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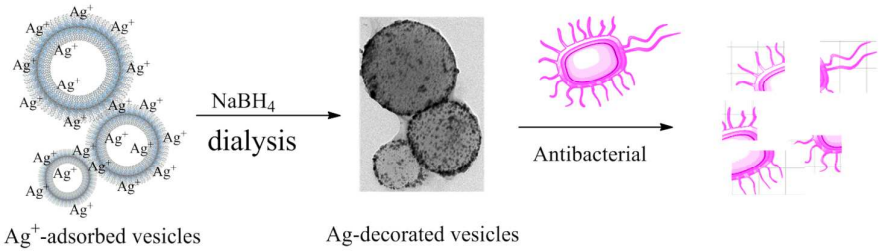

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ARTICLE

Decoration of homopolymer vesicles by antibacterial ultrafine silver nanoparticles

Cite this: DOI: 10.1039/x0xx00000x

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Received ooth January 2014,
Accepted ooth January 2014

DOI: 10.1039/x0xx00000x

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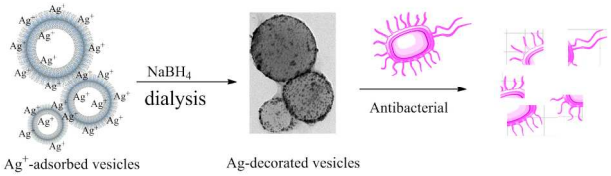
Presented in this article is the proof of principle that silver nanoparticles can be decorated on homopolymer vesicles. The homopolymer vesicles are prepared by self-assembly of poly(2-(2-ethoxyethoxy)ethyl acrylate) (PEEA) in water/tetrahydrofuran (THF) solvent mixture. The –COOH end groups on the surface of the homopolymer vesicle facilitate the growth of ultrafine silver nanoparticles because of the electrostatic interactions between negatively charged carboxyl group and positively charged Ag^+ ions, which were *in situ* reduced by sodium borohydride ($NaBH_4$). Those silver-decorated PEEA homopolymer vesicles exhibit good antibacterial activity against both Gram-positive and Gram-negative bacteria. This strategy may be extended to prepare more economic antibacterial agents and catalysts.

Introduction

Recently much attention has been paid to polymer vesicles or polymer micelles self-assembled by block copolymers for applications in drug, antioxidant agents and macromolecules delivery,[1-3] bioimaging,[4, 5] catalysis,[6] and antibacteria,[7-10] *etc.* In contrast, vesicles prepared by homopolymers received less attention.[11] During the last decade, polymer self-assemblies built up by amphiphilic homopolymers were reported because of the simple synthetic procedures and promising potential applications of various self-assembled nanostructures.[12-22] Recently, our group put forward a new homopolymer vesicle with a gradient membrane structure and a new mechanism of hydrogen-bonding induced homopolymer self-assembly.[17, 21] It has been confirmed that the membranes of homopolymer vesicles consist of both hydrophilic and hydrophobic moieties, which is different from traditional block copolymer vesicles and gives potential for further applications such as depositing gold nanoparticles for catalysis and water remediation.[22]

Metal nanoparticles have wide applications in catalysis, sensors and antibacterial materials because of their excellent optical property, catalytic activity, and so on.[23] Silver nanoparticles (AgNPs) have great toxicity to a broad range of bacteria and can efficiently kill both Gram-positive bacteria and Gram-negative bacteria such as *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).[7, 8, 17, 24-27] However, the agglomeration is one of the biggest problems in preparing AgNPs. Once the AgNPs agglomerate to micro-particles or bigger aggregates, its antibacterial activity declines sharply.[28] Therefore, templates such as polymer micelles, polymer vesicles and microgels have been used to prevent the aggregation of nanoparticles.[7, 8, 26, 29] For example, we recently prepared AgNPs with a narrow size distribution and remarkable antibacterial activity using block copolymer vesicles as the template.[7, 30] However, compared with block copolymers, homopolymers are much easier to synthesize. Therefore, it seems promising to prepare silver nanoparticles using homopolymer vesicles as template.

Herein, we report an antibacterial homopolymer vesicle decorated by ultrafine silver nanoparticles (AgNPs@vesicles), as shown in Scheme 1. First, the Ag^+ ions are adsorbed in the PEEA vesicle membrane; second, the addition of reducing agents such as $NaBH_4$ leads to the *in situ* formation of silver nanoparticles to form silver-decorated PEEA homopolymer vesicles, which show good antibacterial activity against both Gram-negative and Gram-positive bacteria.



Scheme 1. Preparation of silver-decorated homopolymer vesicles and its antibacterial mode.

Results and discussion

We have recently reported that PEEA homopolymer could easily self-assemble into vesicles with a gradient

bilayer membrane in water, which has been confirmed by transmission electron microscopy (TEM) and dynamic light scattering (DLS), *etc.*[17] The thermo-responsive PEEA homopolymer vesicles were found to have the trend to aggregate and form 'vesicle clusters' in aqueous solution.[17] In this paper, we further studied the effect of the drop rate of water on the size of the vesicle clusters by DLS (Fig. 1). The hydrodynamic diameters (D_h's) of PEEA vesicle clusters can be tuned within a wide range from 300 nm to one micron when the drop rate of water into THF varies from 1.2 mL/min to 0.05 mL/min. Throughout the whole process, the polydispersity index (PDI) was kept as low as 0.1 except for the case of 0.05 mL/min (but the PDI is still lower than 0.25).

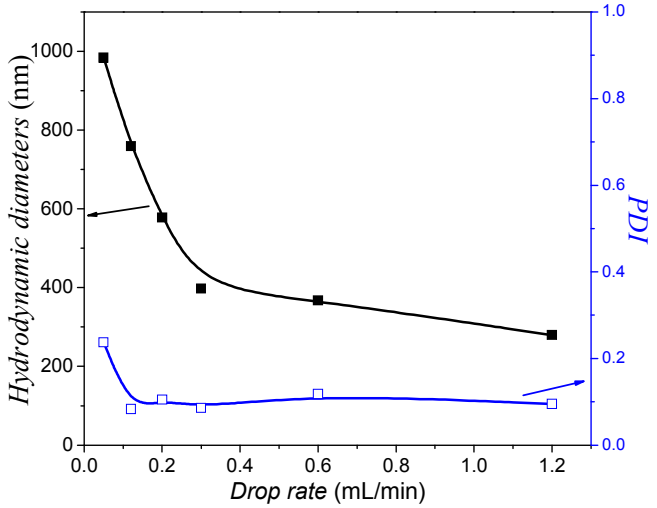


Fig. 1 Hydrodynamic diameters and PDI's of PEEA homopolymer vesicle clusters measured by DLS. The vesicles were prepared at different drop rates of water into THF during self-assembly, and then dialyzed against water.

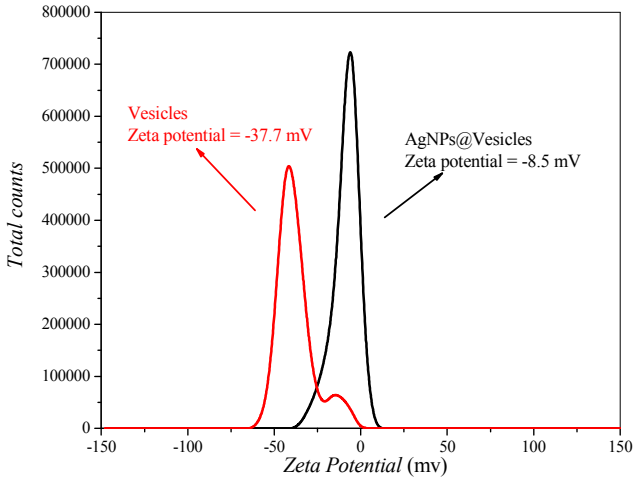


Fig. 2 Zeta potential distributions of PEEA vesicles and AgNPs@vesicles at 25 °C.

As for the structure of PEEA vesicles, the –COOH end groups of PEEA homopolymer are on the edges of the bilayer membranes of PEEA vesicles due to the charged nature and more hydrophilicity than that of the oligo(ethyleneoxy) side chains at higher temperature, which results in the highly negative Zeta potential (–37.7 mV) and ensures the long-term stability of PEEA vesicles (Fig. 2).[17] Thus the homopolymer vesicles can mediate the growth of silver nanoparticles by using –COOH groups on the bilayer membrane. Furthermore, the silver nanoparticles were stabilized by charge and polymer vesicles.

After *in situ* deposition of silver nanoparticles, the zeta potential of PEEA homopolymer vesicles becomes –8.5 mV at 25 °C and pH 7.2, suggesting significant consumption of –COOH groups (Fig. 2). The residual –COOH groups and oligo(ethyleneoxy) side chains (OEs) can stabilize the vesicles, which are very stable at 25 °C for a few months.[17]

To confirm the formation of silver nanoparticles, UV-vis studies were carried out because usually there was a characteristic peak at 400~450 nm for Ag(0) nanoparticles.[7, 30] Fig. 3 shows the UV-vis spectra before and after the deposition of nano-sized silver particles. The sharp peak at 415 nm after deposition indicates the existence of Ag(0) nanoparticles while there is no obvious absorbance before deposition of silver nanoparticles.

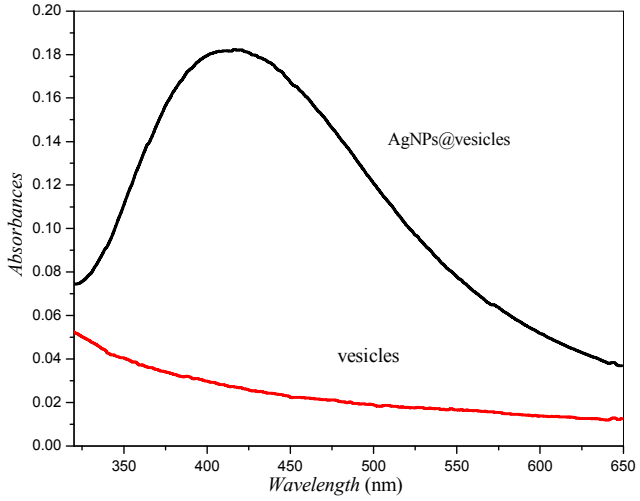


Fig. 3 UV-vis spectra of PEEA vesicles and AgNPs@vesicles at 25 °C. The PEEA vesicle solution was diluted to the same concentration as the AgNPs-decorated PEEA vesicle solution.

The silver nanoparticles surrounding the vesicle membrane help to clearly visualize the morphology of vesicles in TEM images (Fig. 4). Each Ag nanoparticle and the vesicle profile can be clearly observed from Fig. 4. It is noteworthy that these homopolymer vesicles used as the template have a D_h of 983 nm measured by DLS.

However, the diameter of Ag-decorated vesicles varied from 300 to 500 nm measured by TEM images (Fig. 4). This is because of the aforementioned aggregation effect of thermal responsive vesicles.[17] Actually, TEM study further confirmed this aggregation of PEEA vesicles to form vesicle clusters (Fig. 4A-B). The mean diameter of silver nanoparticles can be calculated from TEM as 7.1 ± 1.5 nm (Fig. 4C).

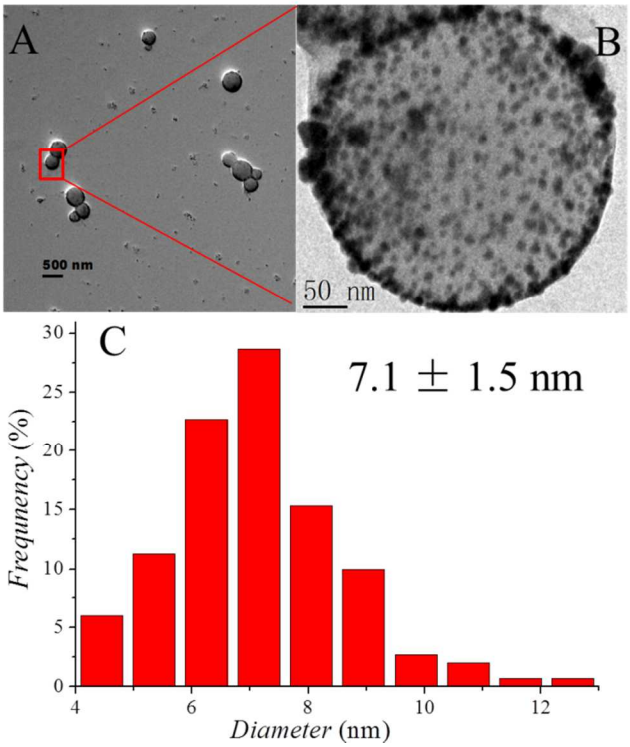


Fig. 4 (A-B) TEM images of Ag-decorated PEEA vesicles (and vesicle clusters) at different magnifications. (C) Statistical mean diameter of Ag nanoparticles calculated by TEM image.

Table 1. MIC test of Ag-decorated PEEA homopolymer vesicles against *E. coli* and *S. aureus*.[a)]

C_{Ag}/(μg/mL)	*E. coli*	*S. aureus*
32.0	-	-
30.0	+	+
27.9	+	+
25.7	+	+
21.4	+	+
10.7	+	+

[a)] "+" for bacteria growth, "–" for no bacteria growth; C_{Ag}: conc. of Ag

These silver nanoparticles are expected to enable to have effective interaction with the cell membrane due to their excellent dispersity in water, large specific surface area and less anionic charge of self-assembled nanostructures, which is important for the disintegration of the cell membrane through electroporation and/or the sinking raft model.[27, 31, 32] In addition, the larger size of the vesicles allows themselves to readily disintegrate the cell wall compared, which may translate to effective antimicrobial activities.

To test the antibacterial activity of the Ag-decorated vesicles, *E. coli* and *S. aureus* were selected as Gram-negative and Gram-positive bacteria, respectively (Table 1). Both the minimum inhibitory concentrations (MICs) of Ag-decorated PEEA vesicles against *E. coli* and *S. aureus* were 32.0 μg/mL, indicating good antibacterial activity of the silver-decorated vesicles.[7, 8, 24-27, 30-32]

In principle, the MIC values of the silver-decorated PEEA vesicles should be lower than 32.0 μg/mL considering the small size of Ag nanoparticles.[28] However, the antibacterial activity may also depend on the structure of PEEA vesicles at different temperatures. Because the Ag@vesicles were prepared at relatively low temperature, some OEs were hydrated to form vesicle coronas and in turn shielded the –COOH on the vesicle membrane,[17] which caused the Ag nanoparticles deposited on the surface of the membrane and partially covered by some OEs. Though the antibacterial test carried out at 37 °C, some embedded Ag nanoparticles could not move to the surface of the vesicles and the dehydrated OEs overcasted them more closely. The existence of OEs cuts off the contact between Ag nanoparticles and bacteria, leading to the higher MICs of silver-decorated PEEA vesicles. On the other hand, the aggregation of individual vesicles to form vesicle clusters may also attenuate the antibacterial activity. Anyway, the current MIC value of silver-decorated homopolymer vesicles indicates good antibacterial activity, which may be further improved in the future.

Conclusions

In summary, our proof-of-principle experiments confirm that homopolymer vesicles can be successfully decorated by ultrafine silver nanoparticles (ca. 7.1 ± 1.5 nm) based on the electrostatic interactions between Ag^+ ions and carboxyl groups. Those silver nanoparticles showed long term stability and good antibacterial efficiency against both *E. coli* and *S. aureus*. This strategy for preparing antibacterial ultrafine silver nanoparticles-decorated homopolymer vesicle may be extended to prepare more economic and efficient antibacterial agents, and to fabricate cheap and effective catalysts, *etc.*

Experimental section

Reagents

Silver nitrate ($AgNO_3$, AR), Luria-Bertani (LB) Agar, LB broth, Sodium borohydride ($NaBH_4$, AR) and phosphotungstic acid (PTA, AR) were purchased from Aladdin. The bacteria used were Gram-negative *E. coli* of ATCC35218 strain and Gram-positive *S. aureus* of ATCC29213 strain, which were purchased from Nanjing Bianzhen Biotechnology Co., Ltd. Other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China) used as received. PEEA was synthesized according to our recently published protocol.[17]

Preparation of AgNPs-decorated PEEA vesicles

In order to achieve the *in situ* reduction and formation of AgNPs on the membrane of vesicles, 500 μL of aqueous $AgNO_3$ solution (0.94 mg/mL, 2.78×10^{-3} mmol) was added into 4.0 mL of PEEA vesicle solution (300 μg/mL, 1.77×10^{-4} mmol) to reach a molar ratio of 6:1 (Ag^+/–COOH), resulting in the adsorption of Ag^+ by –COOH because of the electrostatic interactions. After stirring for 1 h, the *in situ* reduction of monovalent Ag^+ to zero-valent AgNPs was carried out via adding $NaBH_4$ solution (prepared on site, 2:1 molar ratio relative to the amount of $AgNO_3$ used). The PEEA vesicle solution turned brown immediately. Then the AgNPs-decorated PEEA vesicle solution was dialyzed against deionized water to remove side products.

Antibacterial test

The antibacterial property of AgNPs@vesicle solution was determined by measuring the MICs against *E. coli* and *S. aureus*, respectively. Briefly, bacteria cells were grown overnight at 37 °C in a LB medium to a mid-log phase and diluted to 10^5-10^6 colony forming units (CFU) mL^{-1}. Various concentrations of AgNPs@vesicle solutions (128.0, 64.0, 60.0, 55.7, 51.4, 42.8, 30.0, 27.9, 25.7, 21.4 and 10.7 μg/mL) were prepared by diluting with LB broth. A total of 100 μL of each diluted AgNPs@vesicle solution from the serial dilutions was added to microtiter plates, followed by addition of 100 μL of bacterial suspension, to give a final inoculum of 5×10^5 CFU/mL. The total volume of mixture in each plate was 200 μL. A blank sheet without polymer vesicles was used as the control to compare antibacterial activity. The plates were incubated at 37 °C for 8 h, and the MICs were determined by the minimum concentration of Ag in antimicrobial agent at which no visible growth of microbes is observed. The experiment was repeated twice independently.

Characterizations

DLS. DLS was used to determine the D_h and polydispersity of vesicles in aqueous solution. The D_h's of the PEEA homopolymer vesicles were characterized by ZETASIZER Nano series instrument (Malvern Instruments ZS 90). The scattering angle was fixed at 90°. Data processing was carried out using cumulant analysis of the experimental correlation function and analyzed using Stokes-Einstein equation to calculate the hydrodynamic diameters of PEEA homopolymer vesicles.

UV−vis Spectroscopy. The UV−vis spectra of the aqueous PEEA vesicles and the silver-decorated vesicles (AgNPs@vesicles) solutions were acquired using a UV759S UV–vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd.). All the samples were analyzed using quartz cuvettes.

TEM. The AgNPs@vesicle solution was diluted at ambient temperature. The copper grid was surface-coated to form a thin layer of amorphous carbon. The AgNPs@vesicle solution (10 μL) was then dropped onto the carbon-coated grid and dried at ambient environment overnight. The sample was prepared without staining. Images were recorded by a JEOL JEM-2100F instrument at 200 kV equipped with a Gatan 894 Ultrascan 1k CCD camera.

Acknowledgements

J.D. is supported by National Natural Science Foundation of China (21074095, 21174107 and 21374080), Shanghai 1000 Plan, the Eastern Scholar program, Ph.D. Program Foundation of Ministry of Education (20110072110048), Fok Ying Tong Education Foundation (132018), and the open fund for characterization at Tongji University (0002013031).

Notes and references

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