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Graphical Contents Entry

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A novel green synthesis approach for polymer nanocomposites decorated with silver nanoparticles and their antibacterial activity

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A novel, low-cost and direct green synthetic strategy was developed for the preparation of polymer composite spheres composed of silver nanoparticles (AgNPs) decorated on the polymer colloids in a raspberry-like fashion. The polymer colloids were employed as both the reductant and the substrate support. The environmentally benign reaction system consists of AgNO₃, polymer spheres and ethanol solvent, without any additional reducing agent or stabilizers. The obtained composite spheres are surprisingly stable. The size range of the formed AgNPs is from several to tens of nanometers, dependent on the reaction time, AgNO₃ concentration, and the amount of polymer spheres. A four-step mechanism was proposed for the formation of AgNPs-coated polymer spheres firstly. These composites exhibit an excellent antibacterial activity, which is dependent on both the concentration of the composite spheres and the size of coated AgNPs.

Introduction

Developing polymer composite spheres composed of metal nanoparticles decorated on the polymer particles have received significant attention since they could make new materials with novel properties originating from the combination of the inorganic components and the polymer, and applications in photonics, electronics, medical imaging, drug delivery and catalysis.¹⁻¹⁰ In this composite structure, the immobilization of metal nanoparticles onto spherical substrates in raspberry-like fashion provides benefits not only in the recycling of the nanoparticles but also in maintaining their size effects.^{5, 8} Many different approaches have been employed for the incorporation metal nanoparticles into the polymer spheres, including so-called in situ and ex situ approaches.^{1, 11-13} However, these syntheses very often require materials that are not environmentally benign. Utilization of nontoxic chemicals, environmentally benign solvents, and renewable materials are some of the key issues that merit important consideration in a green synthetic strategy.¹⁴⁻¹⁶ The much more attractive green processes do not require additives such as surfactants or reducing agents at all. Recently, some polymers containing functional groups such as conjugated conductive polymer, nonelectrolyte homo-polymer, polyelectrolyte and dendrimers have been reported to reduce metal ions into particles.¹⁷⁻¹⁹ However, the reported metal nanoparticles synthesized by polymer mediated approach are often embedded within the polymer matrix or being covered with organic molecules.

And the *in situ* formation of metal nanoparticles decorated on the external surface of the polymer colloids mediated by only polymer colloid support itself has not been reported.

The global rise in the resistance of pathogens to conventional antibiotics has created an intensive search for alternatives with antimicrobial properties. The use of silver as an antimicrobial agent is well recognized.²⁰⁻²⁹ However, one problem that needs to be solved in practical application of AgNPs is the strong tendency of aggregation owing to van der Waals attraction, which reduces the antibacterial efficiency greatly. Further, the colloidal silver nanoparticles have shown toxicity toward mammalian cell, although not fatal.³⁰ Therefore, immobilization of AgNPs onto polymer colloidal spheres is advantageous because it resists the particle aggregation and inhibits direct uptake of the nanoparticles by cells. A further advantage is that the immobilization of AgNPs onto the sphere structures prevents the release and disposal of the nanoparticles themselves. This property may be highly desirable for the nanoparticles to cross biological membranes and other barriers. In this paper, a new type of silver nanoparticlesdecorated polymer composite spheres was successfully synthesized in raspberry-like fashion by a direct environmentally friendly green method. The previously-reported chemically reactive poly(glycidy) methacrylate) (PGMA) colloidal spheres³¹ were used as both the reductant and the substrate support without adding any additional reducing agent or stabilizers. The silver precursor was reduced by the hydroxyl groups on the PGMA sub-microspheres, which comes

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59 60 from the ring opening of epoxy groups of the PGMA and the hydrolysis of sulfate radicals.^{32, 33} The reduction of AgNO₃ by PGMA spheres was enhanced considerably with the introduction of ethanol which saturated the external surface of the polymer colloids firstly. The formed AgNPs coated on PGMA colloidal spheres (PGMA@AgNPs) were very stable, without the aggregation or the loss of AgNPs for long time (8 months till now). Size of AgNPs is controllable by adjusting reaction time, AgNO₃ concentration, the amount of PGMA spheres used, which ranges from several to tens of nanometers (>50 nm). Further, the antibacterial activity of the PGMA@AgNPs composite spheres was also investigated using Escherichia coli (E. Coli.) bacteria, which is found to be dependent on both the concentration of the composite spheres and the size of the formed AgNPs used.

Experimental

Preparation of raspberry-like PGMA@AgNPs composite spheres

Highly monodisperse poly(glycidyl methacrylate) colloidal particles with diameter of 448 ± 9 nm were successfully synthesized by a modified surfactant-free polymerization method.³¹ Briefly, 15 mL of inhibitor-free glycidyl methacrylate (GMA) (≥ 97.0%, Sigma-Aldrich, St Louis, MO, USA) was dispersed in 150 mL of Nanopure water (Barnstead, 18 M Ω cm) purged with N₂ by stirring vigorously at 1,200 rpm for 30 min at room temperature. The mixture was refluxed and heated to 90 °C following dropwise addition of 0.5 g of potassium persulfate (KPS) (99+%, Sigma-Aldrich, St. Louis, MO, USA). The flow of nitrogen was then decreased through a nitrogen tank pressure regulator to minimize the stripping of monomer from the reaction mixture. The stirring rate of 1,200 rpm and temperature of 90 °C were kept constant until the end of the reaction. After 2 h of reaction, the reaction mixture was terminated by bubbling oxygen for 30 min. Upon completion of the reaction, the product was allowed to cool to room temperature. 25 mL of the PGMA spheres were dialyzed with Cellulose Ester Dialysis membrane (MWCO 3,500-5,000, Spectrum Laboratory, Inc, Rancho Dominguez, CA) for 24 h and centrifuged at 5,000 rpm (Eppendorf centrifuge 5804, VWR International, Radnor, PA, USA) for 30 min. After three successive centrifugation-decantation-redispersion cycles with 190-proof ethanol (95%, Sigma-Aldrich, St. Louis, MO, USA), 25 mL of purified PGMA spheres dispersed in ethanol were obtained.

To synthesize the raspberry-like PGMA@AgNPs composite particles, 25 mL of purified PGMA spheres in ethanol was firstly heated to 90 °C while stirring at 1,200 rpm. Then 3 mL of 364 mM silver nitrate (99+%, Sigma-Aldrich, St. Louis, MO, USA) in the solvent mixture of Nanopure water and ethanol was added into the PGMA dispersion solution (39 mM of the final concentration of AgNO₃). The reaction mixture was refluxed while kept at 90 °C with stirring for 36 h. To study the formation mechanism of the composite particles, the dispersion solutions were taken out at different time points. To investigate the effect of AgNO₃ concentration, four more solutions were adopted at the final concentrations of 10, 20, 60 and 120 mM respectively. For the evaluation of effect of PGMA spheres amount, different volumes (6.25, 12.5, 25, 50 and 75 mL) of the dialyzed PGMA spheres in water were experienced 58

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centrifugation-decantation-redispersion with ethanol for three times respectively and finally re-dispersed in 25 mL of ethanol. The reaction procedure was the same as above-mentioned with AgNO₃ at constant final concentration of 39 mM.

Antibacterial activity

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In order to start the initial liquid culture of bacteria to be used in the antibacterial test, 100 µL of competent E. Coli. (Strain DH5-Alpha, New England Biolabs), were added to 5 mL of LB medium. E. coli. were then cultured at 37 °C with shaking (250 rpm) overnight, and bacteria concentration was then determined by measuring OD_{600} in a glass cuvette with Eppendorf BioSpectrometer. OD₆₀₀ of 0.1 is considered equivalent to approximately 10⁸ cells/mL. To test the bactericidal effect of PGMA@AgNPs composite particles, 10⁶ E. coli bacteria were added in 100 mL of LB medium supplemented with different concentrations of PGMA@AgNPs composite particles of 1, 10, 100, 250 and 500 µg/mL respectively in 250 mL flasks. The composite particles consist of AgNPs in the diameter of $10.2 \pm$ 2.1 nm, which were synthesized with 25 mL PGMA sphere ethanol solution and the precursor AgNO3 at the final concentration of 39 mM at 90 °C for 10 hours. To monitor the bacterial growth, the flasks were shaken at 37 °C, and every 30 min, 0.5 mL of culture was taken out from each flask to measure OD₆₀₀.

To test the effect of size of AgNPs on the bacterial inhibition, the adopted composite spheres consisting of AgNPs in different diameters were prepared by the reaction of 25 mL PGMA sphere ethanol dispersion solution with AgNO₃ at the final concentration of 39 mM for 5, 10, 15, 24 and 36 h, resulting in the mean diameters of the generated AgNPs of 8.0, 10.2, 13.6, 15.2 and 16.3 nm respectively. Similar bacterial growth experiments were performed with these composite particles. The antibacterial efficiency can be calculated as % inhibition = $(B-A)/B \times 100$, where A is the absorbance of surviving bacteria in the tested sample, and B the absorbance of surviving bacteria in the control group without supplementing any AgNPs composite spheres under the same experimental conditions.

For the plate test, $10^5 E$. *coli* cells were inoculated onto the LB agar plate supplementing 500 µg/mL PGMA@AgNPs composite particles synthesized from the reaction of 25 mL PGMA sphere ethanol solution and the precursor AgNO₃ at the final concentration of 39 mM at 90 °C for 10 h. Same amount of E. coli were inoculated on the control LB agar plate without supplementing any composite particles. These plates were allowed to grow for 18 h in a 37 °C incubator.

Characterization

The structure and morphology of the raspberry-like PGMA@AgNPs composite particles were visualized using a JEM-1400 transmission electron microscope (TEM) (JEOL, Peabody, MA, USA) at an accelerating voltage of 120 kV. High-resolution TEM (HRTEM) images and size distribution of the AgNPs were determined by a JEOL JEM2100F highresolution analytical transmission electron microscope (JEOL, Peabody, MA, USA) equipped with energy-dispersive X-ray spectroscopy (EDX) at an accelerating voltage of 200 kV. Samples were prepared by placing 50 μ L of the sample solution onto carbon film coated copper grids (Electron Microscopy Sciences, Hatfield, PA, USA) followed by drying at room temperature. All UV-Vis spectra were recorded using an Agilent 8453 UV-visible diode array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The crystalline

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Fig. 1 (a) TEM image of PGMA@AgNPs composite spheres (Inset is the size distribution of AgNPs); (b) HRTEM image of AgNPs; (c) EDX spectrum of AgNPs; and (d) XRD pattern of PGMA@AgNPs composite spheres.

structure of PGMA@AgNPs was studied by X-ray diffraction (XRD), which was carried out using a Shimadzu XRD-6000 diffractometer operating with a Ni CuK α radiation source filtered with a graphite monochromator (0.154 nm). The samples were pressed in a sample holder. The X-rays were generated at 40 kV and 40 mA power and XRD scans were recorded at 2 θ from 10 to 80° with a 2°/min scan rate. The Fourier transform infrared (FTIR) spectra of the spheres were recorded with a PerkinElmer Spectrum Two FT-IR spectrometer (Waltham, MA, USA).

Results and discussion

The morphology and structure of PGMA@AgNPs composite spheres

Silver nanoparticles were successfully synthesized and coated on the chemically reactive PGMA sub-microspheres host in a raspberry-like fashion by a green polymer sphere supportmediated approach. TEM image establishes the unique morphological characteristics of the polymer sphere support and the size and shape of the silver nanoparticles. As shown in Fig. 1, silver nanoparticles were successfully synthesized and coated on the chemically reactive PGMA sub-microspheres support in a raspberry-like fashion by a polymer sphere support-mediated green approach. PGMA spheres with the diameter of 424 \pm 27 nm are coated with AgNPs of 15.2 \pm 2.7 nm in diameter in a raspberry-like fashion (Fig. 1a). The inset graph in Fig. 1a shows the size distribution of the coated AgNPs, which turns out to be relatively narrow with relative standard deviation of 17.8%. Here it is worth mentioning that the size of PGMA colloid support becomes smaller during the reaction from the original diameter of 448 ± 9 nm, which can be attributed to the ethanol etching effect. The crystallinity of the AgNPs was checked by high-resolution TEM (HRTEM) in Fig. 1b. The lattice plane spacing was 0.24 and 0.198 nm, which were indexed to the (111) and (200) planes of the facecentered-cubic (fcc) crystal structure of silver. Fig. 1c shows the Energy-dispersive X-ray (EDX) spectrum of green synthesized AgNPs. The peak at ~3 eV is the silver from the AgNPs. In addition, the dominant peak at ~0.28 eV is related



Fig. 2 (a) Photographs of PGMA@AgNPs composite spheres solutions as a function of reaction time. Control 1: the product of AgNO₃ reacting with ethanol; Control 2: the product of AgNO₃ reacting with purified PGMA spheres in DI water; (b) TEM images of PGMA@AgNPs composite spheres indicating the size evolution of AgNPs; (c) LSPR absorption spectra as a function of reaction time

with the carbon, and the peak at ~0.5 eV with the oxygen, which are both from the polymer sphere support. X-ray diffraction (XRD) pattern of the synthesized AgNPs-coated polymer composite spheres is shown in Fig. 1d. The observed 2θ values at 38.3° , 44.5° and 64.6° corresponding to the (111), (200) and (220) reflections respectively can be indexed to the fcc structure, which is in good agreement with HRTEM image. It indicates that silver nanoparticles were successfully synthesized and coated on the PGMA colloidal spheres. Lastly, the resulting composite colloids could be stable for months even under ambient light illumination. No color change or the loss of AgNPs from the polymer spheres were observed after 8 months storage.

Size evolution of AgNPs on PGMA spheres

To better understand the nucleation and growth of silver nanoparticles coated on the PGMA spheres, the dispersion solutions were taken at different time points in a standard synthesis and the samples were then recorded by camera and analyzed using UV/Vis spectrophotometer and TEM. After the addition of Ag precursor, the milky polymer sphere dispersion solution was kept whitish for some time, and then a tint of light yellowish color appeared. A gradual yellowish coloration of the solution became deepened as a function of reaction time, indicating the formation of AgNPs (Fig. 2a). No black solid particles were found at the bottom of the container, indicating no aggregation of silver nanoparticles. The localized surface plasma resonance (LSPR) absorption peak of AgNPs was dependent on the morphology, size as well as the size distribution of the nanoparticles, which could be analyzed by UV/Vis spectrophotometer. As shown in Fig. 2c, no LSPR absorption band was found for the sample synthesized within the first 5 and 10 hours due to the interference of PGMA spheres, although a light yellowish color was observed. However, the LSPR band at around 400-470 nm with the maximum absorption peak of 430 nm was observed after 15 h.

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The size evolution of AgNPs supported on PGMA spheres was shown in the TEM images of Fig. 2b with the precursor AgNO₃ at the final concentration of 39 mM and the dialyzed PGMA sphere dispersion solution of 25 mL. AgNPs were loosely distributed on the polymer spheres. The formed nanoparticles are spherically shaped, but the diameter of the AgNPs increases with time proceeding. The diameter of AgNPs increases to 8.0 ± 2.9 nm within the initial 5 h of the reaction time, and then increases to 10.2 ± 2.1 nm for the next 5 h reaction. The particle size gradually increases to 16.3 ± 3.7 nm after the whole reaction process of 36 h. And the number surface coverage of AgNPs on the PGMA spheres was almost constant throughout the whole reaction period (Fig. 2b). To deeply understand the formation mechanism of AgNPs reduced by the chemically reactive polymer spheres, studies were performed on the changes of formed particle diameter with reaction time at other 4 different precursor AgNO₃ final concentrations (10, 20, 60 and 120 mM) with the constant amount of polymer sphere dispersion solution and the reaction temperature of 90 °C. As can be seen from Fig. 3, the mean diameter of the formed AgNPs increases gradually at all AgNO₃ concentrations studied. At the lowest concentration of Ag sources of 10 mM studied, the AgNPs size changes from 4.1 ± 1.3

nm to 13.8 ± 6.2 nm when the reaction time increases from 5 to 36 h. But, the mean diameter of the formed AgNPs grows from 27.4 ± 7.5 nm to 51.5 ± 15.5 nm at the largest AgNO₃ final concentration of 120 mM.

Effect of AgNPs precursor concentration

The concentration of silver nanoparticle precursor, AgNO₃, available in the reaction system is important in controlling the particle size. Experiments were done to investigate the relationship between the Ag precursor concentration and the diameter of the resulting Ag nanoparticles. Fig. 4 shows the more AgNO₃ available in the system, the larger the AgNPs size at different time periods. After 10 h of reaction time, the AgNPs grow up to 4.4 ± 2.0 nm at the AgNO₃ concentration of 10 mM. However, when the concentration reaches 120 mM, a larger size of AgNPs of 32.3 ± 5.4 nm was obtained. On the other hand, after 24 hours of reaction time, the AgNPs of 7.9 \pm 2.3 nm were formed when the concentration of $AgNO_3$ is kept at 10 mM in the reaction mixture. However, larger particles of 15.2 ± 2.7 and 48.7 ± 17.1 nm were formed for the AgNO₃ concentrations of 39 and 120 mM in the system, respectively. The pictures of the final composite particles dispersion (36hour reaction) show a light yellowish color at a low range of AgNO₃ concentrations studied (10 \sim 20 mM), but brown color at a high range of AgNO₃ final concentrations ($60 \sim 120$ mM). These observations suggest that the concentration of AgNO₃ had an influence on the reduction rate and ultimately the population and size of the formed AgNPs.

Effect of the amount of PGMA spheres

We also investigated the effect of the amount of PGMA spheres as reducing agent and the scaffold support on the formation of silver nanoparticles. Fig. 5 shows that the size of the formed AgNPs increases with increasing the volume of the PGMA sphere ethanol dispersion solution available in the reaction system with AgNO₃ concentration kept constant. When the volume of PGMA spheres increases from 6.25 to 75 mL while keeping the final concentration of AgNO₃ constant at 39 mM,







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Fig. 6 (a) Schematic of the formation mechanism of PGMA@AgNPs composite spheres in ethanol solvent and (b) the related chemical reactions; (c) The Fourier Transform Infrared spectra of hydrolyzed PGMA spheres and PGMA@AgNPs spheres.

the size of the formed AgNPs changes from 6.6 ± 3.1 to 9.9 ± 2.9 nm, 7.3 ± 1.3 to 15 ± 4.1 nm, and 12.1 ± 1.9 to 25.4 ± 6.1 nm after 5, 15 and 36 h of reaction respectively. The results studied correlated with the reaction kinetics. As expected, a relatively low amount of PGMA spheres corresponded to slow reaction from Ag⁺ to Ag⁰, which only produced a small amount of Ag atoms on the PGMA spheres, thus a small size of AgNPs formed finally. In contrast, a high amount of PGMA spheres available in the reaction system greatly accelerate the reaction, generating a large amount of Ag atoms supply for the synthesis of AgNPs. As a result, Ag tended to grow in the larger diameter. The color of the formed composite particle dispersion changes from light yellow to dark brown with increasing the volume of PGMA sphere solution.

Mechanism of the formation of PGMA@AgNPs composite spheres

To gain deeper insight into the formation mechanism of PGMA@AgNPs composite spheres, two control experiments were performed. Control 1 is the addition of AgNO₃ solution

into 25 mL ethanol with the final concentration of 39.0 mM, and then was refluxed at 90 °C for 36 h. Control 2 is the addition of AgNO₃ aqueous solution at the final concentration of 39.0 mM into 25 mL of the purified PGMA sphere dispersion solution in DI water, and then was refluxed at 90 °C for 36 h. Fig. 2a shows the pictures of the as-prepared products for Control 1 and Control 2, without any observable color changes of the mixed solution indicating no formation of AgNPs in either of these systems. It suggests the formation of AgNPs is impossible from the reduction of AgNO₃ by just the ethanol, and the reduction of AgNO3 by PGMA spheres was enhanced considerably with the introduction of ethanol. The role of ethanol in the reaction system is not as the reductant, but is to saturate the external surface of the polymer spheres. Here four-step-mechanism was proposed for the formation of PGMA@AgNPs composite spheres: solvent saturation, reduction of Ag^+ to Ag^0 , AgNPs nuclei formation and adsorption onto the PGMA colloidal sphere surface, and the growth of AgNPs (Fig. 6a). Step 1: Saturation of external surface of PGMA spheres by ethanol solvent. PGMA spheres soaked in the solvent and preheated at 90 °C for a while, and then AgNO₃ ethanol solution was added. During the preheating in ethanol, the external surface of PGMA spheres becomes saturated with ethanol solvent molecules, which has an important effect on the location of the reduction process. The hydrophobic nature of PGMA allows the penetration of ethanol to the surface of the PGMA spheres. Water, however, cannot penetrate the PGMA surface. Step 2: reduction of Ag^+ to Ag^0 . During the synthesis of PGMA spheres by the surfactant-free emulsion polymerization with potassium persulfate as the initiator, the end-capped sulfate groups were partially hydrolyzed to the hydroxyl groups by the reaction with water and hydrogen ions. In the meanwhile, the epoxy groups at the surface of the PGMA spheres were partially transformed to hydroxyl groups under acidic condition and high temperature. The hydroxyl groups of PGMA spheres function as reducing agent, Ag ions are reduced to Ag atoms. And the hydroxyl groups are transformed to carbonyl groups, and finally to carboxylic groups due to the oxidation of the hydroxyl groups by silver ions. Step 3: AgNPs seeds formation and adsorption onto the PGMA sphere external surface. When the concentration of silver atoms reached supersaturation, a situation that drives the system far from the minimum freeenergy configuration, they start to nucleate to form AgNPs seeds. The soften PGMA external surface with carboxylic groups saturated by ethanol is prone to adsorption of the silver nanoparticle seeds. Step 4: the growth of AgNPs. The seeds of the silver nanoparticles attached to PGMA spheres serve as the growing nuclei for the further growth of AgNPs when more Ag ions reach the PGMA surface. The AgNPs grow in size with the coalescence of the formed silver atoms. With an increase in aging time, large-sized silver nanoparticles formed. In the meanwhile, the external surface of PGMA sphere shrinks resulting in the smaller size compared to the original sphere.

The chemical reactions involved are shown in Fig. 6b. The presence of hydroxyl groups on the PGMA spheres can be ascribed to the hydrolysis of sulfate end-capped functional groups and the ring opening of the epoxy groups. The sulfate chain end groups of the PGMA spheres hydrolyzed and transformed to hydroxyl groups firstly. In the meanwhile, the oxygen in the epoxy groups was protonated by hydrogen ions forming more hydroxyl groups. A characteristic peak from hydroxyl group is found at 3,396 cm⁻¹ from FTIR spectrum of PGMA spheres (Fig. 6c). Then redox reaction occurred

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Fig. 7 Antibacterial analysis of PGMA@AgNPs composite spheres in *E. Coli* bacterium. (a) Photograph of bacterial colonies grown in the silver-free LB agar medium as a control; (b) Photograph of bacterial colonies grown in the LB agar medium with 500 μ g/mL PGMA@AgNPs composite spheres; (c, d) Dose-dependent antibacterial activity of PGMA@AgNPs composite spheres shown with its inhibitory effect on bacterial growth; and (e, f) Effect of the size of AgNPs on the antibacterial efficiency of PGMA@AgNPs composite spheres.

between the hydroxyl groups and silver ions. The reduction led to the formation of AgNPs, and the carboxylic groups were formed from the oxidation of hydroxyl groups via intermediate carbonyl groups. FTIR spectrum obtained for the PGMA@AgNPs spheres in Fig. 6c clearly shows the formation of carboxylic groups with two characteristic peaks: 2917 cm⁻¹ for carboxylic acid O-H bond stretching and 1711 cm⁻¹ for the stretching vibration of C=O bond. And the intensity of broad alcohol O-H stretch peak (3416 cm-1) for PGMA@AgNPs is much smaller compared to the hydrolyzed PGMA spheres, which proves the transformation of the hydroxyl groups into carboxylic groups during reducing reaction process of silver ions.

7 The antibacterial activity of PGMA@AgNPs composite spheres

The antibacterial properties of the as-prepared PGMA@AgNPs composite spheres were evaluated with *E. Coli.* strain DH5-Alpha as a model bacterium. Fig. 7a and b display the photographs of bacterial colonies grown when 10^5 of *E. Coli* cells were applied to LB agar plate in the absence and presence of PGMA@AgNPs composite spheres and cultured at 37 °C overnight respectively. The results clearly indicate that the polymer composites with AgNPs totally inhibit microbial growth, leading to a clear plate without any bacteria growth in the presence of 500 µg/mL composite particles. The bacterial growth kinetics was monitored with liquid culture of *E. Coli.* (starting with 10^6 bacteria) in 100 mL LB medium supplemented with different concentrations of the polymer composites coated with AgNPs in different diameters. Fig. 7c and d show the dose-dependent bacteria inhibition effect of the PGMA spheres decorated with AgNPs in the diameter of 10.2 \pm 2.1 nm, which were synthesized with 25 mL PGMA sphere ethanol solution and the precursor AgNO₃ at the final concentration of 39 mM. The higher the concentration of the composite particles in solution, the more effective the inhibition of the bacterial growth. There is no obvious effect of inhibiting bacteria growth with 1 or 10 μ g/mL of the composite spheres. However, when the concentration increases to 100 µg/mL, the bacteria exponential growth stage delays. The bacteria growth is totally inhibited by 500 µg/mL of the composite polymer spheres and above. On the other hand, the size of the AgNPs coated on the polymer spheres could also affect the bacterial inhibition result. The E. Coli bacteria were treated with 150 µg/mL of the composite spheres with AgNPs in different mean diameters of 8.0, 10.2, 13.6, 15.2 and 16.3 nm respectively for 14 h. Fig. 7e and f show the trend of bacteria inhibition effect dependent on the diameter of the coated AgNPs. Compared to the control experiment, the bacteria exponential growth stage delays at any size of AgNPs with 150 µg/mL of the composite spheres. When the diameter of the coated AgNPs reaches to 13.6 nm, the growth of bacteria is completely stopped with 100% inhibition rate. But the antibacterial efficiency decreases when the diameter of the coated AgNPs increases to 15.2 nm, 16.3 nm and above, with the inhibition rate of 97% and 86% respectively. The mechanism for such size-dependent antibacterial property needs further investigation.

Conclusions

In summary, the as-synthesized PGMA@AgNPs composite spheres in a raspberry-like fashion is of high purity since this direct green synthesis method introduces no more reducing agent or stabilizing material into the reaction system except polymer colloids support. The combination of PGMA spheres and ethanol offers a unique approach to manipulate the reduction rate of Ag precursors to produce composite particles decorated with AgNPs in different diameters. Further, the antibacterial activity of the composite particles was found to be dose- and size-dependent. The simple and environmentally benign process and the wide tunability of compositions, and thus the special functions make the as-prepared composite particles promising candidates for applications in biomedicine, catalysis, photonics, and so on.

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Notes

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