

Supplementary materials

Effects of Metallic and Carbon-Based Nanomaterials on Human Pancreatic Cancer Cell Lines AsPC-1 and BxPC-3

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Interference Analysis

In order to exclude the influence of nanomaterials on the result of MTT and LDH cytotoxicity assays interference analysis was performed. The performed analysis revealed a statistically significant influence of Ag nanoparticles at the concentrations of 2 mg/L and 5 mg/L on the results of colorimetric LDH reaction.

Firstly 90 µL of complete growth medium RPMI 1640 (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% foetal bovine serum (FBS, Gibco) and 1% of antibiotic – antimycotic mix (Gibco) was added to the 96-well microplate. Subsequently 10 µL of 10x concentrated nanomaterials suspensions was added in order to obtain final concentration as follows: 0.1 mg/L; 0.5 mg/L; 1 mg/L; 2 mg/L; 5 mg/L for metallic (Ag, Au, Pt) nanoparticles and 10 mg/L; 20 mg/L; 50 mg/L; 100 mg/L; 200 mg/L for carbon-based nanomaterials (GO, ND, C₆₀(OH)₄₀) and incubated together in humidified atmosphere containing 5% of CO₂ at for 37°C for 24h.

The MTT and LDH interference analyses (Thermo Fisher Scientific) were carried out similarly to the cytotoxicity tests but without the presence of cells. For MTT interference (Figure S1) analysis MTT reagent (Thermo Fisher Scientific) was dissolved in phosphate-buffered saline (PBS, Sigma Aldrich, St. Louis, MO, USA) in a concentration of 5 mg/mL and 10 µL was introduced into each well. After 4 h of incubation in 37°C, the culture medium was removed, and formazan crystals were dissolved in 200 µl of solubilization detergent (Iso-propanol, Triton X-100, 0.01N HCl). Afterwards, microplates were centrifuged (10 min, 200 x g) and 100 µl of supernatant was transferred to a new plate. Spectrometer readings were performed at a wavelength of 570 nm in a microplate reader.

MTT - interference analysis

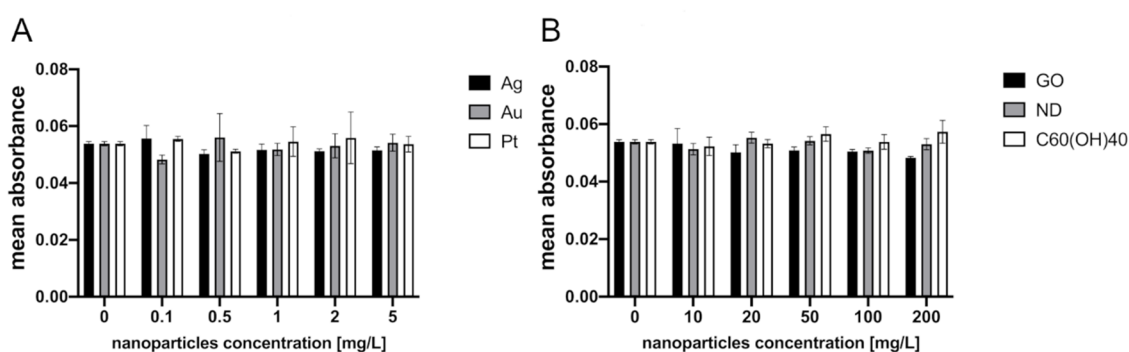


Figure S1. MTT interference analysis. Interactions of MTT reagent and nanomaterials presented as the mean absorbance from 3 replications with standard deviation. A statistically significant difference between a group and the control was marked as * $p < 0.05$. Capital letters refer to different groups of nanomaterials: (A) metallic NPs, (B) carbon-based nanomaterials.

For LDH (Thermo Fisher Scientific) interference analysis (Figure S2) nanomaterials were incubated for 24 h in the prepared microplates. Subsequently, microplates were centrifuged (10 min, 200 \times g) and 50 μ L of supernatant was transferred into a new plate. One hundred microlitres of LDH reaction mixture was added to the probes and incubated for 30 minutes at room temperature, protected from light. Spectrophotometer readings were performed at a wavelength of 490 nm (reference wavelength: 690 nm) in a microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

LDH - interference analysis

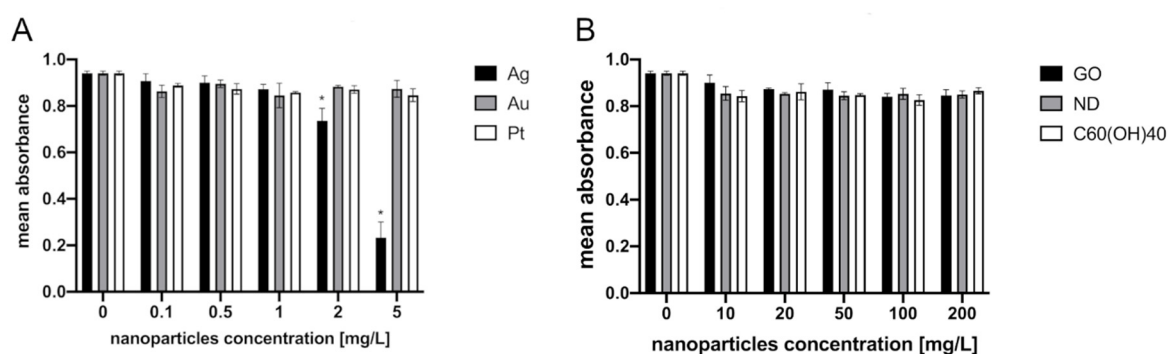


Figure S2. LDH interference analysis. Interactions of LDH reagent and nanomaterials presented as the mean absorbance from 3 replications with standard deviation. A statistically significant difference between a group and the control was marked as * $p < 0.05$. Capital letters refer to different groups of nanomaterials: (A) metallic NPs, (B) carbon-based nanomaterials.