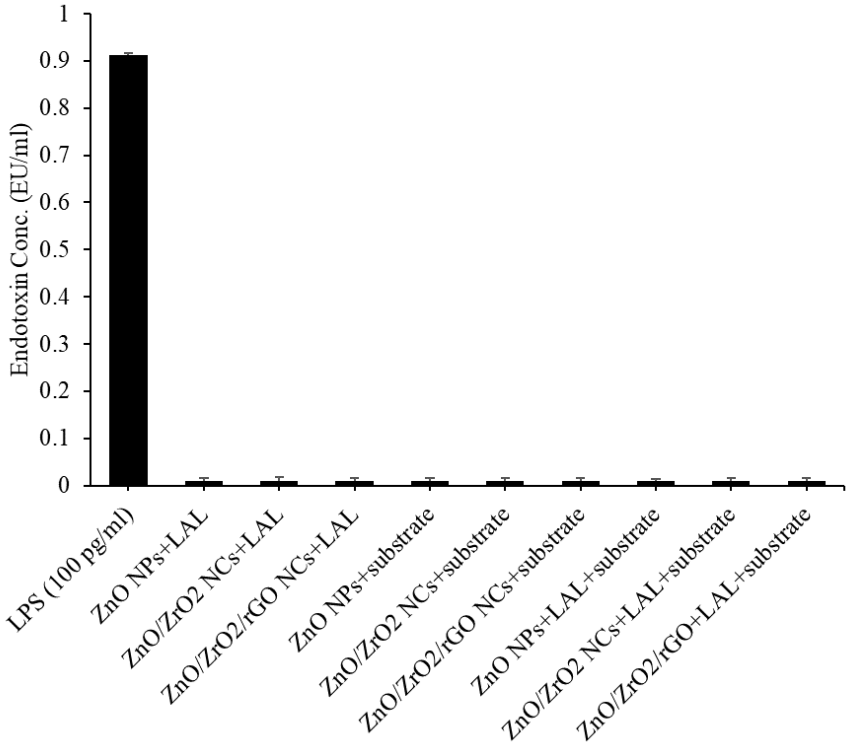

Figure S1. Endpoint chromogenic limulus amebocyte lysate (LAL) assay for endotoxin detection in prepared ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs**.** A concentration of 50 μg/ml of ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs were incubated with LAL (containing enzyme), or substrate, or both (LAL+substrate). After the completion of incubation time, absorbance of the substrate was measured at 405 nm. If endotoxin is present in the sample, a yellow color should develop only in the complete reaction mixture (NPs/NCs+LAL+substrate), not in other two mixtures. The absorbance of the enzymatically cleaved p-nitroaniline part of the substrate peptide was measured at 405 nm by a microplate reader (Synergy-HT).

Results showed that there is no endotoxin contamination in synthesized ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs.

Hydrodynamic size and zeta potential examination

Hydrodynamic size and zeta potential of green prepared ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs was examined in culture media (DMEM) (Figure S2).

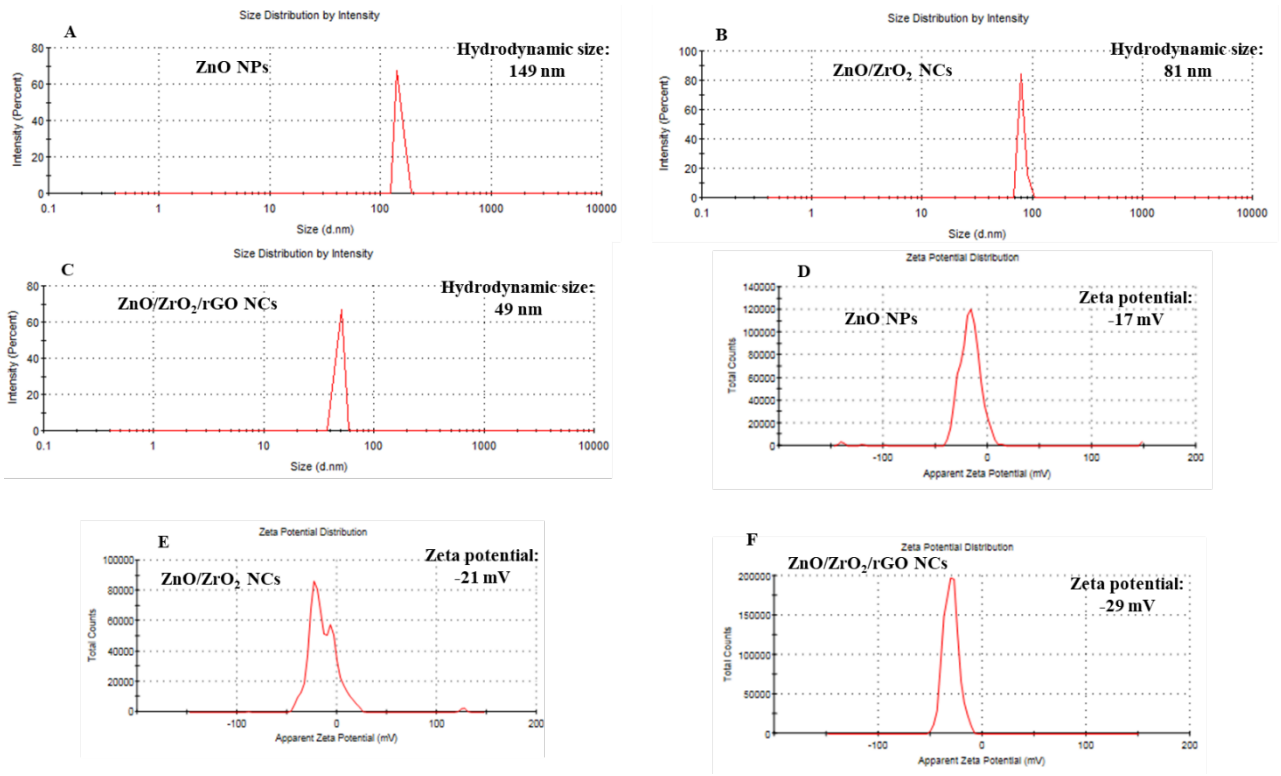

Figure S2. DLS characterization of green prepared ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs. (A-C) Hydrodynamic size of ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs. (D-F) Zeta potential ZnO NPs, ZnO/ZrO_2 NCs, and ZnO/ZrO_2/rGO NCs.