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Fabrication of antibacterial surface via UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles

Zhang Yuan†, Yongchun Zhao†, Weihu Yang†, Yan Hu†, Kaiyong Cai†, Peng Liu†, b*, Hongyan Ding c

†Key Laboratory of Biorheological Science and Technology of Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, China

bState Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

cJiangsu Provincial Key Laboratory for Interventional Medical Devices, Huaiyin Institute of Technology, Huaiian, Jiangsu Province, 223003, China

*Corresponding author: Fax: +86-23-65102877; Tel: +86-23-65102507, E-mail: liupeng79@cqu.edu.cn

Abstract

In this study, a simple one-step co-deposition process was introduced to fabricate antibacterial surfaces for synthetic materials via UV-inducing dopamine polymerization combined with in situ formation of Ag nanoparticles. The successful formation of a composite coating was demonstrated by scanning electron microscopy. Antibacterial experiments including antibacterial rate test and bacteria adhesion were evaluated in vitro. The results confirmed the excellent antibacterial property of this surface modification process. In conclusion, the approach presented here provides a simple and effective approach to construct antibacterial surfaces for synthetic materials to meet the requirements of daily life and industry application.

Keywords: Surfaces; UV-inducing polymerization; Dopamine; Nanoparticles; Antibacterial property

1. Introduction

Bacterial persistence, attachment, accumulation and colonization on materials surfaces have serious impacts in daily life and industry application [1, 2]. For food packaging, the persistence of bacteria increases the risk of pathogen contamination and reduces the shelf-life of perishable food products. For water purification, biofouling arises from the undesired formation of biofilm on the membrane surface via the adhesion, growth and metabolism of microorganisms, particularly bacteria, which greatly increases the cost of operation and maintenance. Marine equipment is susceptible to bio-corrosion, which is directly associated
with the colonization of indigenous bacteria on surface.

To improve the performance of synthetic materials, one needs to induce them antibacterial property. In this respect, surface modification plays an important role by providing a means to enhance antibacterial property without affecting the bulk attributes of materials.

Recently, a facile surface coating method based on polydopamine (PDOP) has attracted great interest due to its ease and generality, as well as its applicability to almost any substrate [3, 4]. However, the current PDOP coating method exhibits some critical limitations. The main drawback is that the deposition of PDOP coating is a time-consuming process, which may take from 24 hours to a few days (the very slow kinetics of the process). This limits the scope of applications of PDOP coating. Levkin’s group from German and Zhou’s group from China reported respectively that dopamine polymerization can be triggered by light irradiation [5, 6]. Under UV irradiation, dopamine was first oxidized and rearranged/further oxidized into different quinone structures, which finally participated in the polymerization step to form polydopamine [5]. The light-inducing dopamine polymerization can greatly shorten the deposition time of PDOP coating, which takes from 30 minutes to several hours.

Here, we report a new approach to generate antibacterial surfaces on synthetic materials via UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles. Under UV light, dopamine was polymerized to form PDOP coating. PDOP was capable of reducing Ag ions to nanoparticles and stabilizing them, a process involving metal coordination, electron and electrostatic interactions [7]. Ag nanoparticles was formed in PDOP matrix in a very short amount of time (about 10 minutes), without the presence of any other reducing agent.

2. Materials and methods

Microscopic cover glass (1 mm thick, 1 cm×1 cm) was obtained from Jinglun Co. (Shanghai, China). Polyethylene film (0.1 mm thick, 1 cm×1 cm) was supplied by Hongda Ltd. Co. (Jiangxi, China). Stainless steel foil (1.8 mm thick, diameter of 15 mm) was purchased from Fuxin Non-ferrous Metal Co. (Baoji, China).

3, 4-dihydroxyphenylalanine (dopamine) was purchased from Sigma Chemical Co. (MO, USA). Silver nitrate (AgNO₃) was purchased from Shenbo Chemical Co. (Shanghai, China). Firstly, a droplet of 2 mg/mL dopamine hydrochloride tris-buffer solution (pH=8.5) was dropped onto polyethylene film and rotated at a
speed of 2,000 rpm for 20 s. Then, AgNO₃ was deposited by successively spin-coating AgNO₃ solution on the substrates (2000 rpm for 20 s). The procedure was performed according to a previous report [8, 9]. Thus, one cycle deposition contains two layers of dopamine and AgNO₃. The processes were repeated until a desired (dopamine/AgNO₃)ₙ coating was obtained, where n refers to the number of bilayers. AgNO₃ solutions with four concentrations (10, 20, 40, 80 mM) were used in the preparation process respectively. In addition, pure dopamine without AgNO₃ solution was used to fabricate coating. The same procedure was used for other substrates (glass and stainless steel). Furthermore, the samples were subjected to UV radiation at 254 nm for approximately 10 min using a UV-lamp to form PDOP coating with co-deposition Ag nanoparticles. The field-emission scanning electron microscopy (FE-SEM) (Quanta 200, Philips-FEI Corporation, Netherlands) was employed to observe surface morphology of the samples. To quantify the film thickness, dopamine/AgNO₃ multilayer films were deposited on silicon wafers and measured using a spectroscopic ellipsometer (M-2000, Woollam, USA).

After stored for 1 day and 28 days, samples were used for antibacterial test. In this study, Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) purchased from ATCC were used to evaluate the antibacterial effects of the samples. Antibacterial rate test referred to a Chinese Standard GB15979-2002, all samples were exposed to 20 μL (10⁶ cells/mL) bacteria suspension (S. aureus or E. coli) and incubated at 37 °C for 6 h. Then, the samples were ultrasonically detached in 1 mL of PBS solution for 3 min. Next, 100 μL of the bacteria suspension was spread onto agar plates. The bacteria were incubated at 37 °C for 12 h. The number of colonies formed units (CFUs) was counted. The antibacterial rates were calculated based on the following formulas: 

\[ C\% = \frac{(A - B)}{A} \times 100\% \]

Where C indicates antibacterial rate; A is the CFUs of control group; and B is the CFUs of experimental group. Each measurement was performed three times. Native substrates were used as controls.

The bacteria adhesion onto different substrates was observed by using confocal laser scanning microscopy (CLSM, TCS SP5, Leica, Germany). 40 μL bacteria suspension (10⁶ cells/mL, S. aureus or E. coli) was spread onto different substrates and cultured at 37 °C for 6 h. The samples were then rinsed with PBS for 3 times, followed by adding 0.5 mL of glutaraldehyde solution (4 wt%) and incubated at 4 °C for 30 min. The adhered bacteria onto substrates were stained with Hoechst 33258 at 4 °C for 5 min and washed with PBS. Then, the representative photographs were taken with CLSM. Finally, the number of adherent bacteria was
performed by analyzing all bacteria in 6 individual fields of 3 samples per sample type.

3. Results and discussion

3.1. UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles

In this study, UV-assisted PDOP coating and co-deposition Ag nanoparticles were demonstrated on different substrates, namely glass, polyethylene, and stainless steel. The rationale to select glass, polyethylene, and stainless steel as substrates is that they have been widely used in daily life and industrial production. Silver (Ag) nanoparticles has attracted increasing attention due to their strong antibacterial effect towards a broad range of bacteria [10-13]. The metal-binding ability of catechol and amino groups present in the dopamine structure was exploited to complex Ag ions. Ag ions and dopamine were used to fabricate multilayer films through the formation of metal-ligand coordination bonds, which ensure that multiple spin coating steps can be performed. Then, Ag nanoparticles were formed by reduction and located in PDOP matrix under UV light.

![Fig. 1. Thickness of multilayer films as a function of bilayer number.](image)

A spectroscopic ellipsometer was used to quantify the film thickness with increasing numbers of spin-coating steps and different amounts of silver salts. The results were shown in following Fig. 1. For one and two cycles, namely (dopamine/AgNO₃)₁ and (dopamine/AgNO₃)₂, we could not detect the film thickness. It is related to the fact that the substrate surface is not fully covered by dopamine/AgNO₃ during the first several-layer coating. With the increasing numbers of spin-coating cycles (from 3 to 5), the film thickness increased. In addition, for 5 bilayer films, with the increase of AgNO₃ concentration, the film thickness
increased, which attributed to a stable and constant film formed on the substrate. Therefore, five cycles of alternative deposition of dopamine and AgNO₃, namely \((\text{dopamine/AgNO}_₃)_5\), can meet the requirement of fabrication and was chosen as optimized parameter for following experiments. In detail, for \((\text{dopamine/AgNO}_₃)_5\) coating, the film derived from 10 mM AgNO₃, the thickness was 28.39±0.18 nm. The film thickness of 20 mM samples was 36.82±0.20 nm. For 40 mM samples, the film thickness was 45.54±0.24 nm. The film thickness of 80 mM samples was 52.50±0.33 nm.

![SEM images](image)

Fig. 2. SEM images of (a), (f) and (k) native; (b), (g) and (l) 10 mM Ag treated; (c), (h) and (m) 20 mM Ag treated; (d), (i) and (n) 40 mM Ag treated; (e), (j) and (o) 80 mM Ag treated glass, polyethylene, and stainless steel substrates.

The UV-triggered dopamine polymerization and Ag nanoparticles deposition were investigated by SEM. As seen in Fig. 2, the size and the number of the Ag nanoparticles were related to the AgNO₃ concentration. The higher concentration of Ag ions resulted in a higher number of Ag nanoparticles and in larger size. UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles took only 10 minutes because precursors of PDOP and Ag nanoparticles were introduced on substrates via spin-coating method instead of traditional soaking method.

3.2. Antibacterial tests

The antibacterial rates of pure PDOP and PDOP-Ag (10, 20, 40 80 mM) treated substrates were quantitatively determined after 1 and 28 days (Fig. 3). After 1 day, for glass, polyethylene, and stainless steel substrates, we found that 40 and 80 mM Ag was sufficient to show significant killing bacterial effect on S.
S. aureus (antibacterial rates ≥90\%) (Fig. 3a, b and c). According to Chinese Standard GB15979-2002, when the antibacterial rate is equal or greater than 90\%, the materials show antibacterial effect. Meanwhile, Ag with four concentrations (10, 20, 40, 80 mM) showed significant killing bacterial effect on E. coli (antibacterial rates ≥90\%) (Fig. 3a, b and c). After 28 days, for glass and stainless steel substrates, Ag with four concentrations all showed significant killing bacterial effect on both S. aureus and E. coli (antibacterial rates ≥90\%) (Fig. 3d, and f). For polyethylene substrates, Ag with three concentrations (20, 40, 80 mM) showed significant killing bacterial effect on S. aureus (antibacterial rates ≥90\%) (Fig. 3e). Meanwhile, the antibacterial rates of all four concentrations Ag on E. coli was greater than 90\% (Fig. 3e). After stored for 1 day and 28 days, glass, polyethylene, and stainless steel substrates treated by pure PDOP films, showed weak antibacterial effect (antibacterial rates <35\%), which were not high in comparison with PDOP-Ag composite films treated substrates (i.e., antibacterial rates >90\%). The antibacterial property of PDOP-modified substrates could be attributed to the bactericidal property of protonated amine groups of PDOP [14]. Keeping in mind that Ag nanoparticles are potentially toxic in large amounts, the use of a low concentration is preferred. 40 mM Ag was sufficient to show significant killing bacterial effect (antibacterial rates ≥90\%) and used to carry out the further study.

Fig. 3. Antibacterial rates against S. aureus and E. coli: pure PDOP and PDOP-Ag (10, 20, 40, 80 mM) treated glass, polyethylene, and stainless steel substrates after stored for 1 day (a), (b) and (c); 28 days (d), (e) and (f).
Bacteria adhesion on a substrate is the first sequential response when they come into contacting with a material surface, which is crucial for regulation of successive behaviors including proliferation, colonization, and formation of biofilm. Therefore, we investigated the adhesion of S. aureus and E. coli onto native and 40 mM Ag treated polyethylene substrates via CLSM. Large amounts of colonies of S. aureus and E. coli were formed onto the surfaces of native polyethylene substrates (Fig. 4a and c), respectively. Nevertheless, only few colonies were formed onto 40 mM Ag treated polyethylene substrates (Fig. 4b and d). Furthermore, the number of bacteria on samples was measured. After statistical analysis, the number of S. aureus and E. coli on polyethylene substrates without silver ions was 788±48 and 850±71, respectively. While the number of S. aureus and E. coli on 40 mM Ag treated polyethylene substrates was 55±11 and 22±6, respectively. These results suggest that UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles could inhibit the adhesion of bacteria.

Fig. 4. CLSM images of bacteria adhesion onto different substrates: native and 40 mM Ag treated polyethylene substrates cultured with S. aureus (a) and (b); cultured with E. coli (c) and (d).
4. Conclusions

In summary, we fabricated antibacterial surface by UV-inducing dopamine polymerization combined with co-deposition Ag nanoparticles. This co-deposition strategy provides a simple and cost-effective manner, which is suitable for formulation of new types of bactericidal materials. The study provides an alternative approach for the fabrication of antibacterial surfaces on synthetic materials in various fields.

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Reference

Highlights

- Antibacterial surface was fabricated by a simple one-step co-deposition process.
- The modified substrates showed excellent antibacterial property.
- The study affords an efficient method for the fabrication of antibacterial surface.