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Mussel-inspired superhydrophobic surfaces with enhanced corrosion resistance and dual-action antibacterial properties

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ABSTRACT

In this study, a multilayer antibacterial film was assembled onto 316L stainless steel via mussel-inspired depositions of polydopamine (PDA) and silver (Ag) nanoparticles followed by post-modification with 1H, 1H, 2H, 2H-perfluorodecanethiol. The resulting surface exhibited excellent superhydrophobicity with hierarchical micro/nanostructures that were constructed by both PDA and Ag nanoparticles. The crystal structure and chemical composition of these surfaces were investigated using X-ray photoelectron spectroscopy (XPS) analysis. Potentiodynamic polarization measurements revealed that the corrosion resistance of the as-prepared surfaces were sequentially increased after each step of the fabrication process. Compared with the surface covered with only Ag nanoparticles, the superhydrophobic surfaces exhibited substantially enhanced antibacterial activity against the Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, resulting from the synergistic antibacterial actions of the superhydrophobic surface and Ag nanoparticles. The superhydrophobic surface exhibited lower cytotoxicity, compared to the surface covered with Ag nanoparticles.

1. Introduction

Bacterial adhesion has been long identified as a notable threat in the medical device industry, and it can cause serious infections and even device failure. To solve this problem, a great deal of attention has focused on the research and design of antibacterial surfaces. As one of the oldest antibacterial materials, the use of antibiotics has been reduced because it can pollute the environment and promote bacterial resistance [1,2]. Another major class of antibacterial materials have been developed based on the incorporation of metallic particles of silver, copper or zinc [3–5]. Among them, the metallic silver (Ag) nanoparticles have been widely used to develop antibacterial materials, with excellent abilities to kill bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6–11]. The metallic Ag nanoparticles can release Ag ions which destroy the permeability and respiration functions of cell membranes and attack the DNA replication progress, leading to cytoplasm leakage and even cell death [12].

Superhydrophobic surfaces are another effective method for antibacterial applications. Inspired by the lotus leaf and striders in nature, superhydrophobic surfaces have water contact angles >150° and water sliding angles <10°. A superhydrophobic surface is typically obtained by combining hierarchical micro/nanostructures and low surface energy materials [13–15]. When in contact with water, an air film can be trapped within the micro/nanostructures on the superhydrophobic surfaces. After decades of exploration, superhydrophobic surfaces have been found to have great potential in applications such as self-cleaning [16], anti-dragging [17], anti-icing [18], water-collecting [19], and anti-corrosion [20]. Recently, superhydrophobic surfaces have also been also proposed for usage in antibacterial applications [21–24]. For example, Privett et al. prepared a superhydrophobic xerogel coating by combining nanostructured silica colloids and fluorinated silane [24]. The results revealed that the adhesion of *S. aureus* and *P. aeruginosa* were reduced by ~2 orders of magnitude vs. the control surface. The unique properties of superhydrophobic surfaces can effectively reduce bacterial adhesion rather than directly killing the bacteria [25–26]. This antibacterial property mainly results from the reduction of protein adhesion due to the air film between the bacterial cells and material surfaces [27].

Superhydrophobic surfaces can be used for antibacterial applications as a new strategy but also suffer from the problem of weak durability [28]. The easily destroyed micro/nano structures and the surface chemistry lead to a loss of the superhydrophobic surface and lead to bacterial colonization on the wetted surfaces. Therefore, other antibacterial materials have been incorporated into superhydrophobic surfaces in

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order to prolong the antibacterial performance. A potential strategy has been proposed to introduce antibacterial metallic nanoparticles into the micro/nanostructures of the superhydrophobic surfaces [29–30]. Heinonen et al. prepared superhydrophobic surfaces based on a roughed alumina layer with and without Ag nanoparticles on austenitic stainless steel AISI 304 2B surfaces [31]. The antibacterial test results indicated that the superhydrophobic silver-containing surfaces exhibited better antibacterial properties compared with those without Ag nanoparticles. The number of viable bacteria on silver-containing surfaces was an order of magnitude lower than that on a surface without Ag nanoparticles. In recent years, some studies have utilized Ag nanoparticles to directly construct superhydrophobic micro/nanostructures [32,33]. The superhydrophobic surface has been demonstrated to be able to control the release of Ag ions, thereby improving the sterilization efficiency of Ag ions and enhancing the antibacterial effect.

In this work, a multilayer superhydrophobic antibacterial film was assembled onto 316L stainless steel via mussel-inspired sequential depositions of polydopamine (PDA) nanoparticles and Ag nanoparticles, followed by hydrophobic post-modification. The micro/nano hierarchical morphology required for surface superhydrophobicity was obtained by both PDA and Ag nanoparticles. The corrosion resistance of 316L SS surface after each step of the assembling process was investigated by potentiodynamic polarization measurements. To evaluate the antibacterial performance, adhesion of Gram-positive bacteria (*E. coli*) and Gram-negative bacteria (*S. aureus*) onto bare steel, the Ag-deposited surface, and the superhydrophobic Ag-deposited surface were compared. The coupled effects of surface superhydrophobicity and Ag nanoparticles on the antibacterial activities were revealed from multiple aspects including not only the biofilm thickness and coverage on the surfaces, but also the optical density and concentration of the released Ag ions in culture medium to study the bactericidal effect on planktonic bacteria cells. The cytotoxicity of the Ag-deposited surface and the superhydrophobic Ag-deposited surface were also compared by CCK-8 assay.

2. Experimental section

2.1. Materials

The 316L stainless steel was cut into specimens with a size of 10 mm × 10 mm × 3 mm, which were used as the substrates. Tris (hydroxymethyl) aminomethane and dopamine hydrochloride were purchased from Aladdin Industrial Corporation. Silver nitrate ($AgNO_3$), ethanol and acetone were purchased from Sinopharm, and 1H, 1H, 2H, 2H-perfluorodecanethiol (PFDT) was obtained from Sigma–Aldrich. All chemicals were used as received and without further purification.

2.2. Preparation of superhydrophobic surfaces

The 316L stainless steel specimens were polished until 1500 grit by abrasive papers, followed by sequential cleaning with acetone, ethanol and deionized water in an ultrasonic bath, and then were dried at room temperature. The steel specimens were immersed in a Tris–HCl buffer solution (10 mM, pH = 8.5) containing dopamine hydrochloride at a concentration of 2 mg/mL, then the solution was mechanically stripped for 24 h. The samples were washed with deionized water after being removed from the solution, and were then immersed in $AgNO_3$ solution (5 mg/mL) for 5 h. After being removed from the $AgNO_3$ solution, the samples were cleaned with ethanol and then were immersed in sealed ethanol solution containing PFDT at a volume ratio of 500:1, followed by drying at room temperature. Before the final immersing process, the ethanol solution was purged with nitrogen for 20 min. The sample surfaces after the separate assembly processes were hereinafter named as BS (bare surface), PDS (PDA-deposited surface), ADS (Ag-deposited surface) and SS (superhydrophobic surface).

2.3. Surface characterization

The morphology of the as-prepared surfaces were observed using a field-emission scanning electron microscope (FE-SEM, FEI Quanta 250) and an atomic force microscope (AFM, Bruker, Multimode). The surface chemistry compositions were characterized by X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi). The wettability of the surfaces was measured by a goniometer (Dataphysics OCA20). The static water contact angles (WCA) were measured by the sessile drop method with a 4 μL water droplet on the as-prepared surfaces at room temperature. The surfaces were tilted until the 10 μL water droplet just rolled off, and the inclined angles were recorded as the sliding angles (SA).

2.4. Electrochemical measurements

The anticorrosion performance of these samples were compared by potentiodynamic polarization measurements, which were performed on an electrochemical station (PARSTAT 2273) in 3.5 wt% NaCl solutions at room temperature. The measurement system adopts the conventional three-electrode system consisting of the as-prepared samples as the working electrodes, the platinum electrode as the counter electrode and the saturated calomel electrode (SCE) as the reference electrode. The open circuit potential (OCP) were measured for 30 min until stable, followed by performing the polarization curves with a sweep rate of 0.5 mV/s.

2.5. Antibacterial activity tests

The *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) strains were obtained from the Institute of Metal Research, China Academy of Science, Shenyang, China. The *E. coli* and *S. aureus* strains were cultured in Lysogeny broth (LB) solid medium and incubated at 37 °C overnight in an incubator. The overnight solid bacterial culture was diluted by phosphate buffer saline (PBS) and then inoculated into the LB broth. The concentration of the bacteria in broth was approximately 10^6 cells mL^{-1}. Subsequently, the samples were placed into 24-well plates with 2 mL of the diluted bacterial suspension, then the plates were incubated at 37 °C for 1 or 3 days, respectively. After incubation, the samples were taken out and washed with phosphate buffer saline (PBS) 3 times to remove the planktonic bacteria on the surfaces.

2.6. Morphological characterization of bacteria

After being incubated for 1 or 3 days, the samples were washed by PBS and fixed with a glutaraldehyde solution for 10 h, followed by continuous dehydration in 50, 60, 70, 80, 90, 95 and 100% ethanol (v/v) for 10 min. The morphology of the bacteria on the treated samples was observed under a scanning electron microscopy (SEM, FEI Quanta 250). The optical density at 600 nm (OD_{600}) values of the incubation solution after 1 or 3 days were measured with an ultraviolet spectrophotometer (Bio Mate3S, Thermo Fisher).

2.7. Live/dead biofilm staining

The samples were washed with PBS after being taken out from the bacterial culture media and were air dried in the super clean bench. Subsequently, the air-dried samples were stained in dark with SYTO-9 and PI (propidium iodide) dyes in PBS for 20 min. The live/dead staining tests were observed under a confocal laser scanning microscopy (CLSM) (Model C2 Plus, Nikon, Tokyo, Japan) as described in previous works [34,35]. In 3D CLSM images, the live bacteria cells were stained as green fluorescence while dead cells were stained as red fluorescence. The biofilm thicknesses were also measured in 3D CLSM images. The biofilm coverages were calculated by ImageJ software (National Institutes of Health, Bethesda, MD, USA).