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Controlled Delivery of Functionalized Gold Nanoparticles by pH-Sensitive Polymersomes

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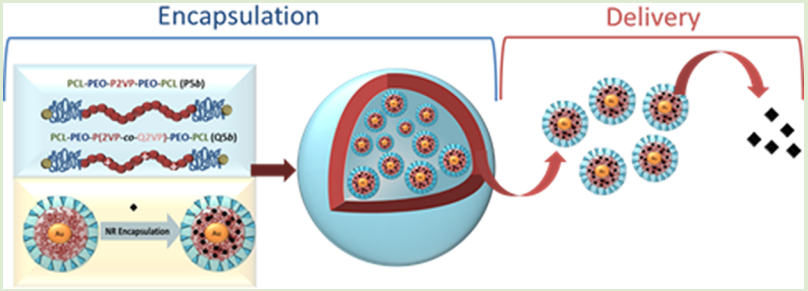
Supporting Information



ABSTRACT: The present study reports on the development of composite gold nanoparticles (AuNPs)/polymersome formulations, based on pH-responsive biocompatible polymer vesicles integrating prefunctionalized AuNPs, doped with a hydrophobic model probe for improved multimodal drug delivery. The polymer vesicles were prepared from an amphiphilic pentablock terpolymer poly(ε-caprolactone)-*b*-poly(ethylene oxide)-*b*-poly(2-vinylpyridine)-*b*-poly(ethylene oxide)-*b*-poly(ε-caprolactone) (PCL-PEO-P2VP-PEO-PCL), consisting of a pH-sensitive and biodegradable P2VP/PCL membrane, surrounded by neutral hydrophilic PEO looping chains. Additionally, partial quaternization of the P2VP block has been performed to introduce cationic moieties. Water-dispersible AuNPs carrying a hydrophobic molecule were encapsulated in the hydrophilic aqueous lumen of the vesicles, and the release was monitored at pH conditions simulating physiological and tumor environments. The complex delivery of the cargos from these vesicles showed improved and controlled kinetics relative to the individual nanocarriers, which could be further tuned by pH and chemical modification of the membrane forming block.

The self-assembly of amphiphilic block copolymers is an attractive route to obtain new, functional materials, with a variety of potential applications in the biomedical field.[1] Among numerous supramolecular assemblies, polymer vesicles or polymersomes have found extensive use in drug delivery for their ability to simultaneously incorporate hydrophobic particles into vesicle walls,[2] along with hydrophilic species in the aqueous cavities.[3] Additionally, polymer vesicles present enhanced robustness and controlled membrane permeability relatively their lipid analogues. Furthermore, stimuli-sensitive polymersomes are particularly valuable as programmable delivery systems in which the release of drugs can be readily modulated by exerting appropriate stimuli.[4]

Recently, there is increasing interest in hybrid AuNP/polymer materials as delivery nanocarriers,[5] sensors[6] and bioimaging agents.[7] As the most stable of all metal nanoparticles, AuNPs are highly attractive because of their distinct surface plasmon resonance (SPR) bands, the ease of incorporating functional ligands to create monolayer protected nanoparticles[6] and their biocompatibility.[5] Surface-decorated AuNPs[8] or hydrophobic AuNPs embedded in the hydrophobic compartment of various nanocarriers[9] are currently widely used for preparation of hybrid nanoparticles. However, the therapeutic value of surface-modified AuNPs is controversial under in vivo conditions due to the cleavage of the Au−ligand bonds by thiol-exchange reactions with cysteines on the surface of proteins[10,11] in the bloodstream, altering the pharmacokinetic profile.[12] Thus, the development of novel nanocarriers, able to protect, integrate/deliver AuNPs and benefit from their synergistic multifunctionality on a single platform, is highly desirable in basic biomedical research and may provide a robust tool for in vivo therapy monitoring.

In the past, a large number of pH-responsive vesicles from diblock AB[3a−c] or triblock ABA[3b,d] polymers have been investigated, while the study of ABC copolymers[3e,f] or even more elaborated structures, is a rather recent development. Linear terpolymers comprising more than three segments different in nature, termed ABCBA pentablock terpolymers, have appeared recently, increasing the diversity of nanostructured polymeric materials. So far, the focus for these systems has been based on their association toward reversible hydrogels,[13] while vesicle formation from pentablock terpolymers remains sparingly explored.

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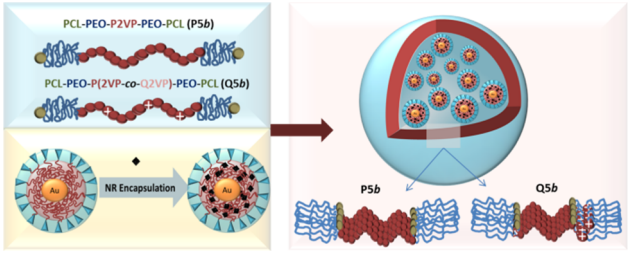
In this communication, we report a novel drug delivery system based on pH-responsive polymersomes incorporating functionalized AuNPs, doped with a hydrophobic model probe (Nile Red, NR). NR was chosen as a model molecule due to its highly hydrophobic character, and thus low membrane permeability into aqueous media. The polymersomes were prepared from self-assembly of amphiphilic pentablock terpolymers consisted of a pH-sensitive poly(2-vinylpyridine)[14] (P2VP) central block, bearing at both ends hydrophilic poly(ethylene oxide) (PEO) blocks and end-capped with hydrophobic poly(ε-caprolactone) (PεCL) blocks (PCL_{46}-*b*-PEO_{199}-*b*-$P2VP_{598}$-*b*-PEO_{199}-*b*-PCL_{46}), denoted as P5*b*. Additionally, partial quaternization of the 2VP block, leading to PCL_{46}-*b*-PEO_{199}-*b*-$P(2VP\text{-}co\text{-}Q2VP)_{598}$-*b*-$PEO_{199}$-*b*-$PCL_{46}$ pentablock terpolymer, denoted as Q5*b*, was performed for the introduction of cationic moieties.[15] We envisaged that the polymersomes will be formed by a pH-sensitive biodegradable P2VP/PCL membrane surrounded by PEO "stealth" looping chains (Scheme 1). The aim of this work was 2-fold: (1) to explore the encapsulation of AuNP in the lumen of polymersomes and their delivery profile and (2) to evaluate the release kinetics of a highly hydrophobic probe (NR) from the AuNP-in-polymersome complex nanovehicle, overcoming the drawbacks of bare gold nanocarriers, that is, insufficient protection in biological fluids and low drug encapsulation capacity.

Scheme 1. Illustration of the Novel Multimodal System Composed of Pentablock Polymer Vesicles Incorporating Functionalized AuNPs, Loading NR, in Their Aqueous Cavity



Bifunctional ligand (mercaptooctanoic acid, MOA) was assembled on the AuNPs surface via S−Au linkage and conjugation with biomarker molecule (folic acid, FA) was performed in order to enhance hydrophilicity and impart selective tumor targeting (SI). The AuMOA-FA core−shell nanostructure is composed of a gold core, a hydrophobic alkyl inner layer and a hydrophilic FA periphery.[16,17] Because these hierarchical nanostructures can be considered as water-dispersible nanovehicles for hydrophobic molecules, NR was subsequently entrapped in the alkyl "nanopockets" (Scheme 1).

These AuNPs are designed as sufficiently hydrophilic to be well dispersed in water, thus they have been further incorporated in the aqueous cavity of pH-sensitive polymersomes. The key feature of our strategy is the usage of a water-dispersible nanocarrier (AuNPs) to integrate hydrophobic molecules into the aqueous lumen of a vesicle. Additionally, owing to their high electron density, AuNPs can be clearly distinguished in complex environments such as tissue, so the release of AuNPs themselves would be useful for in vivo applications as imaging agents. The dual delivery of the NR probe and AuNPs from polymer vesicles was monitored by tuning pH conditions relevant to in vivo applications.

The polymer vesicles were prepared by the film hydration method (SI). Representative TEM micrographs of the assemblies derived from the pentablock terpolymers are shown in Figure 1A,B. As can be seen in the micrographs,

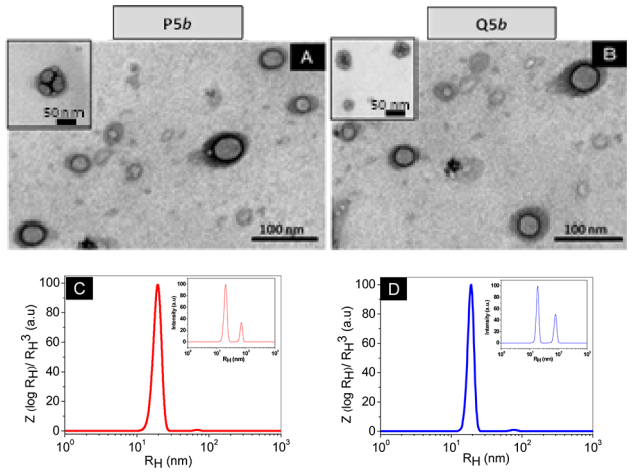


Figure 1. TEM micrographs of (A) P5b and (B) Q5b polymers in PBS at pH 7.4. Volume weighted size distribution of hydrodynamic radius measured by DLS at 90° for (C) P5b and (D) Q5b 0.2 mg/mL in PBS at pH 7.4. Insets: Intensity weighted size distributions.

the samples formed spherical or "ring-shaped" hollow structures either independent or, in some cases, clustered. The brighter centers of the particles should correspond to the locations of the PEO aqueous cavities, and the dark rings represent the collapsed P2VP/PCL membrane (Scheme 1).

The average particle hydrodynamic radius (R_H) for the polymer vesicles was also determined by DLS. The pentablock assemblies displayed a bimodal distribution of particle sizes with a dominant peak around 24 nm, for P5*b* and 18 nm for Q5*b*, respectively, as a result of increased hydrophilic content (inset Figure 1C,D). Besides, light scattering by bigger particles (peak around 70 nm) was also detected. Since the size distribution graphs are based on the scattering intensity, the number of the smaller particles constitutes the major fraction of the pentablock vesicular structures (Figure 1C,D). The particle size of the small nanoparticles derived from DLS readings was more closely examined by angular dependence of the decay rate (Figure S1). Morever, SLS analysis (Figure S2) enabled determination of the radius of gyration R_G for these assemblies, value that coincided with the R_H from DLS measurements, characteristic for vesicular morphology.[18]

The potential of these vesicles for drug delivery applications has been further explored by encapsulation of functionalized AuNPs of uniform size distribution (D_H 3.4 ± 0.9 nm, Figure S4) in the film hydration step. The loading ability of the vesicles was determined by comparing the absorbance and the fluorescence intensity of the final polymer/payload solution relative to the hydration solution, for AuNPs and NR, respectively (Figure S5). Before the encapsulation in the polymer vesicles, the NR-loaded AuNPs show the strong characteristic fluorescence signal at ~630 nm (Figure S5C,D), indicating that the dye resides in the hydrophobic alkyl "nanopockets", and not adsorbed to the gold surface.[19] Upon vesicle encapsulation, both the absorbance and the fluorescence

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intensity are strongly reduced, illustrating the entrapment of AuNP into the polymer vesicles. Moreover, no shift in the payload spectra was observed, implying that the nanoparticles retained their integrity upon vesicle encapsulation. The successful incorporation of AuNPs in the polymer vesicles was also confirmed by TEM (Figure 2).

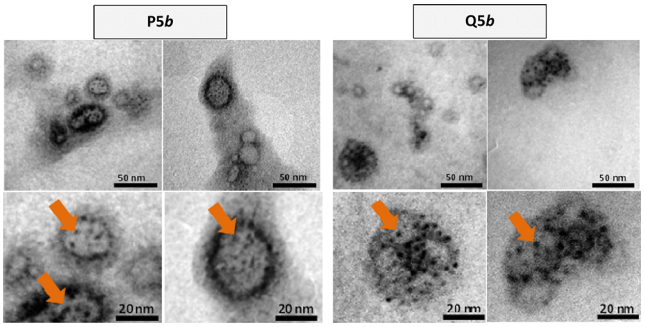

Figure 2. TEM micrographs of AuNPs encapsulated into the P5b and Q5b polymers in PBS at pH 7.4.

The AuNPs are clearly observed in the vesicle lumen, as indicated by the arrows. Moreover, these morphologies are identical to those observed for the bare vesicle systems with a slight size increase upon loading. Some empty vesicles were observed and the presence of nonencapsulated AuNPs, even though not detected by TEM, cannot be ruled out. The average diameter of the AuNP was 3.2 ± 0.8 nm, similar to the pure AuNPs demonstrating nanoparticle incorporation into vesicles without aggregation. Apparently, the repulsive electrostatic interactions between negatively charged surface ligands (Figure S3A) were able to restrict aggregation, even in the confined space of the vesicular cavity.

The release of AuNPs and NR from pentablock vesicles was monitored by UV and fluorescence measurements, respectively (SI, Figures S6 and S7). Figure 3 shows the release kinetics of

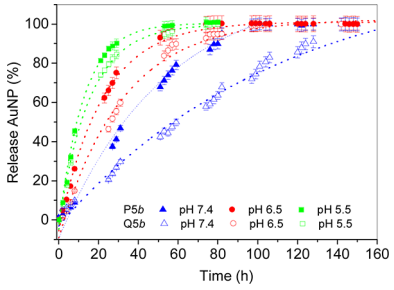

Figure 3. Release kinetics of AuNPs from P5b (solid symbols) and Q5b (open symbols) vesicles at pH 7.4 (blue), 6.5 (red), and 5.5 (green).

AuNPs from P5*b* and Q5*b* vesicles at pH 7.4, 6.5, and 5.5, relevant to in vivo circulation, tumor, and lysosome environment, respectively. The release of AuNPs at physiological pH proceeds in controlled stepped fashion without significant burst effect. Vesicles formed by the Q5*b* copolymer show a significantly delayed leakage of the payload relative to the nonquaternized counterpart. It seems that the neutral polymersomes act as "tankers" for the metal nanocarrier, with a continuous AuNP permeation through the vesicular wall, while the cationic vesicles behave as "barge" reservoirs, because after membrane diffusion, the anionic AuNPs attract to the P2VP membrane/PEO corona interface[3f] through electrostatic interactions, enhancing retention. Moreover, the in vitro AuNPs release behavior from polymersomes was also pH-dependent with a faster release rate at acidic pH. This enhanced diffusion of metal nanocarrier could be ascribed to a dual effect: weakening ionization of the AuNP carboxylic functions (Figure S3A) and a decrease of particle size (Figure S3B).

In the following, the release of the hydrophobic probe from the composite AuNP/polymer vesicle system was monitored (Figure S5). As a control experiment, NR was encapsulated into the pentablock vesicles and the delivery kinetics was evaluated. Figure 4 depicts a comparative picture of the dye

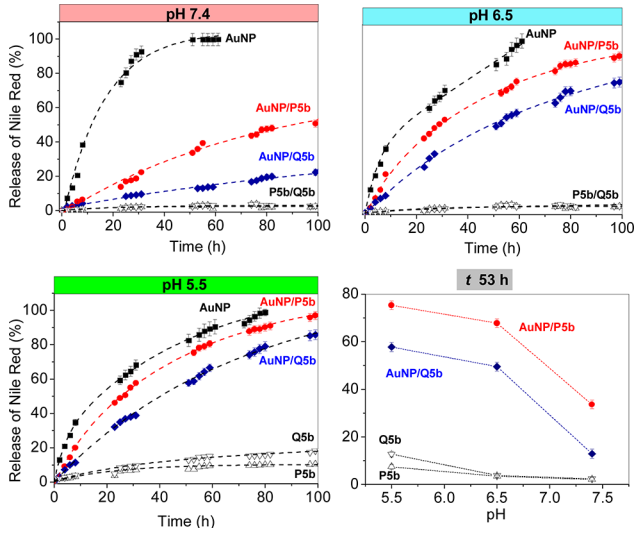

Figure 4. Release kinetics of NR from bare AuNPs (squares, black), P5b (up triangles), Q5b (down triangles) vesicles and from AuNP/P5b (circles, red) and AuNP/Q5b (diamonds, blue) composite systems at pH 7.4, 6.5, and 5.5 and pH-dependent release from bare and composite vesicles at t = 53 h.

delivery from bare AuNP, polymersomes and AuNP/polymersome nanocarriers, at pH 7.4, 6.5, and 5.5. As observed, the results from the composite vesicles are intermediate between the bare AuNP and bare polymersome, which is extremely slow (less that 8% for 5 days at pH 7.4). More importantly, incorporation of NR into the hydrophobic pockets of AuNPs and protection of this complex nanocarrier within polymer vesicles results in a prolonged release relative to the AuNP carrier alone (Figure 4). The release kinetics of AuNP (Figure 3) is faster relative to the dye kinetics through the AuNP-in-polymer vesicle complex systems in all pH conditions. Provided that NR leakage from the polymersome is extremely slow, it seems that the drug release proceeds in a successive fashion, that is, first of soluble molecule (AuNP) by transmembrane diffusion followed by a slower release of the hydrophobic dye.

As shown in Figure 4, NR release from the polymersomes was also pH-dependent, with faster release rate upon pH decrease, which again follows the same order of bare AuNP kinetics (Figure 3). Release times from pH-responsive vesicles were expected to be shorter in acidic microenvironment via membrane wall swelling by P2VP protonation and/or PCL hydrolytic degradation, leading to a faster leakage of metal nanocarrier, followed by NR encapsulate. Thus, the presence of AuNPs within the vesicles can be used to further tune the release rate of hydrophobic payload, at pH values of biological

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significance. Apparently, the escape of AuNPs into an aqueous environment boosts the release of hydrophobic dye, which cannot easily escape single-handedly the vesicle wall. This finding can be useful for tuning the rate of delivery of hydrophobic drugs within physiological aqueous media.

In conclusion, we have demonstrated for the first time a complex nanovehicle constituted of pH-sensitive ionizable polymersomes, encapsulating multifunctional water-dispersible AuNPs, carrying a highly hydrophobic model probe (NR). It was shown that the release of AuNPs can be controlled by tuning pH and degree of ionization of the polymeric vesicle. In addition, the delivery of the hydrophobic drug (NR) from the AuNP-in-polymersome system proceeded in a controllable stepped fashion, that is, AuNPs escape from polymersome followed by NR delivery. Thus, it was clearly shown that highly hydrophobic drugs can be delivered from polymersomes in a controlled manner only through this complex procedure. The AuNPs-in-polymersome nanovehicles seem to display several potential advantages with respect to traditional drug delivery systems, as they can combine the properties of polymersomes (high capacity, membrane permeability tuning, stealth properties, environmental responsiveness, robustness) with those of gold nanocarriers (multifunctionality, imaging, etc.). This novel strategy offers new possibilities for simultaneous multiple drug delivery (i.e., also through glutathione-mediated release) and monitoring in theranostic applications.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, DLS and SLS analysis of polymers, AuNP characterization, and UV and fluorescence spectra at different time points. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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