Exocytosis of gold nanoparticles (AuNPs) in macrophages was evaluated in vitro varying their size and surface chemistry. PEGylated AuNPs showed the highest rate of exocytosis in macrophages regardless of their sizes while positively charged AuNPs remained longer in the cells than others.

Nanoparticles that can be injected into the body hold tremendous potential to detect and treat disease. Despite efforts to improve their targeting efficiency, significant quantities of systemically administered nanoparticles are cleared by the organs of the mononuclear phagocytic system (MPS), increasing the likelihood of unintended acute or chronic toxicity. Generally, nanoparticles are taken up in the MPS via endocytosis into Kupffer cells of the liver sinusoid and macrophages of the splenic red pulp, where they are trapped in the lysosomes of those cells for a relatively long time without elimination from the body. Previous studies have mostly focused on cellular uptake and organ distribution of nanoparticles depending on their size, shape and surface charge. However, there has been little effort to engineer the exocytosis of nanoparticles taken up in the macrophages that may be responsible for their systemic elimination and biosafety.

Here, we assess exocytosis of monodisperse gold nanoparticles (AuNPs) in macrophages in vitro. AuNPs were selected in this study due to their excellent biocompatibility and easy tunability of size and surface chemistry. We investigated the influence of size and surface chemistry on the process by which macrophages direct the AuNPs out of the cell membrane and into the extracellular space.

For 20-nm and 40-nm AuNPs, 20 mL of 1.0 mM HAuCl₄ was brought to a boil in a 100 mL bottle and 1.6 mL and 1.0 mL of 38.8 mM tri-sodium citrate was added, respectively. The solution color changed from clear to bright red and dark red over 5 min. Boiling and stirring were continued for 15 min. For negatively charged surface, as-synthesized (citrate-coated) AuNPs were used. For neutrally charged and PEGylated surface, 1 mL of 1 mM Au as-synthesized AuNPs were functionalized with 70 μL of 0.01 M cysteine solution and 25 mg of mPEG(2K)-SH, respectively. For positively charged surface, 400 μL of 213 mM cysteamine solution was added to 40 mL of 1.42 mM HAuCl₄ solution. After stirring for 20 min at room temperature, 10 μL and 30 μL of 10 mM NaBH₄ solution was added for 20-nm and 40-nm positively-charged AuNPs, respectively. The mixture was vigorously stirred for 25 min at room temperature in the dark. All formulations of AuNPs were finally coated with serum proteins in 10% Fetal bovine serum (FBS) for 1 h to prevent their aggregation and mimic in vivo situations. After synthesis and surface modification, hydrodynamic size of the AuNPs was characterized using dynamic light scattering (DLS).

In order to test their stability in a biological solution, hydrodynamic sizes of protein-coated AuNPs were monitored in cell growth medium at 37 °C over a 6-h period using DLS.

In order to evaluate their exocytosis in macrophages, U937 cells were grown in RPMI 1640 medium with 10% FBS. For differentiation into macrophages, the non-adherent monocyte-like undifferentiated U937 cells were induced to differentiate by treating them with 40 nM phorbol-12-myristate-13-acetate (PMA). After PMA treatment, the cells were plated onto tissue culture plates. Macrophages were incubated with 500 uMAu of protein-coated AuNPs for 6 h to achieve a substantial nanoparticle uptake. Then, the cells were washed three times with PBS and added with fresh medium. AuNPs exocytosized from the cells were collected with the supernatants at the indicated time points over a 48-h period. Amounts of AuNPs taken up by and exocytosized from the macrophages were measured using inductively coupled plasma mass spectrometry (ICP-MS).

20-nm and 40-nm AuNPs with a variety of surface chemistries (anionic, cationic, zwitterionic, and PEGylated surface) were prepared and further coated with serum proteins. In order to test whether
protein-coated AuNPs are stable in a physiological solution, their hydrodynamic size were monitored every hour over a 6-h period in cell growth medium at 37 °C using a DLS technique. As shown in figure 1, hydrodynamic sizes of protein-coated AuNPs did not change during the tested time regardless of their sizes and chemistries. It indicates that proteins coated on the surface prevent AuNP aggregations and individual protein-coated AuNPs interact with cells.

![Image](https://via.placeholder.com/150)

Figure 1. Change of hydrodynamic size of protein-coated AuNPs with a size of 20 nm (A) and 40 nm (B) in cell growth medium at 37 °C over a 6-h period.

For nanoparticle uptake by macrophages, differentiated U937 cells are treated with protein-coated AuNPs for 6 h. Positively charged AuNPs displayed the highest uptake in the macrophages within the tested time while PEGylated ones did the lowest. The amounts of AuNPs secreted from the macrophages at the indicated time points were plotted in figure 2. PEGylated AuNPs showed the highest rate of exocytosis in macrophages regardless of their sizes while positively charged gold nanoparticles remained longer in the cells than others. These results demonstrate a new strategy to design biosafety nanoparticles for clinical translation.

![Image](https://via.placeholder.com/150)

Figure 2. Normalized exocytosis rate of protein-coated AuNPs with a size of 20 nm (A) and 40 nm (B) in macrophages (U937 cell line).

**CONCLUSION**

Nanoparticles remained long in macrophages have the potential to induce unintended toxicity in normal organs. In this study, exocytosis of AuNPs in the macrophages was evaluated *in vitro* varying their size and surface chemistry. PEGylated gold nanoparticles showed the highest rate of exocytosis in macrophages regardless of their sizes while positively charged gold nanoparticles remained longer in the cells than others. These results demonstrate a new strategy to design biosafety nanoparticles for clinical translation.

**REFERENCES**


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