Gold nanoparticles with a monolayer of doxorubicin-conjugated amphiphilic block copolymer for tumor-targeted drug delivery

Mani Prabaharan,[1] Jamison J. Grailer,[2] Srikanth Pilla,[1] Douglas A. Steeber,[2] Shaoqin Gong [1,3]
[1]Department of Mechanical Engineering, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA
[2]Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA
[3]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53706, USA

Statement of Purpose: Monolayer-protected gold (Au) nanoparticles (NPs) have recently emerged as an attractive candidate for delivering various therapeutic agents into their targets (Ghosh P et al., Adv Drug Del Rev 2008;60:1307–15). These NPs are one of the most promising candidates for cancer treatment because of their functional versatility. Functionalized Au NPs with an outer PEG shell can offer excellent stability in physiological conditions. The PEG shell can also greatly reduce the interaction between the Au NPs and the plasma protein, thereby greatly minimizing the uptake of the Au NPs by the reticuloendothelial system, which can lead to a much longer circulation time in the bloodstream. This will allow the Au NPs to preferentially accumulate in the tumor sites through the leaky tumor neovasculature by the enhanced permeability and retention effect. In addition, monolayer-protected Au NPs targeted to cancer tissue may improve surgeons' ability to identify metastatic lesions because they can significantly enhance the optical and computed tomography (CT) imaging contrast. In this work, we describe the synthesis of folate (FA)-conjugated amphiphilic Au NPs with a poly(L-aspartate-doxorubicin)-*b*-poly(ethylene glycol) monolayer (Au-P(LA-DOX)-*b*-PEG-OH/FA) for tumor-targeted drug delivery. The DOX molecules were conjugated to the hydrophobic poly(L-aspartate) segment by pH-sensitive hydrazone linkage. Au-P(LA-DOX)-*b*-PEG-OH/FA NPs can form stable unimolecular micelles in aqueous solutions due to their unique amphiphilic multi-arms and globular architecture.

Methods: Amine functionalized Au NPs were synthesized by treating Au NPs with 2-aminoethanethiol followed by triethylamine. Au-poly(β-benzyl L-aspartate) (Au-PBLA-NH_2) was prepared by the ring-opening polymerization of β-benzyl L-aspartate using amine functionalized Au NPs. Au-PBLA-*b*-PEG-OH was prepared by reacting Au-PBLA-NH_2 with HO-PEG-COOH in presence of NHS and DCC. Au-PBLA-*b*-PEG-OH/FA was prepared by reacting Au-PBLA-*b*-PEG-OH with FA in presence of DCC and DMAP as the catalysts. Thereafter, the benzyl groups of Au-PBLA-*b*-PEG-OH/FA were substituted with hydrazide groups by reacting Au-PBLA-*b*-PEG-FA with excess of anhydrous hydrazine. The resulting product was then treated with an excess amount of DOX-HCl to obtain Au-P(LA-DOX)-b-PEG-OH/FA copolymer.

Results: Au-P(LA-DOX)-*b*-PEG-OH/FA NPs formed stable unimolecular micelles in aqueous solutions because of its amphiphilic muti-arm and globular core/shell architecture. DLS studies showed that amine functionalized Au NPs showed a narrow size distribution ranging from 5–11 nm with an average particle size diameter of 6.3 nm and a PDI of 0.028. In the case of micelles made from Au-P(LA-DOX)-*b*-PEG-OH/FA NPs, the size distribution was relatively broad, ranging from 24–52 nm, and the average micelle size was 34 nm with a PDI of 0.029. The increased size and size distribution of micelles formed from Au-P(LA-DOX)-*b*-PEG-OH/FA NPs might be due to the presence of a fairly thick P(LA-DOX)-*b*-PEG-OH/FA monolayer on the surface of Au NPs. The morphology of these micelles appeared as well-defined Au core and P(LA-DOX)-*b*-PEG-OH/FA shell structures with a diameter in the range of 10–25 nm (Fig.1). In addition, no aggregation between the Au-P(LA-DOX)-*b*-PEG-OH/FA micelles was observed in the TEM image, confirming that the monolayer of amphiphilic P(LA-DOX)-*b*-PEG-OH/FA copolymer on the Au core prevents the NPs from aggregation. Au-P(LA-DOX)-*b*-PEG-OH/FA NPs showed a much faster DOX release at pH 5.3 and 6.6 than at pH 7.4. This pH-dependent releasing behavior is of particular interest in achieving the tumor-targeted DOX delivery with micelles. The flow cytometry and CLSM studies showed that the cellular uptake of the Au-P(LA-DOX)-*b*-PEG-OH/FA NPs is mainly based on a folate-receptor-mediated endocytosis mechanism that might result in a greater amount of micelles internalized inside the tumor cells.

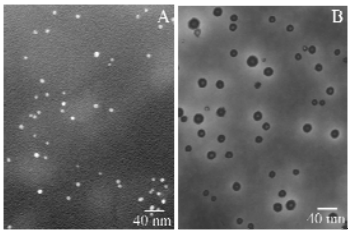

Fig. 1 TEM images of (A) amine functionalized Au NPs; and (B) Au-P(LA-DOX)-*b*-PEG-OH/FA NPs.

Conclusions: FA-conjugated amphiphilic Au-P(LA-DOX)-*b*-PEG-OH/FA NPs was synthesized as a tumor-targeted, anticancer drug delivery nanocarrier. The anticancer drug DOX was conjugated onto the hydrophobic inner shell of the NPs via an acid-labile hydrazone linkage. The release of DOX from the Au-P(LA-DOX)-*b*-PEG-OH/FA NPs depended strongly on the pH values of the medium. It was found that DOX released rapidly at acidic pHs such as those encountered in tumor tissue and the endocytic compartments of the tumor cell due to the hydrolysis of hydrazone linkage. The increased cellular uptake was observed for Au-P(LA-DOX)-*b*-PEG-OH/FA NPs. In addition, the H40-PLA-*b*-MPEG/PEG-FA NPs can be used for photothermal cancer therapy and contrast agent for various imaging modalities (e.g., optical and CT imaging), thereby making targeted cancer theranostics possible.