Surface and size effects on cellular uptake of gold nanoparticles by phagocytic and non-phagocytic cells

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Introduction

Gold nanoparticles (AuNPs) due to their unique properties have been widely used for biomedical application. Understanding the nanoparticles interaction with cells is important for realizing their function. Surface charge[1] and size[2] of nanoparticles are demonstrated greatly affect their uptake by cells. However, few of work considered the effect of cell tpye, especially between phagocytic and non-phagocytic cells. Cell uptake of phagocytic cells such as macrophages is greatly affect the nanoparticles fate in vivo.[3] In this study, we prepare both positively and negatively charged gold nanoparticles with different sizes, and study their cell uptake with both phagocytic and non-phagocytic cells (Fig. 1). This basic research aims to synthetically study the relevance amoung nanoparticle size, surface charge and cell type for cellular uptake, which will be important for understanding the nanopartilces interaction with cells.

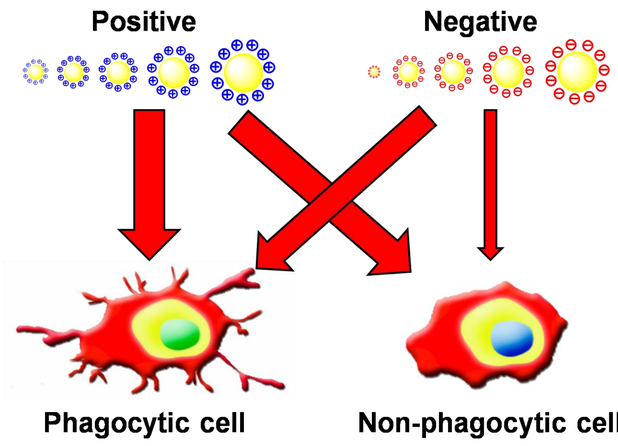

Fig. 1. *Schematic of cell uptake of gold nanoparticles with different size and surface charge by phagocytic and non-phagocytic cells.*

Method

Gold nanoparticles with a diameter of ca. 4 nm are synthesized by using $NaBH_4$ as reducer, nanoparticles with diameters from 16 nm to 58 nm are synthesized by citrate-mediated growth method in boiling water.

Positively and negatively charged AuNPs are prepared by modifying the citrate capped nanoparticles with (mercaptodecyl)trimethylammonium bromide (TMA) and mercaptoundecanoic acid (MUA) respectively (Fig. 2).

All these nanoparticles are purified by centrifugation and filtration with 450 nm membrane. Their physicochemical properties are characterized by UV-Vis spectrum, TEM, DLS, zeta potential.

Cell lines RAW 264.7 and HepG2 are chosen as phagocytic cell and non-phagocytic cell respectively (Fig. 2). Inductively coupled plasma mass spectrometry (ICP-MS) and TEM cell sections analysis are applied to study the cell uptake of gold nanoparticles. Cytotoxicity evaluation is done by both MTT and LDH assay.

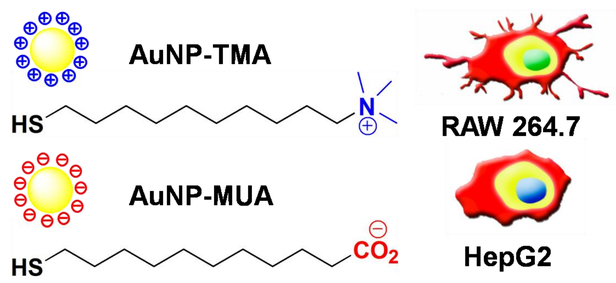

Fig. 2. *Schematic of gold nanoparticles and cells used in this study.*

Results

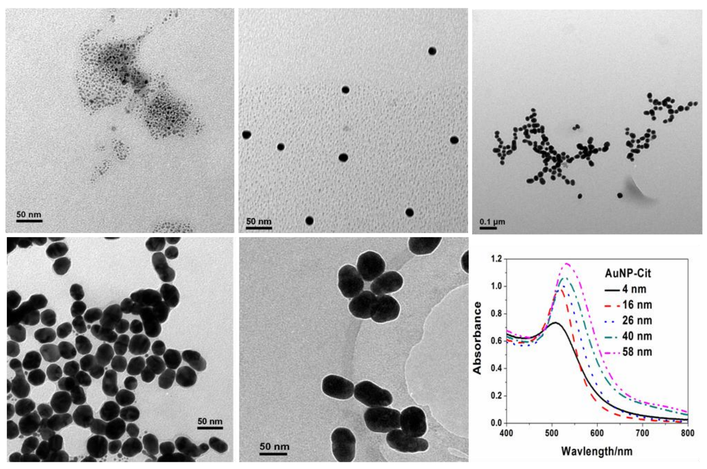

Fig. 3. *TEM images and UV-Vis spectra of as-synthesized gold nanoparticles with different sizes.*

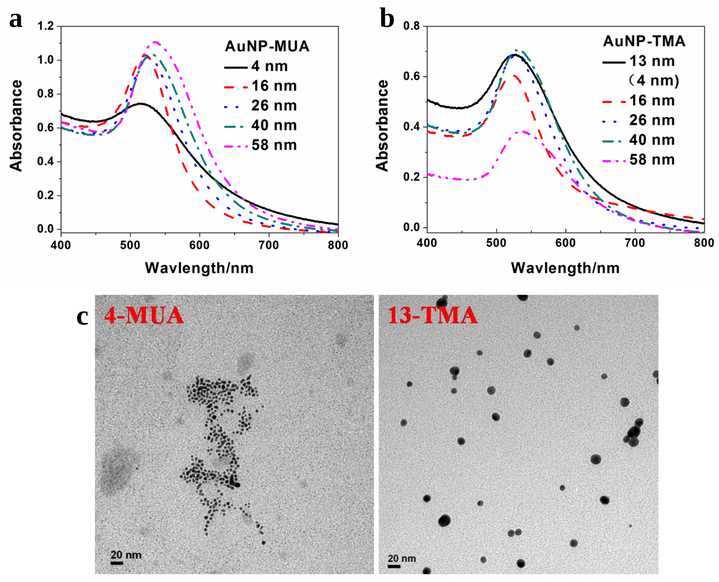

Fig. 4. *UV-Vis spectra of gold nanoparticles modified with negative MUA (a) and positive TMA (b), TEM images of pre-4 nm AuNPs after modified with MUA and TMA (c). Note: after modified with TMA, the AuNP size change from 4 nm to ca. 13 nm, the size of other AuNPs don't change after modification.*

	Dh (nm)	ζ （mV)		Dh (nm)	ζ （mV)
4-MUA	117.2	-9.92	13-TMA	135.5	-12.43
16-MUA	131.1	-11.26	16-TMA	115.2	-14.29
26-MUA	135.8	-9.15	26-TMA	140.0	-14.77
40-MUA	129.4	-10.64	40-TMA	132.7	-18.70
58-MUA	97.2	-9.85	58-TMA	172.7	-18.94

Tab. 1. *Hydrodynamical sizes and zeta potential of gold nanoparticles in cell culture media DMEM with 10% FBS.*

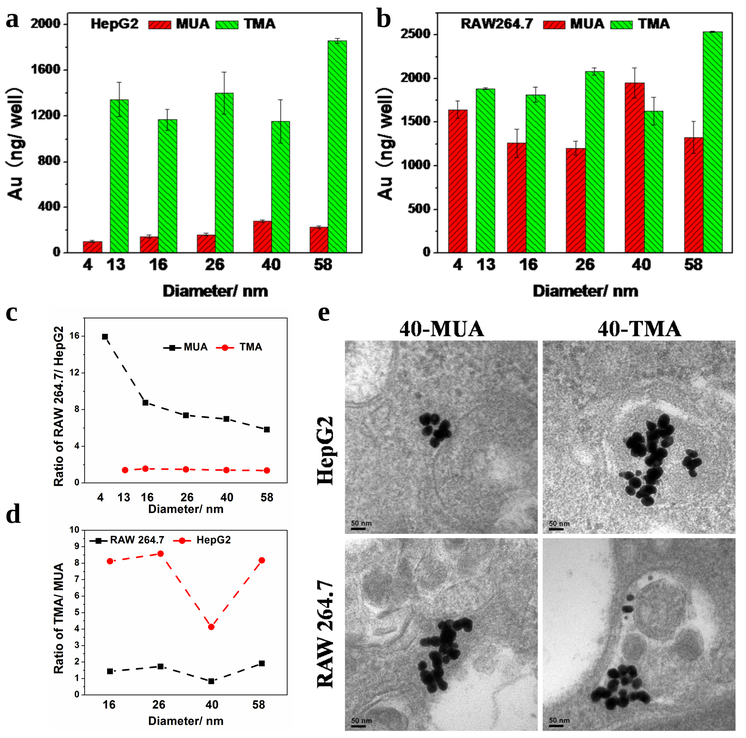

Fig. 5. *ICP-MS measurements for Au contents per well of HepG2 (a) and RAW 264.7 (b) after incubation with different AuNPs at a concentration of 10 mg/L for 12 h, error bars represent mean ± SD ($n \geq 3$), (c) comparison of the uptake difference between two cell types for negative and positive AuNPs with different sizes, (d) comparison of the uptake difference between positive and negative AuNPs with different sizes in two kinds of cells, (e) TEM cell sections of MUA and TMA modified 40 nm AuNPs uptaken by HepG2 and RAW 264.7 .*

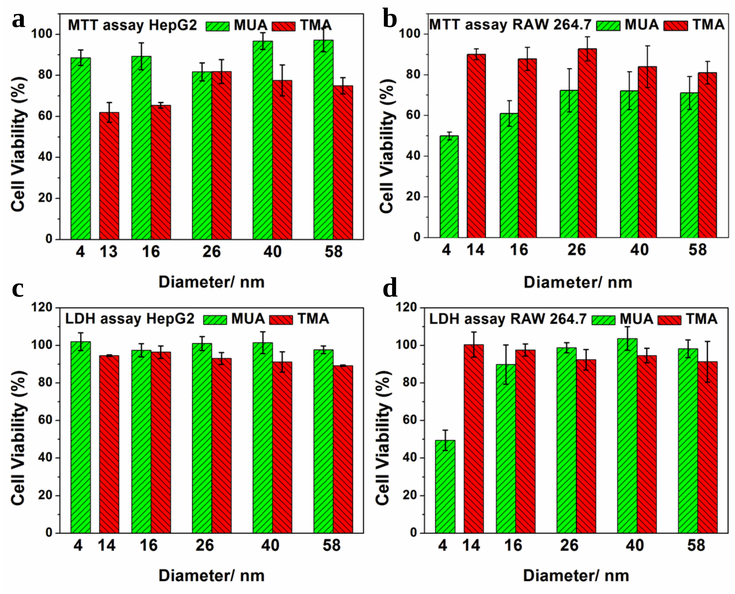

Fig. 6. *Cytotoxicity evaluation of HepG2 (a, c) and RAW 264.7 (b, d) after incubation with different AuNPs at a concentration of 10 mg/L for 24 h evaluated by MTT assay (a, b) and LDH assay (c, d), error bars represent mean ± SD ($n \geq 3$).*

Brief discussion

Larger hydrodynamical sizes and all negative zeta potential results of AuNPs due to form certain aggregation of nanoparticles and serum protein adsorption when measured in culture media (Tab.1) .

The size dependent uptake effect is different between positive (highest for 58 nm) and negative (highest for 40 nm) AuNPs may due to their different aggregate form and endocytosis route (Fig. 5 a, b).

The cytotoxicity results indicate that most of these gold nanoparticles mainly affect the mitochondria activity (reflected by MTT) while have little effect to cell membrane integrity (reflected by LDH). For 4 nm AuNPs, it have much stronger cytotoxity than other larger nanoparticles when they are highly uptaken by the phagocytic RAW 264.7 cells (Fig. 6) .

Conclusion

Here we demonstrate that in all size range the uptake of positive nanoparticles is much higher than that of negative nanoparticles by non-phagocytic HepG2. While the uptake amount of negative nanoparticles is similar to that of positive nanoparticles for phagocytic RAW 264.7. The uptake of negative nanoparticle in RAW 264.7 is significantly higher than that in HepG2, and the difference is increased as size decreased. For positive nanoparticles the difference between the two cells is much less obvious. Cytotoxicity evaluated by both MTT and LDH assay reveals that the cell viability is closely relative to the cell uptake, depends on cell types and also is affected by the applied method. It suggests that nanoparticles interaction with cells is closely related to nanoparticle size, surface charge and cell types.

Acknowledgements

Financial supports from the National Science Fund for Distinguished Young Scholars (51025312), the National Basic Research Program of China (2011CB606203), NSFC-50830106, 21174126 and Open Project of State Key Laboratory of Supramolecular Structure and Materials (SKLSSM 201204) and Research Fund for the Doctoral Program of Higher Education of China (20110101110037) are gratefully acknowledged.

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