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Facile synthesis of novel size-controlled antibacterial hybrid sphere with silver nanoparticles loaded to poly-dopamine sphere

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Silver nanoparticles has proven to own excellent antimicrobial activity, but the problems of aggregation and toxicity limit its practical application. To solve the problems, a kind of poly-dopamine sub-micrometer structure-sphere loaded with silver nanoparticles (Ag@PDS) was fabricated by a facile method. Silver nitrate was directly reduced by poly-dopamine sphere without surface modification of the spheres and additional reducing agent. This whole preparation process is very green, simple and time-saving. As a result, Ag NPs have been fixed and uniformly disperse onto the surface of the PDS, which can effectively prevent Ag NPs from aggregation and oxidization in application. Since the size of the uniform hybrid sphere is easily controlled by adjusting the ratio of ammonia to dopamine, it is convenient to fabricate different size hybrid spheres according to need. And the antibacterial activity of this hybrids show that the bacterial growth has fully been inhibited. Finally, prospect of the organismal level impact of the Ag@PDS hybrid has also been proposed.

Introduction

Antibacterial materials which play critical roles to health care are always widely studied. Silver and antibiotics, to our best knowledge, are two important and popular antibacterial agents. Unfortunately, antibiotics may rise of microbial drug resistance, which results in poor treatment efficacy and significant economic losses. But silver nanoparticles has proven to own excellent antimicrobial activity which is mainly due to the function of deactivating respiratory enzymes and in part by interfering with DNA replication and disrupting the cell membrane. Although the silver nanoparticles has been used in commercial products, it still attract researchers attentions because of its huge practical application and some elusive questions. In recent decades, the effect of silver nanoparticles size, shape, surface charge, solution chemistry, and surface coating to antibacterial properties are widely studied. But, the problems of aggregation and oxidization of silver nanoparticles are still the critical factor that exist and limit the practical application through affect the activity of silver nanoparticles. In order to resolve these problems, several novel strategies have been made through preparing silver nano-composites which often synthesizing the silver nanoparticles on the surfaces of nanowires, nanotubes, nanospheres. However, it is easy to find that these processes are usually complex. Surface modification of the substrate, additional reductant and multi-steps processes are often needed.

Dopamine has attracted very widespread research interest as a hormone and neurotransmitter in many years. Its unique property of forming coating on various bulk substrates have caused the wide interests of researchers. Once found to easily obtain through self-polymerization process of the dopamine at alkaline conditions, poly-dopamine is quickly applied into various interfaces of different fields, such as the surface modification, the enhancement of the electronic properties, the biological application, and so on. Recently, poly-dopamine sphere (PDS) has been fabricated by a facile method at room temperature. This PDS has many advantages, such as the easily controllable size, uniformly spherical shape, extremely good thermal stability. And the inherit catechol and N-H groups enable yields of transition metal-carbon hybrid materials, which probable greatly enhance its application in the field of electrochemistry. The presence of these functional groups endowed PDS with an active surface for absorbing and reducing metal ions. In addition, this sphere owns huge practical application in the field of biomaterial because of its good biocompatibility and non-toxic property. Due to inherit polar group, it also can disperse in many solvent, which make it used as vector of other poor-dispersion nanoparticles.

In this report, we present a novel kind of uniform hybrid spheres that is PDS loaded with silver nanoparticles (Ag@PDS). This whole preparation process is very facile, simple and time-saving, both surface modification of PDS and additional reductants are not needed (Fig. 1). Specifically, the silver nitrate is directly reduced on the surface of PDS with the assistance of ammonia to dopamine, it is convenient to fabricate different size hybrid spheres according to need. In addition, the PDS can well disperse in many solvents, which also results to a well-dispersion of silver nanoparticles because of tight bonding between silver nanoparticles and PDS. Simultaneously, this tight bonding can effectively prevent the aggregation of the silver nanoparticles and possible release of silver ionic. In the hybrid sphere, silver nanoparticles are uniformly distributed on the surface of PDS. This structure has great contribution to the efficient antibacterial activity of Ag@PDS. Moreover, as Ag@PDS has character of good dispersion but poor solubility in solvents, it will dramatically increase its application in the field of antibacterial material by embedding silver nanoparticles into the polymer through solution blending method.

Furthermore, silver nanoparticles (Ag NPs) are increasingly applied in consumer products for their antimicrobial properties and may have adverse impacts on organisms when they enter biotic receptors, though the mechanisms of Ag NP toxicity have not been fully illuminated. In particular, there is debate as to
whether toxicity can be directly attributed either to the nanoparticles themselves or to dissolved ionic silver released from Ag NPs. Liyan Yin’s study shows that the release of silver in the form of nanoparticles may be the primary importance compared to the release of ionic Ag from NP. Our silver nanoparticles loaded to poly-dopamine sphere hybrid have high antibacterial property while possesses controllable size of hundreds of nanometers to micrometers, allowing potentially great biocompatibility and possible tailored size effect for antibacterial applications in biotechnology and biomedical fields. Next, in another paper, we discuss that the possibility of the hybrid applied in infected organisms on the premise of reduction of the Ag NPs toxicity due to the hybrid’s bigger size and better biocompatibility of dopamine, which will further illustrate the toxicity mechanism of Ag NPs nanoparticles.

![Schematic illustration of the procedure for fabricating poly-dopamine sub-micrometer sphere loaded with silver nanoparticles (Ag@PDS)](image)

**Fig.1** Schematic illustration of the procedure for fabricating poly-dopamine sub-micrometer sphere loaded with silver nanoparticles (Ag@PDS)

### Experiment

#### Material

Dopamine-HCl was purchased from Aladdin-reagent Company without further treatment. Silver nitrate, ethanol and ammonia aqueous solution (25wt%-28wt%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Phosphate buffered saline (PBS) was prepared by dissolving 7.9g NaCl, 0.2g KCl, 0.24g KH2PO4 and 1.44g Na2HPO4 into 800 ml of water. The pH was adjusted to 7.40 with 1 M NaOH or 1 M HCl, and the solution was mixed with additional water to 1 L in a volumetric flask.

Bacteria strains Staphylococcus aureus (ATCC6538) and Escherichia coli (ATCC8739) were purchased from The Shanghai Fu Xiang biological Technology Co., Ltd. Tryptic soy agar was obtained from The Shanghai Zhi Yan biological Technology Co., Ltd.

#### Synthesis of poly-dopamine sphere (PDS)

The synthesis is similar to the previous reference. Deionized water (90mL) was mixed with ethanol (40mL) at room temperature, ammonia aqueous solution (NH4OH, 1mL, 25%) was added into the above solution under mild stirring, then stirred for 30 minutes. Dopamine hydrochloride (0.5 g) was dissolved in deionized water (10mL) and then injected into the above mixture solution. The reaction was allowed to proceed for 30 hours. The PDS were obtained by centrifugation and washed with water for three times. The control over the size of PDS would be achieved by adjusting the amount of ammonia aqueous solution.

#### Synthesis of poly-dopamine sphere loaded with silver nanoparticles (Ag@PDS)

The obtained PDS was dispersed in deionized water through stirring and sonication for several minutes, 0.8 g silver nitrate was dissolved in deionized water (10 mL) then dropwise added into the PDS dispersion, and the reduction reaction carried out for 10min in the ice bath with the assistance of sonication. And the Ag@PDS were collected by centrifugation and washed with deionized water for three times. At last, the products were dried at 60 °C in vacuum for further use.

### Characterization

#### SEM patterns were all acquired using a scanning electron microscope (JSM-7401F, JEOL) at an acceleration voltage of 5kV. Energy Dispersive Spectrum (EDS) analysis was also performed on the JSM-7401F JEOL instrument during SEM. The morphology of samples were also characterized by transmission electron microscopy (TEM, JOEL, JEM-2100, with an accelerating voltage of 100 kV) equipped with an Oxford INCA Energy TEM 200 EDX system. The particle size distribution were measured using dynamic light scattering (DLS) (Zetasizer ZS, Malvern Instruments Ltd.). The fourier transformed infrared spectroscopy (FTIR) were recorded on a Perkin-Elmer 1000 FTIR spectrometer. The X-ray diffraction (XRD) recorded on Rigaku D/Max 2550 (Cu Kα, λ=1.5418Å) with a 20 scan configuration in the range 5–90°. The surface chemical composition was analyzed using a Shimadzu-Kratos (AXIS Ultra) X-ray photoelectron spectroscopy (XPS).

#### Bactericidal testing

**Inhibition efficiency**

The antimicrobial activity of the Ag@PDS was determined using inhibition efficiency and the bactericidal experiment was carried out with the Gram-negative bacteria Escherichia coli and the Gram-positive bacteria Staphylococcus aureus. A modified ATCC-100 biodial testing protocol was employed. 100µL E. coli (ATCC8739) or S. aureus (ATCC6538) bacteria suspension was grown in 160mL MHB (Mueller-Hinton Broth, cation adjusted) at 37ºC for 12h. Then the bacteria containing suspension was diluted 50 times to enable the separate bacterial colonies to be distinctly visible and containing ~10⁶ CFU mL⁻¹ colonies. And the algae filament was collected by centrifuge (5000rpm, 10min) from 30 mL of incubated bacteria suspension containing ~10⁶ CFU mL⁻¹ colonies, after that 30 mL phosphate buffered saline (PBS) were added to the algae filament. Then 0.15 mg mL⁻¹ Ag@PDS, PDS, Ag NPs suspension was added to bacteria solution respectively. Each sample contains five repetitions to eliminate accidental factors and get statistical results. Put the suspension into shaker ZHWY-2102C for another 18 h, then 200 µL of final solution was spread out on the plate and incubated at 37 °C for 24 h. The surviving bacteria on the controls and experimental plates were observed.

**Zone of inhibition (ZOI)**

The antimicrobial activities of the Ag@PDS composite spheres were also analyzed by inhibition zone testing. In the inhibition zone test, S. aureus bacteria solution was grown in 160ml MHB (Mueller-Hinton Broth, cation adjusted) at 37°C for 12h. Then 200µL bacteria suspension was uniformly spread over a Luria–Bertani (LB) agar plate. Samples coated with 5mg of Ag@PDS hybrid sphere, PDS, Ag NPs, respectively, and were placed at the center of the agar plate carefully to without touching the other parts and incubated at 37°C for 24 h. Each sample contains five repetitions to eliminate accidental factors and get statistical results. The clear zones in the center of the disks were then photographed and measured with the help of a scale that represented the antibacterial properties of the different composites.
Results and discussion

Microstructure and morphology studies

At first, uniform PDS were synthesized through a facile process which was carried out at room temperature with gentle stirring (Fig.1). This process takes 30 hours in order to obtain uniform shape only after 12 hours and the size was also similar to that of the final products which obtained after 30 hours. But the morphology of the final sphere is much more uniform. And in this synthesized process, the mild stirring plays a key role to obtain uniform spheres. Hundreds of nanometers to micrometers-sized PDS have been successfully synthesized. But the deterioration of dopamine which is easily oxidized probably causes the products of a little different in size (about several to tens nanometers) in the same ratio of ammonia to dopamine under the same experimental condition.

The widespread application of poly-dopamine has also raised the interest of researchers to study the mechanism of dopamine polymerization in recent years (ESI, Fig.S1), but the mechanism, to our best knowledge, has not yet been clearly demonstrated. In this work, we focus on analyzing the structure of PDS. The typical SEM and TEM images of PDS were shown in Fig.2. From the electron microscopy images, it can be clearly found that these organic spheres are uniform. And the diameter of the PDS can be easily controlled by the ratio of ammonia to dopamine. The chemical composition of the sample is determined using the energy dispersive spectrum (SEM-EDS) (ESI, Fig.S2). There are carbon, oxygen, nitrogen and silver elements peaks in the SEM-EDS spectrum taken from Ag@PDS, while there is only carbon and, oxygen and nitrogen peaks taken from PDS. UV-vis measurement (ESI, Fig.S3) shows an absorbance at 280 nm attributed to catechol groups in PDS, which correspond to the size measured by TEM. The size of Ag nanoparticles on the PDS is about 12-18nm, and their shape is spherical. This sphere-structure prevent the aggregation of silver nanoparticles effectively. The chemical composition of the sample is determined using the energy dispersive X-ray spectrum (EDX). As shown in Fig.3d, there are several kinds of peaks in the EDX spectrum taken from the Ag@PDS, which correspond to carbon, oxygen, sulfur and silver elements, respectively. It confirms well the existence of Ag NPs on the surface of the PDS. The elemental atoms results in insert table also confirm that the particles formed on the PDS surface is composed of silver. The dynamic light scattering (DLS) result (Fig.3e) also shows Ag@PDS is about 350nm with narrow size distribution, which corresponds to the size measured by TEM.

Before the measurement of TEM, Ag@PDS was ultrasonicated about 30 minutes, silver nanoparticles were still attached on the PDS and no pure particle was found. It provides evidence that the bonding between silver nanoparticles and PDS is firm and stable. This strong bonding ensures that this nanostructure will not destroy in the process of embedding Ag@PDS into various biomacromolecules. We find that the grow speed of Ag nanoparticles on the PDS is very fast with the assistance of sonication. The time of reaction to obtain the above Ag nanoparticles is only 10 minutes, so this process was rapid and time-saving.

Crystal forms of Ag@PDS

Crystal forms of PDS and Ag@PDS can be identified with XRD patterns (Fig.4). The XRD patterns of the obtained Ag@PDS (Fig.4b) demonstrate the existence of PDS loaded with silver. The broad XRD reflection peak at 23.2° could be attributed to the diffraction of the amorphous structures of the PDS. Five XRD diffraction peaks of Ag@PDS at 20 of 38.1°, 44.4°, 64.6°, 77.5° and 81.6° correspond to the diffraction of the (111), (200), (220), (311), and (222) lattice planes, indicating a face-centered-cube (FCC) phase of the Ag crystal (ICPD file no.04-0783), which evidently reveal that silver ions have been reduced to 0 value of metallic and fixed on the surface of PDS. Besides, the peaks belong to amorphous PDS did not change, indicating that the reduction process did not affect the crystallography structure as
well as the good property of the PDS. All of the TEM, EDX and XRD results demonstrated that the AgNPs were successfully synthesized on the PDS surface by this simple facile reduction process.

**FT-IR analysis of Ag@PDS**

FT-IR spectra was chosen to investigate the surface chemical structure of Ag@PDS as well as the binding interactions between PDS and Ag NPs, and the spectra are illustrated in Fig.5. The spectra of the Ag-based samples Ag@PDS display well-defined characteristic bands that appear also in the spectra of the PDS but slightly shifted and with difference relative intensity, indicating that the reaction process did not affect the surface chemical structure as well as the good property of the PDS. The absorption band at 3379 cm$^{-1}$, which corresponds to the stretching vibrations of –OH&N-H groups of PDS, broadened and gradually shifted to lower wavenumbers 1608 cm$^{-1}$ and 1268 cm$^{-1}$. On the other hand, the peak of C=N&C=C at 1512cm$^{-1}$ almost stay the same. The peak shifting could be due to bond formation between silver and oxygen, catechol group and hydroxyl group were oxidized to quinone group or carbonyl group while Ag$^+-$ was reduced to Ag$^0$. The interaction facilitate the attachment of Ag NPs to the PDS surface, leading to the steric stabilization of the particles. Furthermore, the remaining positively charged PDS could prevent the aggregation of Ag NPs by providing reciprocal electrostatic repulsion.

**XPS study of Ag@PDS**

To better understand the mechanism underlying the formation of the Ag@PDS, the result was further verified by X-ray photoelectron spectroscopy (XPS) analysis to further illustrate the composition of the PDS and Ag@PDS. Fig.6a shows the XPS wide scan of PDS and Ag@PDS, and high-resolution spectra of Ag3d, O1s and C1s, respectively. The scan survey spectra (Fig.6a) of PDS and Ag@PDS surface showed almost the same peak components of C 1s, N 1s (ESI, Fig.S4) and O 1s. In addition, Ag@PDS exhibited two specific peaks with binding energies of 368.0 eV and 374.0 eV in Fig.6b, which were attributed to Ag3d 5/2 and Ag3d 3/2 electrons of Ag0. The spin energy separation was identified as 6.0 eV which indicates silver on the PDS surface is metallic Ag0 in nature; in turn, it further supports the conclusion that Ag NPs has been successfully loaded to the surface of PDS. The XPS binding energy of the silver is somewhat lower than the values reported by Gole et al. This may be partly from the bonding interaction between Ag NPs and the PDS surface.

**Fig. 5 FT-IR spectra of poly-dopamine sphere (PDS) and poly-dopamine sphere loaded with silver nanoparticles (Ag@PDS).**

**Fig. 6 X-ray photoelectron spectra(XPS) of (a) scan survey, (b) Ag3d, (c, d) O1s, (e, f) C1s of poly-dopamine sphere (PDS) and poly-dopamine sphere loaded with silver nanoparticles (Ag@PDS).**

Moreover, only a small amount of energy is required to move atomic oxygen readily through the silver lattice, which makes silver little repulsion to oxygen. The interaction between the AgNPs and supporting PDS will directly lead to the tight adherence of AgNPs to the PDS surface, which is very useful and essential for the practical application.

The C1s high-resolution spectrum of the PDS (Fig.6e) could be curve-fitted with four peak components, having binding energies at 284.2 eV for the sp2C species, at 285.0 eV for the sp3C species, at 286.0 eV for the C=O species, and at 287.7 eV for the C-N species. The C1s core-level spectrum of the Ag@PDS (Fig.6f) has almost the same peak components as that of the PDS but the peaks shifted to 284.5 eV, 285.3 eV, 286.1 eV, 288.1 eV, respectively. The peak for the species of C=O of the PDS is identified as 6.0 eV which indicates silver on the PDS surface is metallic Ag0 in nature; in turn, it further supports the idea that Ag NPs has been successfully loaded to the surface of PDS. The XPS binding energy of the silver is somewhat lower than the values reported by Gole et al. This may be partly from the bonding interaction between Ag NPs and the PDS surface.
decreased, which shows that many oxygen-containing functional groups are reacted or removed due to the interaction of catechol groups with silver.

**Thermal gravity analysis of Ag@PDS**

Thermal Gravity Analysis (TGA) was carried out at the temperature range of 40 °C to 800 °C to examine the thermal stability of PDA and Ag@PDS. As expected, both PDS and Ag@PDS show excellent thermal stability, 51.6% and 52.5% residual weight of PDS and Ag@PDS were observed even at the end of the analysis (800 °C) under N2 atmosphere, respectively and they exhibits almost the same behavior in Fig 7. While difference of TGA curve can be found under air atmosphere, Ag@PDS show relatively slower rate of weight loss, and it reached balance at 625 °C with 6.41% residual weight; while PDS reached balance at a higher temperature of 700 °C with 3.06% residual weight.

**Solubility measurement**

Fig.8 The images of solubility (a) and dispersion (b) measurement of this poly-dopamine loaded with silver nanoparticles (Ag@PDS) (The solvents from left to right are water, acetone, 1, 4-dioxane, chloroform, toluene, and DMF, respectively).

For the feasibility of application, the solubility and dispersibility of Ag@PDS spheres have been further studied (Fig.8). This experiment demonstrated that several common solvents, such as water, acetone, chloroform, toluene, and N, N-dimethylformamide (DMF), were all can’t dissolve these spheres. But due to inherit polar group, PDS owns a perfect dispersion in many solvents after ultrasonic treatment. This characteristic of good dispersion but poor solubility of the spheres make them perfect vectors for Ag nanoparticles, which will results to uniform and stable dispersion of Ag nanoparticles in the composite through import method of solution blending.

**Antibacterial property**

Samples designated as Ag@PDS$_{1.0}$, Ag@PDS$_{1.25}$, Ag@PDS$_{1.5}$, Ag@PDS$_{1.75}$ and Ag@PDS$_{2.0}$ were achieved by adjusting the amount of ammonia aqueous solution with 1.0ml, 1.25ml, 1.5ml, 1.75ml and 2.0ml of ammonia aqueous solution, and keeping the amount of dopamine hydrochloride the same (0.5g). Fig.9 TEM images show that the different sizes of uniform spherical PDS are obtained, and little size PDS is available by adding more ammonia aqueous solution. The diameter of PDS in Ag@PDS$_{1.0}$, Ag@PDS$_{1.25}$, Ag@PDS$_{1.5}$, Ag@PDS$_{1.75}$ and Ag@PDS$_{2.0}$ are 350nm, 310nm, 280nm, 260nm and 230nm, respectively, while the Ag NPs in these samples are almost the same, mainly 12~18nm. And Ag content measured by TGA are 3.35%, 4.43%, 3.85%, 3.86% and 4.01% for Ag@PDS$_{1.0}$, Ag@PDS$_{1.25}$, Ag@PDS$_{1.5}$, Ag@PDS$_{1.75}$ and Ag@PDS$_{2.0}$, respectively. Pure Ag NPs was synthesized according to previous reference 29, whose size was similar to those Ag NPs on the surface of PDS in Ag@PDS.

Fig.10 Images of LB-agar plates which is used in the antibacterial activity experiment of samples against E.coli: (a) control, (b) poly-dopamine (PDS), (c) poly-dopamine sphere loaded with silver nanoparticles (Ag@PDS).
To investigate the antibacterial activity of our new hybrid material, to our knowledge, which is never been reported, its ability to inhibit Gram-negative bacteria E. coli and Gram-positive S. aureus bacteria has been measured. Since after the PDS dispersed, the color of LB liquid media became dark, which result impossible to observe the turbidity of the LB liquid media. So the antibacterial efficacy of Ag@PDS was evaluated by the inhibition rate experiment on the LB-agar plates in this report. After the LB-agar plates solidified, the suspension of bacteria was spread onto the LB-agar plates. Then, the above LB-agar plates were cultured at 37°C for 24 h, and the clear evidence which was shown on Fig.10 confirmed that there was a robust growth of E. coli bacteria with no samples added. No considerable antibacterial activity was observed in disk with PDS added, indicating that PDS were not toxic to the bacteria. In addition, another controlled experiment which suspension consisted of E. coli bacteria and Ag@PDSx was taken under same condition. Fig.10 shows that few bacteria are observed and this strain is inhibited, which means that this new hybrid material has very strong antimicrobial activity to E. coli. In addition, from Fig.11, residual bacteria are fewer with the size of Ag@PDS decreased. Ag@PDS1.75 and Ag@PDS2.0 even exhibit fully inhibition of S. aureus, and the minimum inhibitory concentration (MIC) against S. aureus of Ag@PDS2.0 is 6µg mL−1 in Ag content, which is comparable with pure Ag NPs, suggesting that Ag@PDS nanohybrid is an efficient antibacterial material for both Gram-negative and Gram-positive bacteria. This excellent antimicrobial activity has a close relationship with the novel nano-structure which can prevent the agglomeration of the silver nanoparticles, and the large surface area of PDS also provides high load capacity for silver nanoparticles with the assistance of functional groups.

<table>
<thead>
<tr>
<th>samples</th>
<th>Ag content (µg)</th>
<th>ZOI value (mm/mm)</th>
<th>normalized Ag content (µg)</th>
<th>normalized ZOI value (mm/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDS</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>Ag NPs</td>
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<td>1.81±0.31</td>
<td>200</td>
<td>1.81±0.31</td>
</tr>
<tr>
<td>Ag@PDS1.0</td>
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<td>1.51±0.33</td>
<td>200</td>
<td>1.50±0.32</td>
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<tr>
<td>Ag@PDS1.25</td>
<td>221.5</td>
<td>1.72±0.25</td>
<td>200</td>
<td>1.65±0.23</td>
</tr>
<tr>
<td>Ag@PDS1.5</td>
<td>192.5</td>
<td>1.75±0.40</td>
<td>200</td>
<td>1.78±0.42</td>
</tr>
<tr>
<td>Ag@PDS1.75</td>
<td>193.1</td>
<td>1.83±0.27</td>
<td>200</td>
<td>1.86±0.28</td>
</tr>
<tr>
<td>Ag@PDS2.0</td>
<td>200.5</td>
<td>1.94±0.33</td>
<td>200</td>
<td>1.94±0.33</td>
</tr>
</tbody>
</table>

It can be seen that the average ZOI values (ratio of ZOI diameter to samples diameter) for the Ag NPs with S. aureus was 1.81±0.31, while the corresponding values for Ag@PDS1.0, Ag@PDS1.25, Ag@PDS1.5, Ag@PDS1.75 and Ag@PDS2.0 are 1.51±0.33, 1.72±0.25, 1.75±0.40, 1.83±0.27 and 1.94±0.33, respectively. After normalized the Ag concentration to 200µg, the normalized ZOI value of Ag@PDS1.0, Ag@PDS1.25, Ag@PDS1.5, Ag@PDS1.75 and Ag@PDS2.0 are 1.50±0.32, 1.65±0.23, 1.78±0.42, 1.86±0.28 and 1.94±0.33, respectively. The specific surface area \(s/m=4\pi r^2/\rho\) (unit, m2/g) is inversely proportional to the particle size with the same density, it means smaller Ag@PDS has larger specific surface area which contribute to better antibacterial property.

The normalized ZOI value of Ag NPs at normalized Ag content (200µg) turns to 1.03±0.01, which is very unreasonable to the fact that Ag NPs have excellent antibacterial property. According to reference5, the antibacterial property of Ag NPs increase with the addition increment, but it is not endless, the antibacterial property of Ag NPs will reach the maximum value and then stay the same with the Ag NPs addition increase to some point (extreme point). The maximum value and the extreme point is limited by the size of Ag NPs, specifically, smaller Ag NPs get higher maximum value and higher extreme point partly due to its larger specific surface area (s/m). So the most probable explanation is that the Ag@PDS composites, especially small sized Ag@PDS, have better antibacterial properties in comparison with those of the pure Ag NPs.

According to previous study, the influence of the antibacterial efficiency of Ag NPs might have been derived from the fact that dispersed Ag NPs tend to aggregate and separate from solutions. In our work, Ag NPs have been fixed and uniformly disperse onto the surface of the PDS, which can effectively prevent Ag NPs from aggregation and oxidation in application. Besides, the XPS, FTIR results for the Ag@PDS show surface functional groups such as C−O, O−H which can improve significantly the capacity and dispersion ability of the Ag@PDS. Consequently, the Ag@PDS composites can bind and be in close proximity to the bacterial surface and efficiently inhibited bacteria growth. Therefore, these composites are highly efficient inhibitors of bacterial growth.

**Conclusion**

We presented a novel micro-structure which composed of uniformly PDS and Ag nanoparticles. PDS as a carrier in this structure effectively prevent agglomeration of Ag nanoparticles, and its antibacterial activity has been firstly studied in the report.
This whole preparation process is very facile, simple, green and time-saving, both surface modification of PDS and additional reductants are not needed. In the hybrid sphere, silver nanoparticles are uniformly distributed on the surface of PDS, which prevent the aggregation of the silver nanoparticles effectively. And this hybrid material has high antibacterial activity. Because of the PDS’s good compatibility in the substrates, it will contribute a lot to the stable and uniform distribution of silver nanoparticles in various media. This will increase dramatically the antibacterial application of this hybrid sphere in biotechnology and biomedical fields. In addition, our silver nanoparticles loaded to poly-dopamine sphere hybrid have high antibacterial property while possesses controllable size of hundreds of nanometers to micrometers, which makes its application in biomedical fields possible for reducing the Ag NPs toxicity, and further provides some support to illustrate the toxicity mechanism of Ag nanoparticles.

Acknowledgements
This project is supported by the National Science Fund of China (20974061) and the Shanghai Leading Academic Discipline Project (No. B202). We thank Dr. Limin Sun from Instrumental Analysis Center of Shanghai Jiao Tong University for his assistance in the XPS experiments.

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Facile synthesis of novel size-controlled antibacterial hybrid sphere with silver nanoparticles loaded to poly-dopamine sphere

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Dopamine + Ethanol and water → NH₃-H₂O polymerization → \[ \text{AgNO}_3 \text{ solution in situ reduction} \]

\[ \text{Dopamine} \quad \overset{\text{polymerization}}{\rightarrow} \quad \text{Polydopamine sphere (PDS)} \quad \overset{\text{in situ reduction}}{\rightarrow} \quad \text{Ag NPs} \]

**Fig.1** Schematic illustration of the procedure for fabricating poly-dopamine sub-micrometer sphere loaded with silver nanoparticles (Ag@PDS).

**Fig.2** Antibacterial result of poly-dopamine sub-micrometer sphere loaded with silver nanoparticles (Ag@PDS)

Sub-micrometer structure hybrid spheres with poly-dopamine core and silver-particles embellishment was fabricated by a facile method. This new hybrid sphere owns strong antibacterial activity because of the special structure effectively prevents the aggregation of silver nanoparticles.

**Abstract**

Silver nanoparticles has proven to own excellent antimicrobial activity, but the problems of aggregation and toxicity limit its practical application. To solve the problems, a kind of poly-dopamine sub-micrometer structure-sphere loaded with silver nanoparticles (Ag@PDS) was fabricated by a facile method. Silver nitrate was directly reduced by poly-dopamine sphere without surface modification of the spheres and additional reducing agent. This whole preparation process is very green, simple and time-saving. As a result, Ag NPs have been fixed and uniformly disperse onto the surface of the PDS, which can effectively prevent Ag NPs from aggregation and oxidization in application. Since the size of the uniform hybrid sphere is easily controlled by adjusting the ratio of ammonia to dopamine, it is convenient to fabricate different size hybrid spheres according to need. And the antibacterial activity of this hybrids show that the bacterial growth has fully been inhibited. Finally, prospect of the organismal level impact of the Ag@PDS hybrid has also been proposed.