Supplementary Information

Toxicity assessment of precise engineered gold nanoparticles with different shape
in zebrafish embryos

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Experimental Detail

Materials

Gold chloride trihydrate (HAuCl₄·3H₂O, 99%), cetyltrimethylammonium chloride (CTAC) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Sodium borohydride (NaBH₄, 98%), ascorbic acid (99%) and silver nitrate (99%) was received from Sinopharm Chem. Reagent Co., Ltd (Beijing, China).

Synthesis and Characterization of GNPs

Gold nanorods (GNRs): The seed solution was first synthesis by the blend of HAuCl₄ solution (0.01 M, 0.25 mL) and CTAB (0.1 M, 9.75 mL) in a 15-mL plastic tube with gentle mixing, followed by a fast inversion of a freshly prepared, ice-cold NaBH₄ solution (0.01 M, 0.6 mL). The resultant seed solution was kept at room temperature for 2 h before use. For the growth solution, HAuCl₄ (0.01 M, 2 mL) and AgNO₃ (0.01 M, 0.4 mL) were added into CTAB (0.1 M, 40 mL) in a 50-mL plastic tube. Then HCl (1 M, 0.8 mL) was used to adjust the pH of the growth solution to 1-2, followed by the addition of ascorbic acid (0.1 M, 0.32 mL). Finally, 0.02 mL of seed solution was rapidly injected into the growth solution. The resultant solution was mixed by gently inversion for 10 s and left undisturbed overnight.

Gold nanopolyhedrons(GNHs): The seeds were made by the addition of a freshly prepared, ice-cold NaBH₄ solution (0.01 M, 0.6 mL) into a mixture solution composed of HAuCl₄ solution (0.01 M, 0.125 mL) and CTAB (0.1 M, 3.75 mL) under
vigorous stirring. The resultant solution was kept under gentle stirring for 1 h at room
temperature before use. The growth solution was prepared by the sequential addition
of CTAB (0.1 M, 6.4 mL), HAuCl₄ (0.01 M, 0.8 mL), and ascorbic acid (0.1 M, 3.8
mL) into water (32 mL). The CTAB-stabilized seed solution was diluted 10 times
with water and the diluted seed solution (0.06 mL) was added into the growth solution.
The resultant solution was mixed by gentle inversion for 10 s and then left
undisturbed overnight.

Gold nanospheres (GNSs): The gold nanospheres were produced through
overgrowth of the small GNSs in conjunction with mild oxidation. Briefly, a freshly-
prepared, ice-cold NaBH₄ solution (0.01 M, 0.6 mL) was rapidly injected into the
mixed solution of HAuCl₄ solution (0.01 M, 0.25 mL) and CTAB (0.1 M, 9.75 mL)
under vigorous stirring. The resultant solution was kept under gently stirring for 3 h at
room temperature. Subsequently, the aforementioned solution (0.12 mL) was added
into a growth solution made of CTAB (0.1 M, 9.75 mL), water (190 mL), HAuCl₄
(0.01 M, 4 mL) and ascorbic acid (0.1 M, 15 mL). The reaction mixture was gently
stirred and then left undisturbed overnight at room temperature. The small GNSs as
overgrowth seeds of large GNSs were obtained.

In order to obtain the large GNSs, the small GNSs solution (16 mL) was
centrifugated and washed with water for two times, then the solid was re-dispersed
into water (4 mL). The obtained small nanospheres solution was added into a CTAC
solution (0.025 M, 30 mL). After the sequential addition of ascorbic acid (0.1 M, 0.75
mL) and HAuCl₄ (0.01 M, 1.5 mL), the mixture solution was mildly stirred in 45 °C
for 3 h. Afterwards, the aforementioned solution were centrifuged and re-dispersed in
a CTAB solution (0.02 M, 30 mL). Finally, the large GNSs were obtained by
oxidizing with HAuCl₄ (0.01 M, 0.2 mL), which stayed in 45 °C for 2 h.

All the resultant GNPs were washed and concentrated by twice into water by
centrifugation and re-dispersion for further use in our experiment. To quantify the
metal content in the nanoparticles, the purified nanoparticles was re-suspended in
water and subjected to ICP-MS analysis (Agilent 7500 CE, Agilent Technologies,
USA). The analysis determined the concentration of elements in the samples based on
the intensity of element specific electromagnetic emission of atoms.

Extinction spectra were measured on a 4802H UV/Vis/NIR double beam spectrophotometer (Unico Instrument Co. Ltd., Shanghai, China). The TEM images were performed on an FEI Tecnai F20 microscope with an acceleration voltage of 200 kV. The diameters of the GNPs were measured on their TEM images, with \( \sim 200 \) particles measured per sample.

**Zebrafish culture and toxicity assay of the GNPs**

Zebrafish (Danio rerio) were obtained from China zebrafish resource center (Wuhan, China) and maintained at 28±1°C on a 14 h light/10 h dark cycle. All animal procedures were approved by Shenyang Pharmaceutical University Animal Care and Utilization Committee. Embryos were incubated in E3 embryo medium consisting 5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl\(_2\) and 0.33 mM MgSO\(_4\) at 28 °C and different developmental stages were determined according to the Zebrafish Book.

The collected embryos were randomly distributed in 24-well plates (8 embryos/well; 2 ml egg water/well) and were continuously exposed to nanoparticles from 4 to 80 hpf. At predetermined time point (8, 32, 56 and 80 hpf), embryos were observed under a SOIF XTZ-E stereomicroscope (Shanghai optical instrument factory, Shanghai, China) with digital camera and scored as normal, abnormal or dead; hatched/unhatched. Dead embryos were moved out and two of three of each well content was renewed with freshly prepared GNPs dispersion at 32 and 56 hpf. Each concentration of the tested nanoparticles was evaluated in duplicate wells, in at least three independent experiments (≥60 eggs).

To test whether free CTAB arising from desorption or residual contamination was responsible for the toxicity seen in the CTAB-capped gold NPs solution, the toxicity of CTAB at the corresponding level was compared with NPs solutions and the control (E3) upon the exposure of zebrafish. For the determination of CTAB mass binding on the nanoparticles, the thermogravimetric analysis (TGA, Netzsch STA 449F3) was carried out. As showed in Figure S2, the weight loss of all investigating nanoparticles were observed at 200-370 °C of 2-3% due to the thermal decomposition of CTAB by
a self-combustion process, yielding a highest value in the range of 4 μM of the original sample. Similarly, toxicity of the capping agent CTAB at different concentrations (1- 4 μM) was evaluated. The stock solutions of CTAB were diluted to 1/10, 1/50 and 1/100 concentration using egg water. The collected embryos were randomly distributed in 24-well plates (8 embryos/well; 2 ml corresponding CTAB solutions/well) and were continuously exposed to the compound from 4 to 80 hpf. Each concentration of the tested CTAB was evaluated in duplicate wells, in at least three independent experiments (≥40 eggs).

Data were recorded as mean±SEM from at least three independent experiments. For the embryo/larval bioassays, a one-way analysis of variance (ANOVA) was employed to detect significant differences between the control and treated groups. A $P<0.05$ was considered statistically significant.

**Quantification of metal content in zebrafish**

This assay was performed to identify metal accumulation in zebrafish embryos and larvae that appeared healthy after gold nanoparticles exposure. Accumulation of gold in embryos and larvae could be used as a parameter for understanding the uptake GNPs of zebrafish. Embryos were treated with 3.85 μM, 6.58 μM, 15.7 μM and 1.88 μM of GNRs, GNSs, freshly prepared GNHs and old GNHs as described above. At 8 and 80 hpf, 50 eggs and larvae were transferred to 1.5 ml tubes respectively, washed 5 times in ultrapure water and immersed in liquid nitrogen for 1 min followed by digestion in 200 μl of aqua regia overnight. The digested samples were reconstituted to 2 ml with ultrapure water and centrifuged at 8000 rpm for 10 min then collected supernatant liquor for analyzing by ICP-MS to detect metal content.
**Table S1** The lethality of free CTAB solutions (40 embryos). At these concentrations, the observed lethality due to free CTAB could be ignored since there were no significant differences in survival rates between the pure CTAB and the control group (embryos medium).

<table>
<thead>
<tr>
<th>CTAB Concentration</th>
<th>Control</th>
<th>4x10^{-7}M</th>
<th>2x10^{-7}M</th>
<th>1x10^{-7}M</th>
<th>8x10^{-8}M</th>
<th>4x10^{-8}M</th>
<th>2x10^{-8}M</th>
<th>1x10^{-8}M</th>
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<tr>
<td>80 hpf (%)</td>
<td>22.5</td>
<td>25</td>
<td>22.5</td>
<td>27.5</td>
<td>27.5</td>
<td>27.5</td>
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**Fig. S1** Size distributions of the gold nanoparticles. (a–c) The average length, diameter and aspect ratio of gold nanorods are $(76 \pm 5)$ nm, $(23 \pm 1.8)$ nm and $3.3 \pm 0.3$, respectively; (d) The average diameter of gold nanospheres is $(46 \pm 1)$ nm; The average diameters of freshly prepared gold nanopolyhedras and placed for 6 months gold nanopolyhedras are $(38 \pm 2)$ nm (e) and $(40 \pm 3)$ nm (f), respectively.
Fig. S2 TGA curves of the pure CTAB (a) and prepared several gold nanoparticles (b). The weight loss of all investigating nanoparticles were observed at 200-370 °C of 2-3% due to the thermal decomposition of CTAB by a self-combustion process, yielding a highest values in the range of 4 μM.
Fig.S3 Gold nanoparticles uptake and accumulate within the embryos and larvae to different levels. Preliminary animal exposure data demonstrated an average amount of loading GNRs was highest among all investigating NPs, suggesting higher numbers of GNRs incorporated into embryos and retained within them. GNSs and old GNHs treated embryos showed similar metal content in the body whereas freshly prepared GNHs exposed embryos showed low Au accumulation inside their bodies at 80 hpf.
**Fig. S4** The contrast of nanostructures and colors between new GNHs and old GNHs. TEM images of the freshly prepared GNHs (a) and the GNHs after the storage of 6 months (b). Digital photos of the samples taken before (c) and after centrifugation (d). Before centrifugation, the color of the new GNHs is pink (left), and the color of the old GNHs (right) is bright red. After centrifugation, the supernatant of the new GNHs (left) is colorless, whereas the supernatant of the old GNHs (right) is yellow due to the presence of dissolved Au$^+$. 