Supplementary Materials

The unexpected effect of PEGylated gold nanoparticles on the primary function of erythrocytes

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Materials and methods

TEM and SEM measurement

The morphology and size of PEGylated AuNPs (4.5, 13, 30 nm) were evaluated using transmission electron microscopy (TEM, JEM-200CX, Jeol Ltd, Japan). Briefly, TEM samples were prepared by dropping the diluted gold colloids on carbon-coated copper grids, followed by natural drying; then, the samples were observed on the JEM-200CX microscope operating at 80 keV. Scan at low magnification (10-12,000×) to get an ideal of the overall sample composition. Erythrocytes were pipetted from leukoreduced blood that incubated with different diameter AuNPs at different time points. After washed with PBS, RBCs were diluted to 5% hematocrit with PBS(containing 2% glutaraldehyde) and incubation for 2 hours. Erythrocytes were fixed to mica plates by natural sedimentation due to gravity. Mica plates were then dehydrated in increasing concentrations of ethanol (25, 50, 75, 90, and 100%) for 15 min each. The plates were dried, and coated with gold before viewing under a scanning electron microscope (quanta250; FEI Ltd., USA).

Blood preparation, leukoreduction and storage.

Three bags of whole blood (450 mL ± 10%, type O) was collected by Chengdu blood center and Deyang blood station from healthy volunteer donors into regular triple-bag containers containing CPDA-1 anticoagulant preservative solutions. The blood sampling and this research program were approved by Ethics Committee of Institute of Blood Transfusion, Chinese Academy of Medical Sciences and Peking Union Medical College, prior to the start of the program. All the blood was tested to determine virus (HIV, HBV, HCV etc.) free before use. Whole blood can be preserved for 35 days in CPDA-1 anticoagulant according to FDA and AABB guidelines at standard banking procedures at 4°C.

Whole blood was leukoreduced by passage through a leukoreduction filter(model: KF-WFR-B, Shuanglu medical apparatus & instruments Corp., Chengdu, China). The efficacy of leukoreduction was measured by a complete blood count (BC-5800, Mindray Corp., Shenzhen,

China). Clearance rate of leucocyte was 99.99%, platelet was less than 20×10^9/L, callback rate of RBC was over 90%.

Each bag of whole blood was divided into 12 small PVC blood preservation bags(Laishi blood transfusion equipment CO.,Ltd. Suzhou, China), approximately 30ml per bag. And 12 small bags of blood were equally assigned to four groups, eventually each group contained 9 small bags of blood. Three groups of blood were added different diameter of AuNPs(4.5nm, 13nm, 30nm) to make final concentration $200\mu g$ ml^{-1}.The last group was added isotonic PBS as negative control. Drain off the air in the bag, and storage blood at standard banking conditions. All operations performed under aseptic conditions. And samples were removed aseptically for the analysis at different time points from day 0 up until day 35 of storage.

ICP-MS

The erythrocytes distributions of different Au nanoparticles were assessed by quantitative measurement of Au levels in various erythrocytes. The erythrocytes treated by PEGylated AuNPs were centrifuged (3000g) and collected. Meanwhile, the suspension of erythrocytes were also collected. For each sample, they was digested in aqua fortis (nitric acid: hydrochloric acid = 3:1). After adjusting the solution volume to 2 ml using 2% nitric acid and 1% hydrochloride acid (1:1), ICP-MS assays were performed on Elemental X7 (Thermo Electron) for quantitatively measuring Au content.

Flow cytometry.

To investigate the expression of CD47, RBCs were analyzed by flow cytometry, as described by Rosemary L. Sparrow [1]. In brief, RBCs were washed for three times and diluted to 1×10^9/L with PBS, then RBCs was incubated with FITC-labeled anti-CD47 for 15 minutes. A total of 1×10^5 RBCs were analyzed on a FACScan flow cytometer (BD, Calibur).

FITC-conjugated mouse anti-human monoclonal antibodies to CD47 (clone B6H12) and appropriate isotype negative controls were all purchased from BD.

Measurement of P_{50}

Oxygen dissociation curves (ODCs) of RBCs were determined with Hemox analyzer[2](TCS Scientific Corp., New Hope, PA). The procedure was performed according to the manufacturer's manual. And the P_{50}, defined as the partial pressure of oxygen at which Hb is 50% saturated, was calculated from the ODC by software (TCS Scientific Corp.,). Oxygen-carrying capacity of RBCs was calculated from Oxygen dissociation curves[2], Oxygen-carrying capacity of RBCs was downgraded in the face of decreased P_{50}.
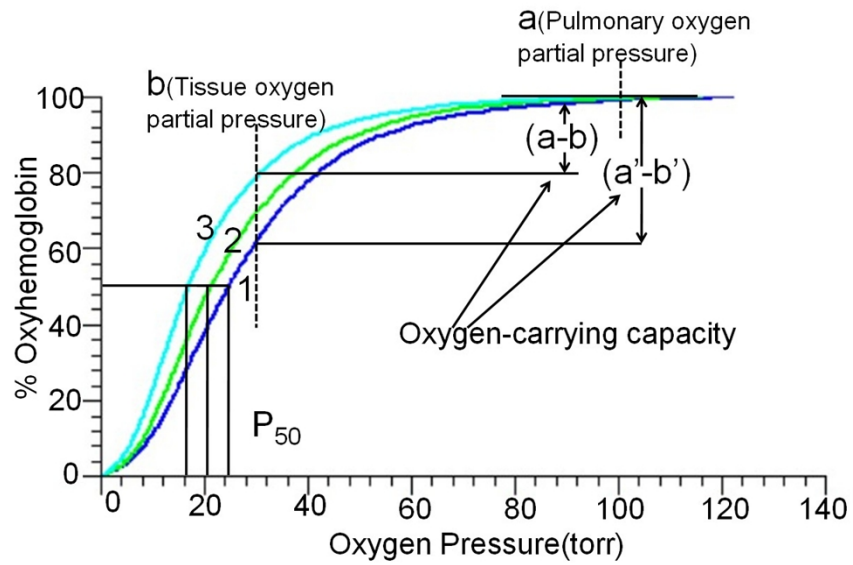
Figure S1. Illustration the relationship between P_{50} and Oxygen-carrying capacity.

Methemoglobin detection.

The percentage of methemoglobin was determined by standard spectral analysis methods described previously[3,4].

Table S1. The effects of different size of PEGylated AuNPs on the oxygen-delivering ability of erythrocytes (mmHg).

Time (day)	ctrl	4.5 nm	13 nm	30 nm
1	26.93 ± 0.21	26.90±0.15	26.87±0.12	26.88±0.18
14	21.85±0.16	21.54±0.27	20.57±0.59*	19.61±0.24*
21	19.19±0.33	18.10±0.19*	18.22±0.76*	18.20±0.16*
35	18.36±0.31	18.84±0.06	18.07±0.29	18.00±0.13
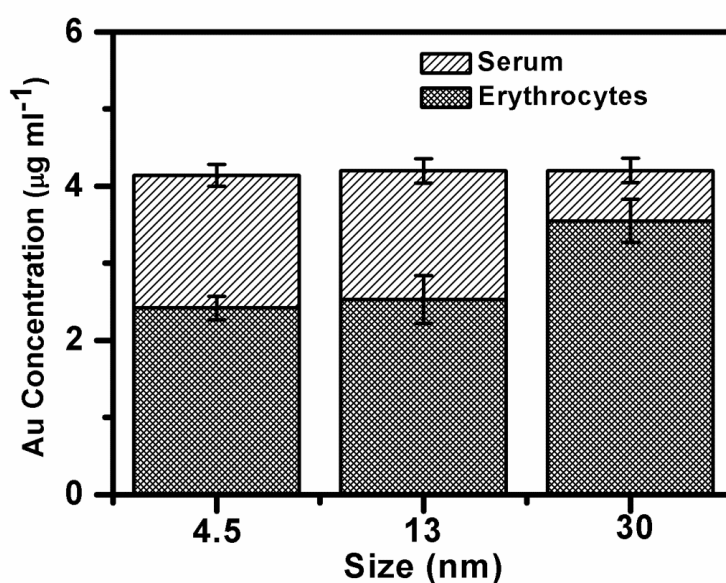
Figure S2. The Au content in the erythrocytes and plasma when treated by PEGylated AuNPs with different size.
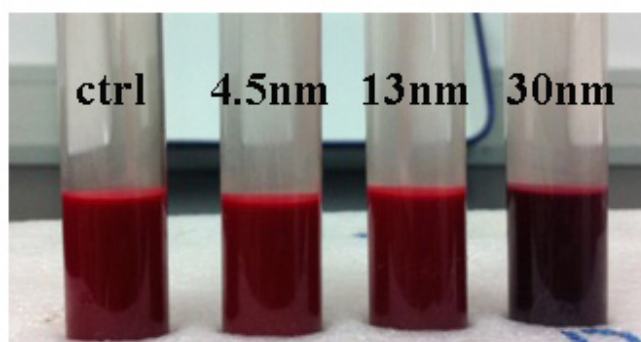
Figure S3. The image of RBCs were treated by PEGylated AuNPs (200 μg ml^{-1}) after 35 days.

References

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