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Stable and efficient loading of silver nanoparticles in spherical polyelectrolyte brushes and the antibacterial effects

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ABSTRACT

A more efficient and convenient strategy was demonstrated to immobilize silver nanoparticles (NPs) with a crystalline structure into the spherical polyelectrolyte brushes (SPB) as an antibacterial material. The SPB used for surface coating (Ag immobilized PVK–PAA SPB) consists of a poly(N-vinylcarbazole) (PVK) core and poly(acrylic acid) (PAA) chain layers which are anchored onto the surface of PVK core at one end. Well-dispersed silver nanoparticles (diameter ~ 3.5 nm) then formed and were electrostatically confined in the brush layer. Ag content is controlled by a repeated loading process. Thin film coatings were then constructed by layer-by-layer depositions of positive charged poly(diallyldimethylammonium chloride) (PDDA) and SPB. The multilayer composites display excellent stability as well as antibacterial performance but not for simple PVK–PAA coated surface. The results show that almost complete bacteria growth including both dispersed bacterial cells and biofilms was inhibited over a period of 24 h. This approach opens a novel strategy for stable and efficient immobilization of Ag NPs in fabrication of antibacterial materials.

1. Introduction

Antibacterial surface coatings or modifications have attracted increasing interests in biomedical and industrial fields because of their important applications in diminish of microorganisms on surfaces of medical devices or in drinking water [1–3]. Layer-by-layer (LbL) assemble technique [4,5] as one of the simplest and most popular methods to construct multilayer films [6], has been widely applied in a variety of areas such as catalysis [7], electronic/optical devices [8–10], and biomimetics [11,12]. Since the composition of each layer can be simply altered through LbL assembly, antibacterial or antifouling properties are able to be realized by introducing some effective materials with bactericidal functionalities [13–15].

Among various antibacterial agents, silver nanoparticles are highly favorable owning to their excellent toxicity to a broad spectrum of microorganisms with low cytotoxicity to human cells [15–18]. In order to fabricate silver-containing films, the stability and durability of the deposited films are required. Some researchers are able to deposit Ag NPs onto the surface of films or membranes by certain surface modifications showing good antibacterial effect [3,19], but the NPs are likely to be eluted from the surface because of weak van der Waals interactions. The loss of Ag particles undoubtedly reduces the antiseptic efficiency dramatically as well as increases the environment contamination. One strategy to resolve this issue is to immobilize Ag NPs into the polymer matrix coated on surfaces. *In situ* synthesis of Ag NPs has been successfully implemented to stabilize Ag NPs into multilayer composites where silver ions adsorbed into organic layers were exposed to a reducing reagent and Ag NPs are formed within the layers of the films [20,21]. Moreover, a cap region is also allowed to prevent Ag NPs from eluting away [22]. However, this method needs post-treatments of the

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films or the membranes which brings lots of difficulties to scale up. In addition, the *in situ* reduction of silver ions within solid films leads to broad size distribution of Ag NPs, which may negatively influence the antibacterial performance. As an alternative, *ex situ* preparation of Ag NPs immobilized multiplayer composites allows a simple way to construct well-structured thin films in large scale [15], but preparation of precursor solution containing Ag NPs is indispensable prior to the construction of multilayer films. So the dispersibility, the stability, and the sustainability of such solutions should be well controlled to avoid the aggregation of NPs [23].

Herein we demonstrated the introduction of silver immobilized spherical polyelectrolyte brushes (SPB) for the fabrication of antibacterial multilayer films. The well-dispersed Ag NPs inside the SPB provide a solution to the above concerns. SPB is composed of a spherical polymer matrix and a polyelectrolyte shell, which has become one of the most attractive materials [24–27]. SPB has been shown to be an ideal accommodation for the synthesis of well-dispersed inorganic nanoparticles with narrow size distribution which has been widely applied as reaction catalysts [28–35]. Due to the electrostatic repulsion among SPB particles, the stability significantly improves and the inorganic NPs immobilized SPB are stable for months. In addition, since metallic ions are captured by polyelectrolyte chains as counterions, almost all of the generated inorganic NPs are distributed within the brush layer and are barely eluted from the polymer matrix [28]. Therefore, silver immobilized SPB is expected to be a promising precursor solution for the fabrication of metal incorporated layer-by-layer thin films.

This study presented a way to prepare silver immobilized PVK–PAA SPB followed by fabrication of antibacterial thin films through LbL assembly. Poly(N-vinylcarbazole) (PVK), as a semiconductive polymer [36–38], displays some activity to inhibit the growth of bacteria with very little damage to human cells [39]. Hence PVK was chosen to serve as the core of SPB followed by covalent grafting of PAA shells onto the surface of PVK core by photo-emulsion polymerization. The stability of PVK particles in aqueous solution was significantly enhanced due to electrostatic repulsions. Furthermore, PAA shells were used for silver ion adsorption through electrostatic attractions. The well-dispersed Ag NPs were then encapsulated in the brush layer of PVK SPB by the reduction of silver nitrate precursors. The antimicrobial coating was then achieved by utilizing multifunctional LBL assembly of the as-prepared Ag immobilized PVK–PAA SPB and PDDA. The silver content is tunable by repeated loading of Ag NPs as well as increasing the number of layers. Such coatings show exceedingly excellent bacteria-killing efficiency and long-lasting antimicrobial performance.

2. Experimental

2.1. Materials

N-Vinylcarbazole (VCz, 98%) from Aldrich was purified by recrystallization from methanol. Styrene and acrylic acid (AA) from Lingfeng Chemical Reagent Co. Ltd. were used after distillation under reduced pressure to remove the inhibitor and were stored in the refrigerator. Sodium dodecyl sulfate (SDS, 99%), $K_2S_2O_8$ (KPS, 99%), $AgNO_3$ (99.8%) and $NaBH_4$ (96%) were purchased from Sinopharm Chemical Reagent Co. Ltd (SCRC), and poly(diallyldimethylammonium chloride) solution (PDDA, MW: 100,000–200,000, 20 wt%) from Aladdin Industrial Corporation are used as received. Photoinitiator HMEM was synthesized and characterized as reported previously [40]. Pyridine, 2-hydroxy-4′-hydroxyethoxy-2-methylpropiophenone (HMP) and methacryloyl chloride (MC) were purchased from J&K chemical Co. Ltd, Technical Choices, Inc. and Ciba Specialty Chemicals Inc., respectively. Water was purified with Milli-Q system (Millipore).

2.2. Synthesis of PVK core

0.1 g N-vinylcarbazole was dissolved in 1 ml toluene to form oil phase since N-vinylcarbazole is crystalline solid at room temperature. Then the oil phase was emulsified in 250 ml 0.4 mg/ml SDS solution with vigorous mechanical agitation (700 rpm) for 1 h. After the addition of 0.001 g initiator KPS, the temperature was adjusted to 70 °C. The polymerization reaction continued for 2.5 h under nitrogen atmosphere. At the end of polymerization, the stirring rate was reduced to 300 rpm, and 1 g acetone solution containing 0.1 g photoinitiator HMEM was added at a rate of 6 s per drop (6 s/d). A thin layer of photoinitiator around the PVK core was formed after 2.5 h. Finally the product was purified through dialysis against DI water for three days.

2.3. Synthesis of PVK–PAA SPB

The purified PVK core was charged into a home-made photoreactor. 3 g AA monomers were added into the reactor and mixed with the emulsion under vigorous magnetic stirring. The whole reactor was degassed by repeated evacuation and subsequent addition of nitrogen for three times. Photo-emulsion polymerization was then initiated by a UV lamp (wavelength: 200–600 nm, power: 150 W) at room temperature. After 2.5 h, the obtained PVK–PAA SPB were purified by dialysis against DI water to remove undesired small molecules.

2.4. Preparation of silver immobilized PVK SPB

PVK SPB were employed as nanoreactors to immobilized Ag NPs onto its surface by the reduction of Ag^+ by $NaBH_4$. In a typical run, 100 ml PVK SPB was charged into a three-necked bottle, then 0.034 g $AgNO_3$ was added in the solution with a stirring rate of 300 rpm. Ag^+ ions were thus confined within brush layers as counterions for 2.5 h. The Ag NPs were prepared by the addition of 5 ml 16 mg/ml $NaBH_4$ solution under nitrogen atmosphere. The reduction reaction continued for 1 h and finally the Ag-NPs immobilized PVK SPB was purified through dialysis against DI water to remove unreacted small molecule residues.

2.5. Repeating immobilization of silver nanoparticles onto Ag immobilized PVK-SPB

100 ml of purified Ag immobilized PVK-SPB (marked as 1#) was charged into a three-necked bottle with the addition of $AgNO_3$. The concentration of Ag^+ was adjusted to 2 mmol/L. After stirring for 2.5 h, the Ag^+ immobilized Ag immobilized PVK-SPB was treated with 5 ml 16 mg/ml $NaBH_4$ solution for 1 h. The product was dialyzed marked as 2#. Finally 2# was used to repeat above steps to obtain the triply Ag immobilized PVK-SPB which was marked as 3#.

2.6. Surface coating of Ag immobilized PVK–PAA SPB by LbL assembly

The microscope glass slides were immersed into piranha solution (3:1 H_2SO_4:H_2O_2) at 80 °C for 1 h, and washed with sufficient DI water. Then the sides were dipped into 0.5 wt% PDDA solution for 10 min, rinsed with DI water, dried by air flow, and dipped into 0.5 wt% Ag immobilized PVK–PAA SPB solution for 10 min, rinsing and drying to accomplished one layer coating. The procedure was repeated for ten cycles to achieve ten layers coating.