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Bioinspired and biocompatible carbon nanotube-Ag nanohybrid coatings for robust antibacterial applications

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ABSTRACT

The design of self-sterilizing surfaces with favorable biocompatibility is acknowledged as an effective approach to deal with the bacterial infections of biomedical devices. In this study, we report an intriguing protocol for the large-scale fabrication of self-sterilizing and biocompatible surface film coatings by using polymer shielded silver nanoparticle loaded oxidized carbon nanotube (AgNPs@oCNT) nano-dispersions. To achieve the antibacterial coatings, the bioinspired positively charged and negatively charged AgNPs@oCNTs were alternately deposited onto substrates by spray-coating assisted layer-by-layer assembly. Then the bacterial inhibitory zones, optical density value monitoring, bacterial killing efficiency and adhesion were investigated; and all the results revealed that the AgNPs@oCNTs thin film coatings exhibited robust and long-term antibacterial activity against both Gram negative and Gram positive bacteria. Moreover, due to the shielding effects of polymer layers, the coatings showed extraordinary blood compatibility and limited toxicity against human umbilical vein endothelial cells. It is believed that the proposed large-scale fabrication of bactericidal, blood and cell compatible AgNPs@oCNT based thin film coatings will have great potential to forward novel operational pathogenic inhibition strategies to avoid undesired bacterial contaminations of biomedical implants or biological devices.

Statement of Significance

Bacterial infection of medical devices has been considered to be a world-wide clinical threat towards patients' health. In this study, a bioinspired and biocompatible antibacterial coating was prepared via the spray-assisted layer-by-layer (LbL) assembly. The silver nanopartilces loaded oxidized carbon nanotube (AgNPs@oCNT), which were coated by functional polymers (chitosan and synthetic heparin mimicking polymers), were prepared via mussel inspired chemistry; and the spray-assisted assembly process allowed the fast construction on devices. Owing to the antibacterial efficiency of the loaded AgNPs, the coating showed robust bacterial killing activity and resistance towards bacterial adhesion. Moreover, since that the AgNPs were shielded by the polymers, the coating exhibited no clear toxicity at blood or cellular level. Benefiting from the universal and large-scale fabrication advancements of the spray assisted LbL coating; it is believed that the proposed strategy can be applied in designing many other kinds of self-sterilizing biomedical implants and devices.

1. Introduction

Bacterial infections have long been considered as an universal problem in hospital treatment, and have caused millions of fatalities every year [1]. In the past decades, antibiotics have been widely used to fight the bacterial infections. Even though considerable successes have been achieved, the misuse of antibiotics has also brought about abundant unexpected consequences, like

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damaging patients' immune system and inducing drug-resistance for bacteria. Researches have demonstrated that most frequently bacterial infections, such as urinary tract, respiratory tract and bloodstream infections, are associated with the bacterial contamination of biomedical implants or devices. Bacterial adhesion and colonization on material surfaces and the subsequent biofilm formation are regarded as the chief culprits for the undesired infections [2]. The current disinfection approaches, such as the use of alcohol or ultraviolet rays, cannot provide long-term antibacterial activities. Therefore, designing self-sterilizing surface with long-term antibacterial activity has been recognized as an efficient strategy to cope with this issue.

Silver metal and compounds exhibit robust wide-spectrum bactericidal activity. Attempts on coating silver in form of ions or nanoparticles on biointerfaces have made significant progresses. As one of the most popular approaches, silver nanoparticles (AgNPs) have been embedded into polymer matrix to form antibacterial coatings [3,4]; while, in this strategy, the intense polymer layer will greatly suppress the antibacterial activity for AgNPs. Moreover, the aging of polymeric coatings will lead to the leaching of AgNPs and then decrease the long-term antibacterial efficiency of the coatings. The combination of AgNPs with other nanostructures has made significant progresses for bacterial control in recent years. Silica nanowires [5], zeolite clay [6], titanium dioxide nanoparticle [7], and many other synthetic nano architectures [8,9], have been used as the supporting matrixes for AgNPs. Employing nano-compounds to load AgNPs for bacterial-resisting applications presents great advantages to facilitate the distribution of AgNPs at the interface.

Carbon nanomaterials, especially the oxidized derivatives of CNT and graphene, are versatile nanoscale building blocks that have demonstrated great promise in biomedical applications, such as tissue engineering, drug delivery, and bio-imaging [10–12]. For AgNPs loading, they are favorable matrices because of their well-defined structures and large surface areas. Some superior antibacterial agents have been obtained by anchoring AgNPs onto oxidized CNT (oCNT) or graphene oxide (GO) surface. Due to the increased surface area and improved colloidal stability, the composites even exhibited better antibacterial activity than bare AgNPs [13–17]. For instance, Mohajerzadeh et al. successfully immobilized AgNPs on CNT arrays and achieved long-lasting efficient killing towards bacteria [17]. As coating materials, 2D AgNPs/GO nanohybrids might yield dense and flat films on materials surface, which were not good for the release of Ag^+. Using nanofibrous AgNPs/oCNT nanohybrids for surface coating was advantageous to create porous pathway and facilitate the locating of AgNPs at the interface. For example, by immobilizing AgNPs/oCNT nanocomposite on ultrafiltration membrane, the modified membrane exhibited porous surface structure and greatly improved antibacterial capability compared to the bare membrane [18,19].

Although that the AgNPs based composites have showed great promise to control bacterial infections, the safety considerations about using AgNPs have been raised recently due to their potential toxicity at cellular level. The direct contact between AgNPs and cell membrane is reported to increase the reactive oxygen species (ROS) to toxic level. Furthermore, the slow oxidation and corrosion of un-protected AgNPs in physiological environment will also increase the concentration of highly cell toxic Ag^+ ions [20,21]. Designing protective coating to shield the exposure of AgNPs and to control the release of Ag^+ ions is developed to address the toxicity problem of AgNPs. For example, Travan and co-workers noticed that chitosan shielded AgNPs did not show any significant *in vitro* cytotoxicity towards eukaryotic stem cells and primary cell-lines [22]. Krishnan et al. also found that PEG shielded AgNP showed no toxicity to endothelial cells and smooth muscle cells [23]. Therefore, for AgNPs@oCNT nanocomposite, a shielding layer was needed to regulate the mammal cell toxicity and to control the release of Ag^+ ions. In the studies by Chaudhari et al., PEG and antimicrobial peptide were applied to achieve polymer shielding on AgNPs/CNT composite. Interestingly, they found that the polymer coated AgNPs/CNT composite showed no toxicity towards eukaryotic cells at low concentrations but was sufficient to inhibit various types of bacteria, such as *Escherichia coli* (*E. coli*), *Salmonella Typhimurium*, *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* [24,25]. In our earlier study, we have proposed a facile and green approach to prepare polymer layer coated AgNPs@oCNT nanocomposite with no obvious cell toxicity [26]. By using mussel inspired polymers, anionic polymers shielded AgNPs@oCNT nanocomposite was synthesized. Compared with the state-of-the-art studies, our mussel inspired antibacterial and biocompatible AgNPs@oCNT nano-dispersions exhibit extremely high stability in psychological solutions, the fabrication process is facile, cheap, and universal; therefore, we believe this novel nanocomposite may serve well as an antibacterial reagent for the surface modification of diverse biological materials or implants.

In this study, we designed a self-sterilizing surface with favorable biocompatibility to efficiently control the potential bacterial infections of biomedical implants or biological devices. Firstly, we applied the mussel-inspired polymer coating strategy to synthesize negatively charged and positively charged nano-dispersions of AgNPs@oCNT, as described in our earlier study [26]; then, multilayer thin film coatings of AgNPs@oCNT were prepared via a spray-assisted layer-by-layer (LbL) assembly. The antibacterial efficiencies of the thin film coatings were systemically investigated in terms of bacterial inhibitory zones, optical density monitoring, bacterial killing ratios and bacterial adhesion. The biocompatibilities at both blood and cell levels were also evaluated in detail. It was verified that the AgNPs@oCNT coatings exhibited robust antibacterial activity, long-term stability, good blood compatibility and limited endothelial cell toxicity. The proposed blood and cell compatible AgNPs@oCNT coatings may have great application potential for the surface modification of diverse biomedical implants and biological devices to avoid undesired bacterial contaminations.

2. Materials and methods

2.1. Materials

Sodium methacrylate (MAANa, 99%), sodium 4-styrenesulfonate (SSNa, 90%), silver nitrite ($AgNO_3$, 99.9%), chitosan (100–200 mPa·s, degree of deacetylation >95%) and polyvinylidene fluoride (PVDF) membrane (M267) were purchased from Aladdin reagent Co. Ltd. (China). Polyethyleneimine (PEI, Mw ~70000), hydrocaffic acid (99%), 4-dihydroxyphenethylamine (Dopamine, DA, 99%) and 4,4′-azo-bis(4-cyanovaleric acid) (99%) were purchased from Sigma Aldrich (USA). The rest agents, if not mentioned specifically, were purchased from Aladdin reagent Co. Ltd. (China) and used as received. Carbon nanotube (multiple walls, outside diameter 8–15 nm, length 15 μm) were obtained from Times Nano. Ltd. (Chengdu, China).

2.2. Bioinspired preparation and characterization of AgNPs@oCNT

The synthesis of mussel inspired polymers, including dopamine grafted sulfonated heparin-like polymer (DASHP) and chitosan (DACS), was performed as reported in our earlier studies, which were also presented along with the characterizations in Figs. S1–S4 (Supporting information) [26–28]. The oCNT was obtained by treating CNT with H_2SO_4/HNO_3 (3/1, v/v). 1 mL 0.1 M $AgNO_3$ solution was then added into 20 mL 4 mg/mL oCNT solutions