## Nanoparticles' interference in the evaluation of in vitro toxicity of silver

## nanoparticles

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## Supplementary data



**Fig. S1** The absorption of LDH with 0.5U mL<sup>-1</sup> by AgNP-PVP-20. LDH was incubated with AgNP-PVP-20 for 0h (a) and 24h (b) followed by centrifugation, respectively. After the 30 min centrifugation at 16000 g at 20°C, the supernatant was mixed with LDH reaction reagents. (c) LDH was incubated with AgNP-PVP-20 for 24h without centrifugation. After incubation, the supernatant was transferred into a new plate and mixed with LDH reaction reagents. Ratio means the LDH activity relative to the untreated control group. \*p<0.05, \*\*p<0.01, significantly decreased compared with the control group.



**Fig. S2** The interference of AgNPs only with MTS solution under abiotic conditions. (a) AgNP-PVP-20. (b) AgNP-CIT-20. (c) AgNP-CIT-110. In Table 3, the different value between Group (c) and (a) was compared with that between Group (g) and (e) to see whether the mixture of AgNPs with MTS can generate formazan. Ratio means the value of (g-c) at 490 nm relative to that of (c-a). \*p<0.05, \*\*p<0.01, significantly decreased compared with the control group.



**Fig. S3** NO production in RAW264.7 cells after 24 h exposure to AgNP-PVP-20. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, significantly different from the control group.

For NO detection, RAW 264.7 cells were seeded in a 96-well plate at  $2x10^5$  cells per well in complete RPMI1640 medium and permitted to grow for 24 h at 37°C in a 5% CO<sub>2</sub> humidified incubator. The following day, the medium was changed with phenol red-free RPMI1640, 500 ng mL<sup>-1</sup> LPS and AgNPs. Cells were incubated at 37°C with 5% CO<sub>2</sub> for 24 h. 100 µL of Griess reagent was added into each well. After shaking, the 96-well plate was placed on a microplate reader (Infinite 200, Austria) for the absorbance detection at 550 nm. The concentration of NO was calculated by NaNO<sub>2</sub> standard curve.