

## Different amino acid modified chitosan nanoparticles for chelating Cu<sup>2+</sup> to suppress the cytotoxicity caused by CuO nanoparticles

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The adverse effects of metal oxide nanoparticles to human beings and environment have received extensive attention recently. It is urgently required to develop a simple and effective method to suppress the toxicity of metal-based nanomaterials. In this study, chitosan nanoparticles (CS NPs) were prepared as a chelation agent and were modified with different amino acid, that was, lysine (named as Ly), glutamic acid (named as Glu), and pristine CS NPs dealed with NaBH<sub>4</sub> (named as CS), to promote the chelating efficiency.



**Figure 1.** Schematic illustration to show the process of suppressing the cytotoxicity. After the CuO NPs are uptaken and Cu<sup>2+</sup> is released, they will be chelated by the CS NPs. Finally, the cytotoxicity of CuO NPs is reduced.



**Figure 5.** Relative viability of three kinds of cells being incubated with 100  $\mu$ g/mL of different chitosan nanoparticles and 25  $\mu$ g/mL CuO nanoparticle for 24 h and 72 h, respectively.





**Figure 2.** Representative SEM images of chitosan nanoparticles(a) modified with lysine, (b) modified with glutamic acid and (c) dealed with NaBH<sub>4</sub>.



**Figure 3.** Representative TEM images of chitosan nanoparticles (a) modified with lysine, (b) modified with glutamic acid and (c) dealed with NaBH<sub>4</sub>.

**Figure 6.** Intracellular ROS level of (a) HepG2 cells, (b) A549 cells, and (c) RAW264.7 cells after being treated with 20  $\mu$ g/mL CuO NPs in the absence and presence of different chitosan particles for 24 h, respectively. Untreated cells and the cells treated with 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 10 min were used as negative and positive controls, respectively..

## Conclusion

Uniform and well-dispersed chitosan nanoparticles were prepared by emulsion crosslinking method. Their chelating efficiency towards Cu<sup>2+</sup> were improved after modified with amino acid. Besides, cytotoxicity and the ROS level brought by CuO nanoparticles were suppressed after adding CS NPs, especially for these modified with lysine.



**Figure 4.** Adsorption capacity of  $Cu^{2+}$  on chitosan nanoparticles with lysine (Ly), Glutamic acid (Glu) and dealed NaBH<sub>4</sub> (CS).

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