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Antibacterial zinc oxide hybrid with gelatin coating

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ABSTRACT

ZnO has been widely investigated as important biomaterials and antibacterial materials. However, the aggregation of nanoparticles and its potential toxicity may hinder its final application. Herein, biocompatible gelatin chains were grafted on the surface of ZnO via mussel inspired method to prevent the aggregation of the ZnO nanoparticles. The in vitro test showed that the gelatin can greatly improve the biocompatibility of ZnO, while the antibacterial properties of ZnO against both *E. coli* and *S. aureus* were maintained.

1. Introduction

Various bacterial infections have been serious problems in clinics, and thus more and more antibiotics or antibacterial peptides have been explored [1]. However, the multi-drug resistance limits the progress of conventional antibacterial agent [2]. Inorganic nanoparticles, due to their excellent properties, have shown much applications in optics, electronics, energy storage, controlled delivery and many other biomedical fields [3–7]. Ag [8], CuO [9], TiO_2 [10], ZnO [11], carbon dots [12] and many other inorganic nanoparticles with antibacterial properties have aroused much attention [13].

ZnO has been widely applied in optical devices [14], sensors [15], imaging [16,17], drug delivery [17,18] and antibacterial fields [19]. Furthermore, ZnO can also works as functional nanofillers in polymer composites. However, its application has been hindered by limited dispersibility and surface modification techniques [20].

Up to now, many methods, such as ring opening polymerization [21], atom transfer radical polymerization [20,22] have been used to tune its surface properties and improve its optical, dispersion and biocompatible properties. Various polymer chains have been connected to the surface of ZnO via “grafting to” or “grafting from”, for example, Poly(*N*-isopropylamide) was grafted on the surface of ZnO to realize its thermal responsive properties and can works as a smart drug carrier [23], poly(methyl methacrylate) was tethered on the surface of ZnO to tune the composites' optical and dielectric properties [22], Poly(lactide) was graft on the surface of ZnO to tune its dispersion and interaction with polymer matrix and thus to improve its mechanical properties [24]. Although much progress has been achieved to graft polymer chains on the surface of ZnO, much more simple or green modification method is still needed. Furthermore, whether the surface grafted polymer brush may have some effect on its biocompatibility or antibacterial properties should be addressed.

Inspired by the adhesion proteins of mussel, Messersmith group proposed a universal surface modification method by dip-coating of objects in the aqueous solution of dopamine [25]. Due to the self-polymerization of dopamine, polydopamine can be formed on various surface, furthermore, the polydopamine can react with various molecules. This green method has been widely used to graft different molecules on various substrates and realize the functionalization of different materials.

In this work, gelatin, a biocompatible polymer, was grafted on the surface of ZnO to form a new hybrid ZnO@Gelatin via mussel inspired method (Scheme 1). Firstly, ZnO nanoparticles were treated with dopamine to form a layer of polydopamine on its surface, and then gelatin was grafted via the reaction between gelatin and polydopamine. The optical and in vitro test showed that the surface modification by gelatin may increase the biocompatibility of ZnO, in addition, the new hybrid also show good antibacterial properties, which have similar antibacterial ability as ZnO, showing its potential application as a biocompatible antibacterial agent.

2. Experimental section

2.1. Materials

Zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) was purchased from Sigma Aldrich. KOH was purchased from Tianjin Damao Chemical Reagent Factory. Methanol and ethanol absolute were purchased from

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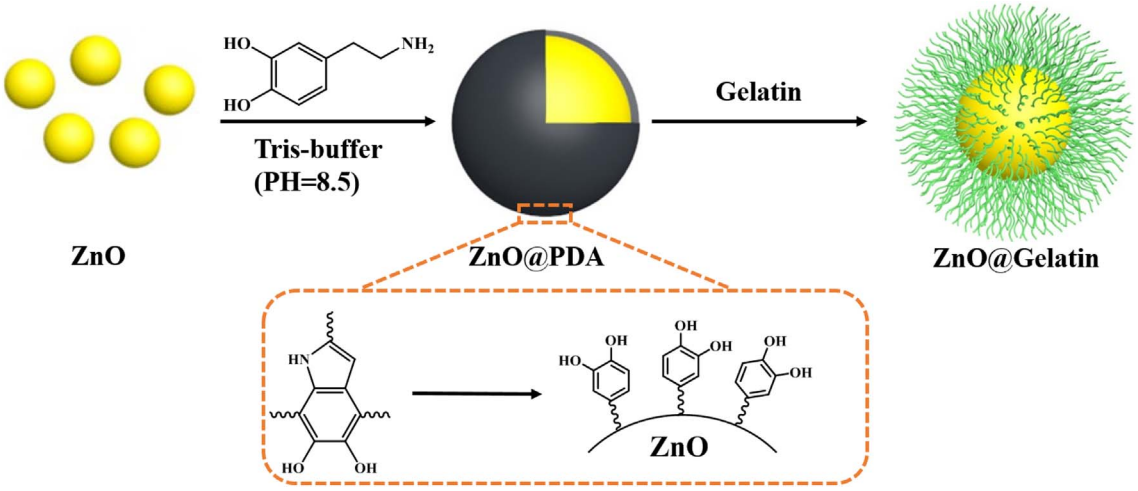

Scheme 1. Synthesis route of zinc oxide with gelatin coating.

XiLong Sciences Ltd. Dopamine, tetrabutylammonium bromide and tris (hydroxymethyl)aminomethane(Tris) were obtained from Energy Chemical Reagent Co. Ltd. Gelatin is purchased from Sinopharm Chemical Reagent Co. Ltd. Nutrient broth, nutrient agar, agar-agar used for antimicrobial studies were purchased from Hangzhou basebio Biotechnology Co., Ltd. PBS Phosphate buffer are purchased from Beijing Solarbio Technology Co., Ltd. All chemicals are used without further purification.

2.2. Synthesis of ZnO NPs

ZnO NPs were synthesized by the modified sol-gel route [26]. Briefly, 2.75 g of $Zn(CH_3COO)_2{\cdot}2H_2O$ and 4.02 g of tetrabutylammonium bromide were added into 150 mL of ethanol. The mixture was refluxed for 1 h, and then 5 mL of KOH (1.7 M) ethanol solution was added. 12 h later, the white precipitates were collected by centrifugation, and washed several times with methanol.

2.3. Preparation of gelatin-functionalized ZnO (ZnO@Gelatin)

ZnO NPs was dispersed in 10 mM Tris-buffer solution (pH = 8.5), and then dopamine (2 mg/mL) was added into suspensions under stirring. After 2 h, polydopamine coated ZnO (ZnO@PDA) were collected by centrifugation, and then washed with demonized water for several times.

ZnO@Gelatin was prepared via the reaction between gelatin and ZnO@PDA. Briefly, gelatin was added into the ZnO@PDA suspensions and stirred at 35 °C for 24 h. The final product ZnO@Gelatin were obtained by centrifugation and washed with water.

2.4. Characterizations

Fourier-transform infrared (FT-IR) spectra were recorded on a Fourier-transform infrared spectrophotometer (Shimadzu IR Prestige-21, Nakagyo-ku, Japan) using potassium bromide (KBr) disks. X-ray photoelectron spectroscopy (XPS) was measured on a X-ray photoelectron spectroscopy (Thermo-VG; ESCALAB 250) with an Al Kα radiation X-ray source. Morphologies and microstructures of different samples were investigated by transmission electron microscopy (TEM, Tecnai G20, FEI, USA). The crystalline structure was measured by X-ray diffraction (XRD, Mini Flex 600, Japan). Thermal gravimetric analyses (TGA) were carried out under N_2 atmosphere from room temperature to 800 °C at a heating rate of 10 °C/min with a thermogravimetric analyzer (Netzsch STA409PC, Selb, Germany). The UV–Vis spectra and photoluminescence spectra were measured by UV–Vis spectrophotometer (Lambda 750 s, PerkinElmer, USA) and Fluorescence spectrometer (Shimadzu, RF-5301 PC, Japan), respectively. The zeta potential and particle size were measured by a High Sensitive Zeta potential and particle size analyzer (Brookhaven, 90Plus PALS, USA).

2.5. Cytotoxicity tests

MTT assay was used to test the cytotoxicity of different samples against 3T3 cells by adapting a previously described method [27]. Briefly, Different amounts of ZnO, ZnO@PDA and ZnO@Gelatin were dispersed in cell culture medium (DMEM) and the concentration of each samples were set to 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0 mg/mL, respectively. After 24 h, the solid samples were removed from the culture medium, which were used for cell culture.

3T3 cell were suspended in DMEM supplemented with 10% (v/v) FBS at a density of 10^4 cells/mL, and 100 μL of cell suspension solution was pipetted into 96-well plates. After incubation at 37 °C under 5% CO_2 atmosphere for 24 h, the medium was replaced by the previously treated DMEM culture medium. After 24 hour incubation, the optical density (O.D.) was read on a multi-well microplate reader at 630 nm. The cytotoxicity for each sample was tested in triplicates. Cells cultured with fresh DMEM were used as Control.

2.6. Antibacterial assays

The antibacterial activities were studied by the bacterial growth kinetic analysis according to reference's method [28]. Briefly, in an aseptic condition, 80 μL of respective bacteria culture was diluted to 8 mL using nutrient broth, followed by growth till mid log phase at 37 °C. And then, 100 μL of bacterial mother cultures were added to 3 mL of nanoparticles suspensions at different concentrations (16, 25, 50, 100, 250 and 500 μg/mL), and then cultured at 37 °C, after a required time, the optical density (O.D.) at 600 nm of each sample were measured by UV–Vis spectrophotometer. Culture without samples was taken as positive control. Each test was measured in triplicates.

3. Results and discussion

3.1. Graft of gelatin onto surface of ZnO

When ZnO nanoparticles were dispersed in dopamine solution, due to the self-polymerization of dopamine, a thin layer of poly(dopamine) was coated on the surface of ZnO, and then, the gelatin was grafted on the surface of ZnO via the reaction between gelatin and polydopamine.