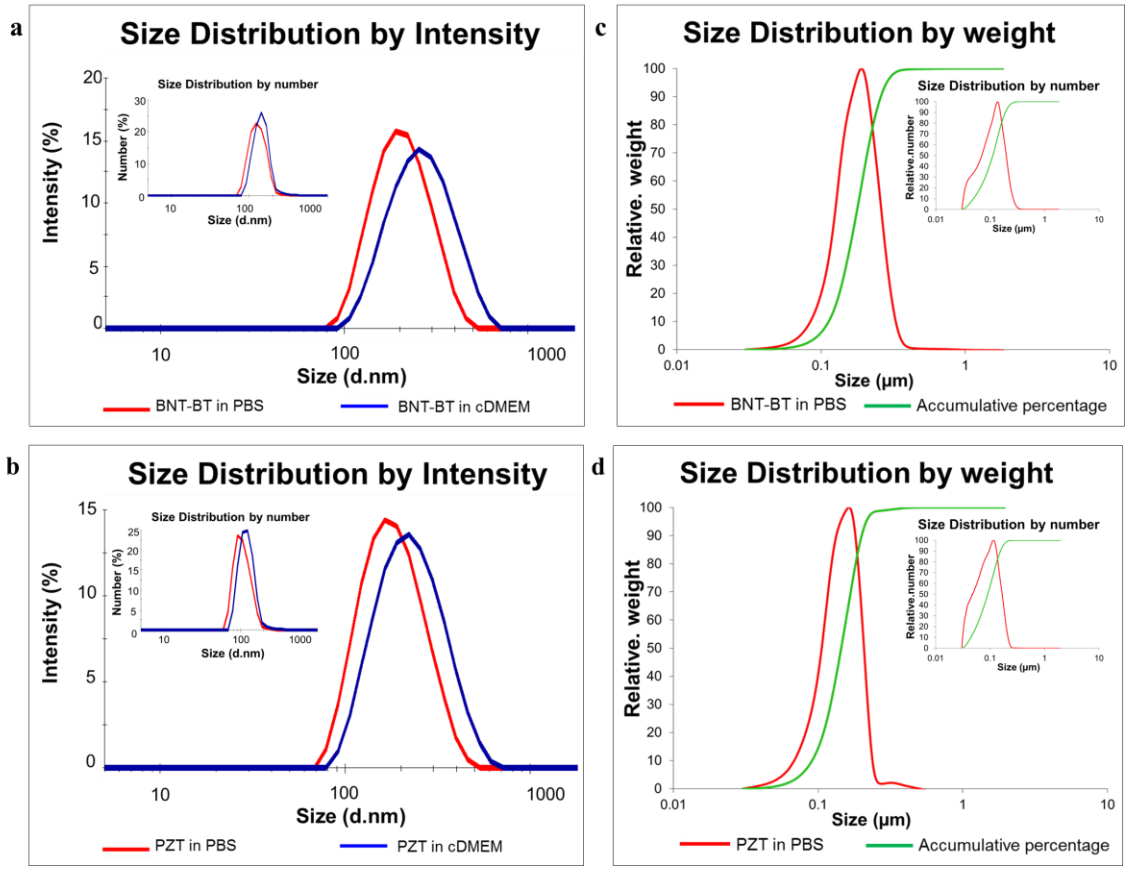
Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Bismuth-based nanoparticles as the environmentally–friendly replacement for lead-based piezoelectrics



Size distribution of BNT-BT and PZT nanoparticles by DLS and DCS.

Figure S1. BNT-BT and PZT size distribution was measured by DLS and DCS. Size distribution by intensity and by number showed a size increase of about 30 nm when the nanoparticles were dispersed in cDMEM, consistent with the formation of a complex protein corona on the surface of nanoparticles.

Nuclear evaluation of A549 cells exposed to BNT-BT and PZT nanoparticles

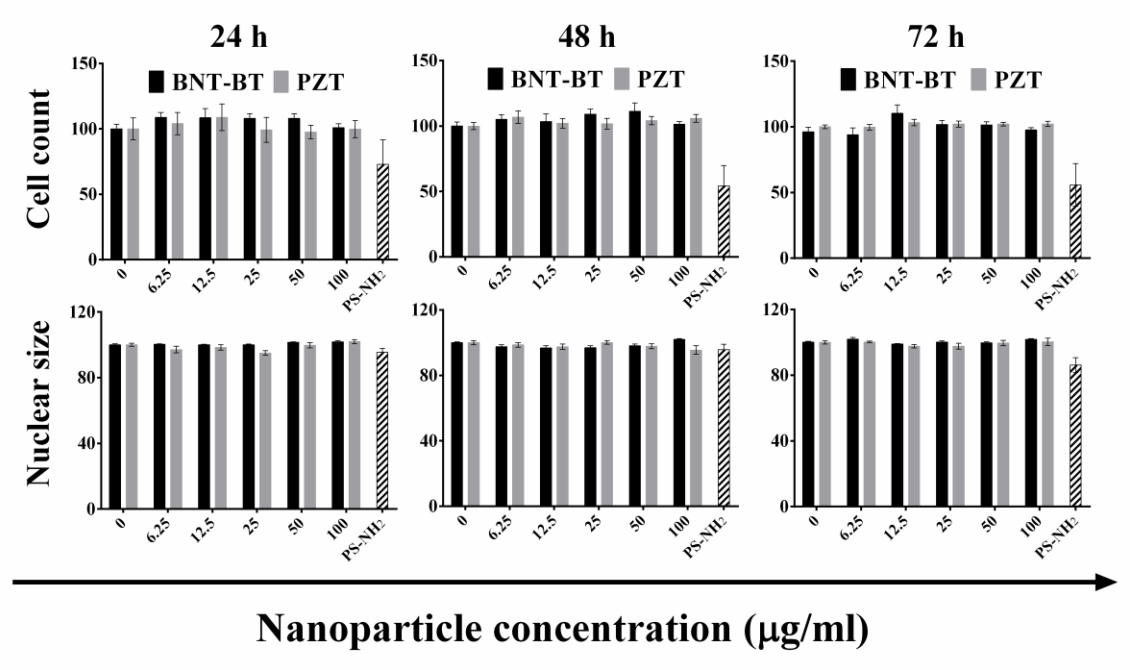


Figure S2a. Nuclear effects after the exposure to BNT-BT and PZT nanoparticles in A549 cells. A549 cells were exposed to increasing concentrations of BNT-BT and PZT nanoparticles in dispersion (6.25 to 100 µg/ml) for 24, 48 and 72h. The nuclear intensity was evaluated by the fluorescent dye Hoechst 33342. The data represent the mean ± SEM of three independent experiments.

A549 cells exposed to BNT-BT and PZT nanoparticles during 24, 48 and 72h.

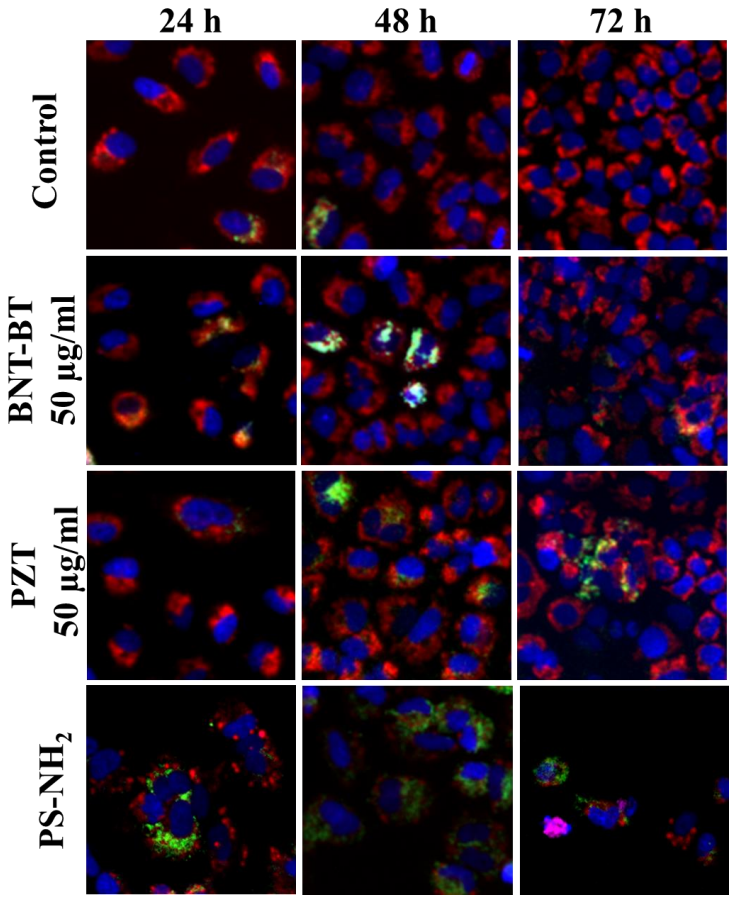


Figure S2b. Representative images of A549 cells exposed to 50 µg/ml of BNT-BT and PZT for 24, 48 and 72h. A549 cells were exposed to increasing concentrations (6.25 to 100 µg/ml) of BNT-BT and PZT nanoparticles for 24, 48 and 72 h. Control cells were exposed to cell culture medium without nanoparticles (Control) and 25 µg/ml PS-NH2 nanoparticles were used as positive control. Images were acquired using high content analysis to assess modification in nuclear morphology (Hoechst 33342), mitochondrial membrane potential (TMRM), acidic compartments (Lysotracker green), and plasma membrane integrity (TOPRO-3). No alterations of cell morphology were found after the exposure to BNT-BT and PZT nanoparticles and a slight increase in the presence of acidic compartments was observed. The control did not how alterations of any of the parameters evaluated, as expected. The positive control PS-NH2 nanoparticles caused a decrease in the number of cells, mitochondrial membrane potential, and membrane integrity together with an increase in nuclear condensation and acidic compartments.

Cell membrane integrity evaluation after the exposure to BNT-BT and PZT nanoparticles

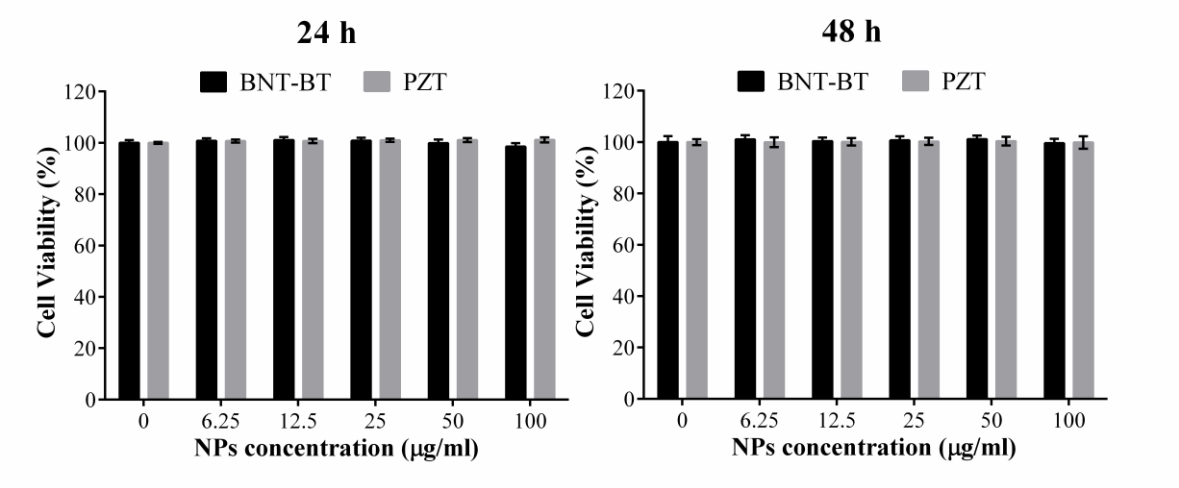


Figure S3a. Cytotoxicity evaluation of BNT-BT and PZT nanoparticles. (a) Cell viability was assessed by the liberation of LDH to the medium. No difference was observed when compare the control against the treated cells.

Mitochondrial activity of A549 cells after the exposure to BNT-BT and PZT nanoparticles

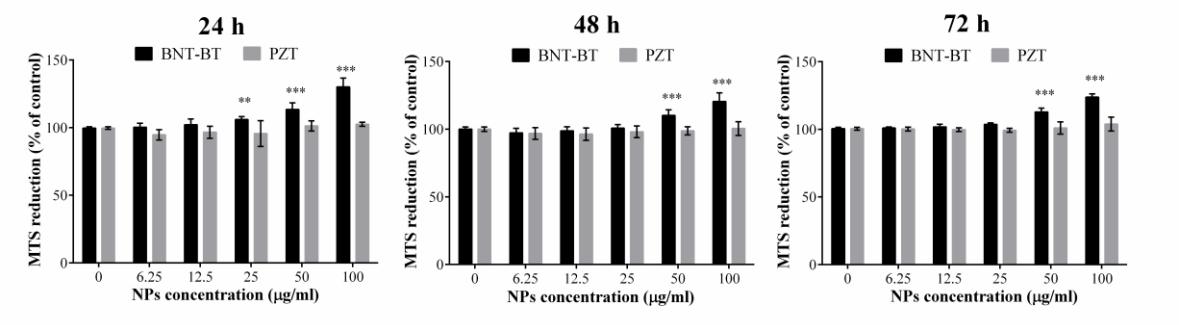


Figure S3b. Cytotoxicity evaluation of BNT-BT and PZT nanoparticles. Tetrazolium salt is converted by mitochondrial enzymes to a dark brown compound which is revealed by its increased absorbance. This method indicate the mitochondrial activity and indirectly quantify the amount of cells that are alive. Significant difference was observed between control cells and cells treated with BNT-BT nanoparticles after 24, 48, and 72 h of exposure at the highest concentrations. ** $p < 0.01$, *** $p < 0.001$.

TEM images of complete cells and organelles.

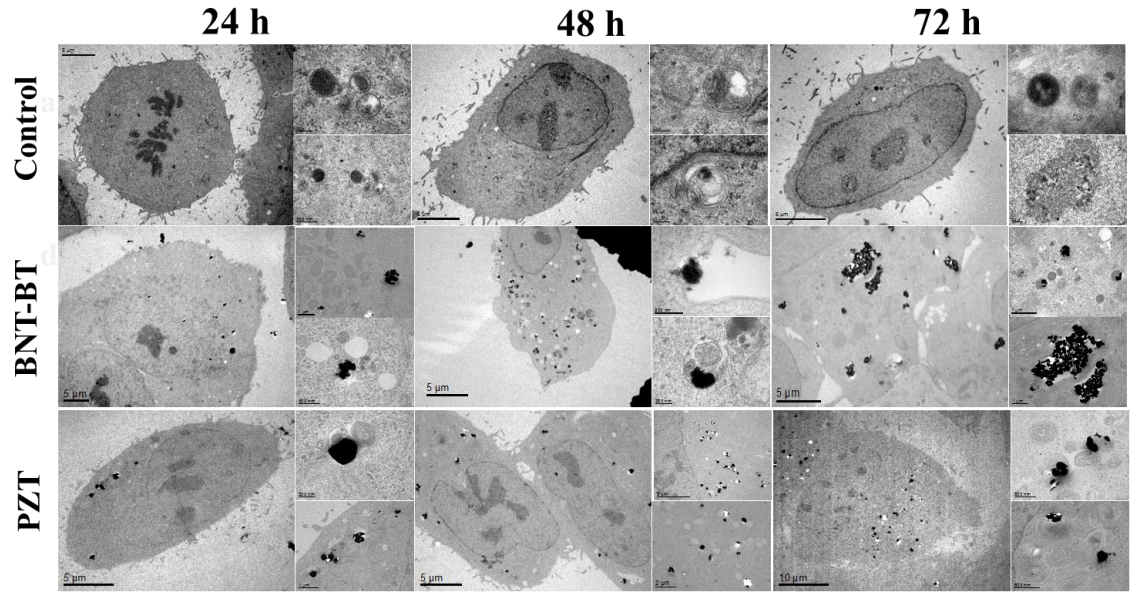


Figure S4. Subcellular localization of BNT-BT and PZT nanoparticles by TEM. BNT-BT and PZT nanoparticles were visualized easily due to their high electric conductivity. Nanoparticles inside vesicles in the cytoplasm of A549 cells are shown at 24, 48 and 72 h. exposure. Nanoparticles were located in lamellar bodies, lysosomes and other endocityc related vesicles in the perinuclear area of the cells. Overall cells appeared healthy without changes in the normal morphology as shown in the images of the complete cell.

BNT-BT and PZT nanoparticles in the cells and in the cell culture medium.

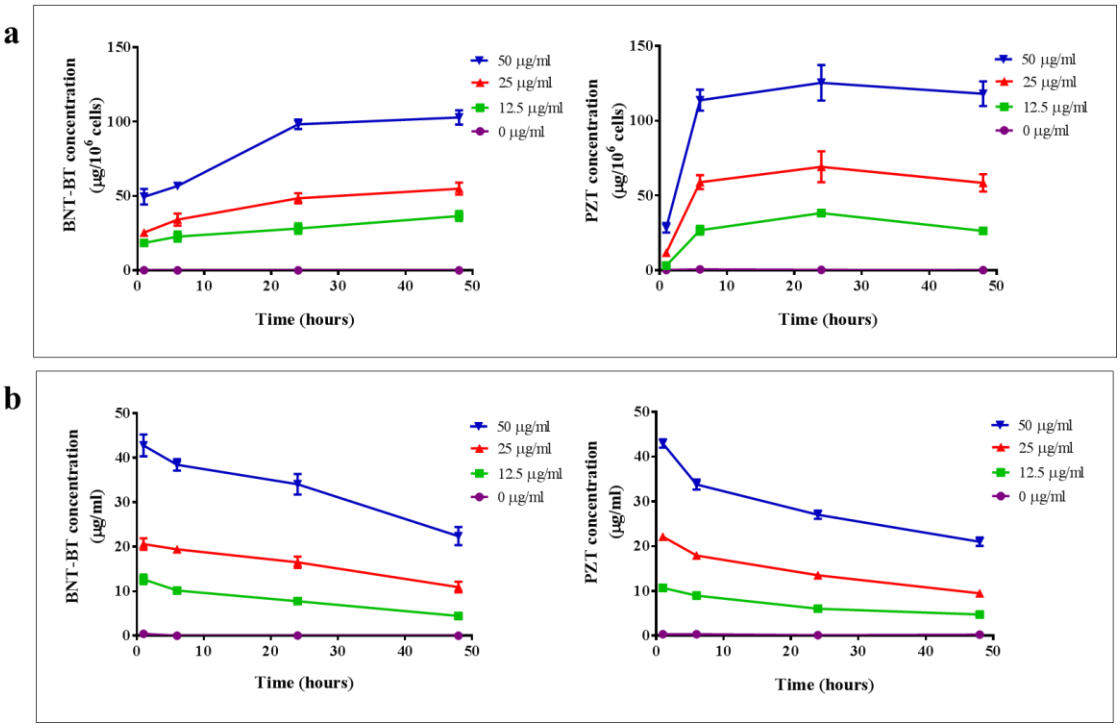


Figure S5. BNT-BT and PZT nanoparticles are uptake by A549 cells on a concentration-dependent manner. A549 cells were incubated during 1, 6, 24, and 48 h with increasing concentrations (0-50 µg/ml) of nanoparticles. Samples were digested and the main metals were quantified in cells (a) and cell culture medium (b) by atomic fluorescence spectrometry for bismuth and atomic absorption spectrometry for lead. The concentration of nanoparticles in the cells was calculated and normalized by cell number. Nanoparticles were uptake by cells in a time – concentration – dependent manner; thus, showing a first order kinetic. The increase of nanoparticles in the cells was confirmed by the decrease in the nanoparticles in the cell culture medium.