Comparative effect of wrapping solid gold nanoparticles and hollow gold nanoparticles with doxorubicin-loaded thermosensitive liposomes for cancer thermo-chemotherapy

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Supporting information

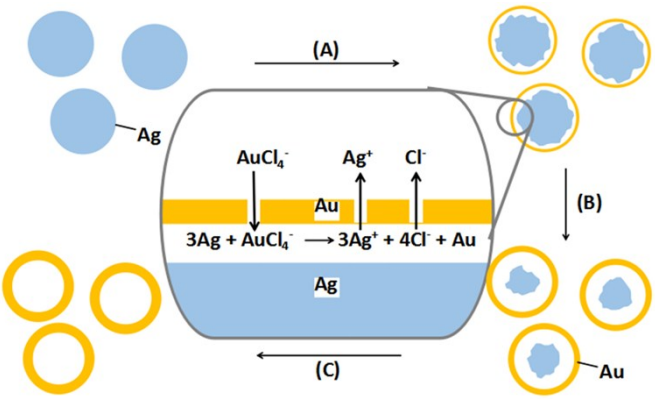
Methods:

Silver template Engaged Replacement Reaction method

The templated galvanic replacement reaction of silver for gold provides a simple and reproducible route to HGNP preparation. Silver nanoparticles (AgNPs) with diameters of the desired HGNPs core size are synthesized first, then are sacrificed by adding a gold salt to the solution; the gold ions are reduced to metal because gold has a greater standard reduction potential than the silver template, which is oxidized to a molecular solution[1] (Scheme S1). The gold plates were onto the outside of the dissolving silver nanoparticles, resulting in an HGNP of controlled diameter, wherein the shell thickness is determined by the relative amount of gold salt to the silver template. The ratio of shell diameter to shell thickness governs the wavelength of the HGN absorbance [1,2].

Silver templates are prepared at 60 ºC in a well-stirred 600 mL solution of 0.2 mM silver nitrate with 0.6 mL 1.0 M sodium borohydride ($NaBH_4$) in the presence of 0.5 mM sodium citrate. The solution is stirred for at least 2 h to allow the $NaBH_4$ to fully hydrolyze. The addition of $NaBH_4$ accelerates the chemical reaction, and the resulting nanoparticles are ca. 15-25 nm in diameter. After cooling to room temperature, larger AgNPs could be grown from these stock sols, if desired. Silver particle growth is initiated by adding 0.5 mL 2.0 M hydroxylamine hydrochloride solution to the silver sol and stirring for 5 min, followed by the addition of 1.25 mL 0.1 M $AgNO_3$ (0-1mL), and stirred overnight. HGNPs could then be made via galvanic replacement chemistry from the template silver sols without the need to isolate the AgNPs.

First, a given silver sol (50 mL) is heated to 60 °C and the necessary amount of 25 mM tetrachloroauric acid ($HAuCl_4$) is added dropwise. Silver (Ag+ /Ag 0.8V, vs. SHE) has a lower redox potential than gold (AuCl4-/Au 0.99V, vs. SHE) and the replacement reaction is : $3Ag(s)+AuCl_4^-(aq)\rightarrow Au(s)+3Ag^+(aq)+4Cl^-(aq)$. Upon the addition of the concentrated $HAuCl_4$, the solution turns from yellow/orange to gray/yellow to blue/gray to blue/turquoise within seconds as the silver and gold are oxidized and reduced, respectively. The reactions are monitored using UV/vis/NIR spectroscopy, and stopped when the 400 nm silver peak vanishes. Once the reaction is complete, the samples are cooled, silver chloride is allowed to precipitate, and the supernatant containing the HGNPs are transferred to another vessel and stored at 4 °C until further use.



Scheme S1. Schematic illustration of the experimental procedure that generates HGNPs from silver templates with various morphologies. The reaction is illustrated in the schematic as follows: (A) Addition of $HAuCl_4$ to a dispersion of AgNPs and initiation of the replacement reaction; (B) The continued replacement reaction of $HAuCl_4$ with the AgNPs; (C) Depletion of silver and annealing of the resultant shells to generate smooth hollow structures. Note that the shape of each silver nanoparticle is essentially preserved in this template-engaged reaction.

Cobalt template Engaged Replacement Reaction method

All glass was cleaned with aqua regia and rinsed with high purity water prior to use to eliminate adsorbents. The cobalt particle synthesis was performed on a Schlenck line[3]. A total of 100 μL of aqueous 0.4 M cobalt chloride ($CoCl_2$), 2mL of 0.05 M aqueous sodium citrate and 2 mL 1 % poly-N-vinylpyrrolidone (PVP) solution were added to 100 mL of water in a double neck round-bottom flask. The clear solution was pumped down for 5 min then exposed to N_2 to deoxygenate the solution. After ca. 2 min under N_2, 1 mL of freshly prepared 0.1 M sodium borohydride ($NaBH_4$) was added to the solution and stirred for 15 min until the color changed from clear to brown and finally to gray, indicating the reduction of Co 2+ ions in solution to Co0 nanoparticles. After 10 min of growth, a total of 400 μL of 25 mM $HAuCl_4$ was injected. After an immediate, slight color change from gray to blue-gray, the particles are exposed to air until the color is completely changed to purple, blue, or green, depending on particle parameters. The gold shell forms within seconds onto the cobalt particle. Upon exposure to oxygen, this cobalt core oxidizes and dissolves, revealing the true color of the HGNPs solution. The prepared HGNPs were centrifuged for 15min at the 10000rpm. The precipitate was collected and re-dispersed for further use.

Effect of PVP on Co nanoparticles templates for Gold Shell Growth

To examine the effect of PVP on the HGNPs synthesis, cobalt nanoparticles were prepared without PVP, while other reagents and steps in the HGNPs synthesis were kept identical. Representative absorption spectra were recorded for comparison.

In order to investigate the stability of the HGNPs in the presence of proteins with and without the protection of PVP, several representative proteins with different physical properties (listed in Table 1) were chosen to carry out this study. The methods in detail are described in the supporting information.

Table .1 Physical properties of proteins

Protein	Source	pI
Fibrinogen	Bovine	5.5
RNase A	Bovine	9.5
Lysozyme	Hen egg white	11.1

Effect of Particle Size and Wall Thickness on Optical Properties

One of the major intents of this size tuning is the control of the optical properties of the HGNPs. In order to tune the plasmon absorption across much of the visible to NIR spectrum, 200, 300, 400 and 500 μL $HAuCl_4$ (25 mM) was added respectively to vary wall thickness and particle size while other reagents and steps in the HGNPs synthesis were kept identical. Representative absorption spectra and HGNPs size were recorded for comparison.

Stability of HGNPs in solutions of proteins with and without the protection of

PVP

In this part, the abilities of adsorbed PVP in preventing the aggregation of gold NPs stabilized by citrate, in solutions of proteins was reported. Many types of proteins tend to non-specifically adsorb on the solid–aqueous interfaces by inducing the aggregation of nanoparticles in the presence of proteins in solutions[4]. In order to investigate the stability of the HGNPs in the presence of proteins with and without the protection of PVP, several representative proteins with different physical properties (listed in Table 1) were chosen to carry out this study. Briefly, equal volumes of HGNPs and a series of protein solution(2.0mg/ml) with PDI ranging from 5.0-11.0 were gently mixed and incubated for 0.5h, 1h, 2h, 4h, 8h and 24h respectively at room temperature. Then the excess protein were removed by centrifugation at 13000 rpm for 10 min (Eppendorf centrifuge 5417c, Germany) and HGNPs were resuspended in 0.01 M PBS (pH 7.4) for two cycles. Finally, the average particle size, the polydispersity coefficient and the flocculation(the multiplication of the particle size and absorption increasement, Δ nm* Δ abs) of HGNPs were measured in triplicates.

The stability of the DOX&HGNPs-TLs

Variance of particle size and drug content were monitored to evaluate the effect of temperature on physical stability of DOX&HGNPs-TLs. The DOX&HGNPs-TLs was placed at 4℃for one month. Particle size and DOX EE were measured according to the method described in 2.6.

Results:

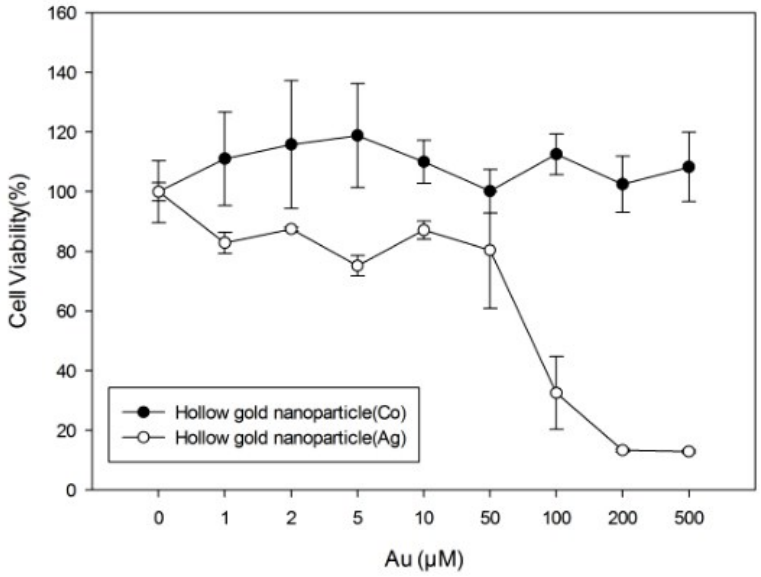


Fig.S1. The cell viability of HGNPs prepared by the Ag template methods and Co template methods on the human fibroblast HDF cells.

The stability of HGNPs with PVP and without PVP

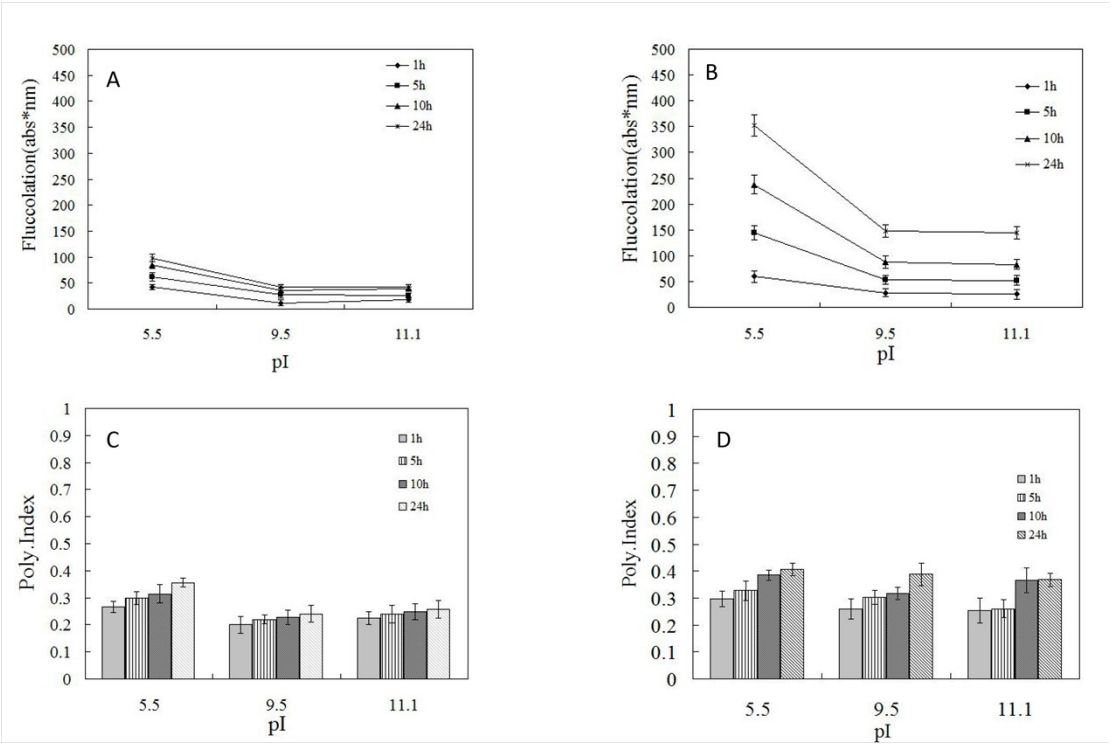


Figure.S2 Stability of HGNPs with and without the protection of PVP in solutions of proteins with different pI values (Table 1) as functions of incubation time. The flocculation(A) and PDI changes(B) of HGNPs stabilized by PVP in different protein solution. The flocculation(C) and PDI changes(D) of HGNPs not stabilized by PVP in different protein solution.

The stability of the DOX&HGNPs-TL

Tab. S1 The stability of DOX&HGNPs-TL at 4°C for one month

	Before	After one month
Particle size	196.8±3.2nm	199 ± 1.7nm
PDI	0.19 ± 0.01	0.20 ± 0.13
Zeta potential	-29.55±2.45 mV	-28.2 ± 0.19mV
EE	89.3±1.37%	88.2±2.21%
Apprearance	Clear solution	Clear solution

Reference:

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