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Ruthenium complexes/polypeptide self-assembled nanoparticles for identification of bacterial infection and targeted antibacterial research

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ABSTRACT

Bacterial infection has been a threat to human health, and so early diagnosis and treatment of bacterial infection is an urgent problem that needs to be solved. In this work, a multifunctional theranostic selenium nanoplatform (Se@PEP-Ru NPs) with early imaging diagnosis and efficient treatment of bacterial infections was designed and constructed. First, the antibacterial peptide UBI29-41 (PEP) was linked to functionalized Selenium nanoparticles (NPs), which enhanced the stability of the antimicrobial peptide and also caused the nanocomposites to specifically target bacterial infection. Ruthenium complexes with good antibacterial activity and fluorescence properties were then coated on to their outer layers. It was worth mentioning that, when the resulting nanoprobe was injected into mice by intravenous injection it was found to be sensitive to sites of bacterial infection for selective fluorescence imaging and targeted therapy. Thus, it can be used to distinguish between bacterial infection, inflammation, and tumor-induced tissue infection with high specificity. In the further antibacterial activity experiments, Ruthenium complexes showed synergistic antimicrobial activity with Se NPs, which indicated that the antibacterial activity of Se@PEP-Ru NPs was the strongest that could promote wound healing. Thus, Se@PEP-Ru NPs appears to be a promising antimicrobial with good biocompatibility, excellent selectivity, and potent antimicrobial activity.

1. Introduction

Antibiotics are the most widely used method for the treatment of infectious diseases caused by pathogens. Unfortunately, high-frequency use of antibiotics has promoted multidrug resistance and reduced the efficiency of antibiotic therapies [1–3]. In order to cope with this phenomenon, the nanoprobes with rapid diagnosis and efficient sterilization have become a hot research topic in recent years. Nanometer-sized particles have the same size range as antibodies, nucleic acids, and proteins in biomolecules [4]. They also have high surface biomimetic function and volumetric ratios, and their properties can be readily modified. This makes NPs a powerful tool for imaging, diagnosis, and therapy [5–7]. Nano-sized antimicrobial materials, e.g. silver, zinc oxide, and other NPs, have become potential candidates for the treatment of drug-resistant bacterial infections [8–10]. In many cases, such materials are still in the research stage due to their unknown mechanism of action. However, nano-selenium is recognized as a safe and beneficial nanoparticle [11]. Selenium NPs, in addition to excellent performance as a nanomaterial, are also having good biocompatibility and low toxicity [12,13]. There are currently only a few reports on the antimicrobial activity of selenium nanomaterials. Webster et al., for example, found that selenium NPs can inhibit the growth of *Staphylococcus aureus in vitro* [14]. In addition, functionalized nano-selenium synthesized by our group have also been shown to have antibacterial activity [15]. However, nano-selenium particles on their own cannot achieve an efficient targeting effect towards bacteria. Multi-functional composite nanomaterials, on the other hand, have been shown to have the potential to establish new combinations of therapeutic drugs for use in targeted drug delivery, imaging diagnosis, and highly effective sterilization. This can greatly improve the efficiency of the treatment and minimize the damage to normal tissues [16–18]. Therefore, for this study, we chose nano-selenium to use as a core loaded with fluorescent and targeting substances, to form a new antibacterial agent with both diagnostic and antibacterial functions.

In addition to opposing bacterial resistance, early and rapid diagnosis is also important for efficient treatment of bacterial infections [19]. The traditional methods presently available (e.g.

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radioactive imaging and urine examination, etc.) are rather limited due to safety concerns and practicability issues with respect to early detection of bacterial infection [20–22]. A simple optical imaging diagnostic technique (fluorescent-probe marker imaging) has already been used for the diagnosis of bacterial infections in the study [23–26]. Kong et al. designed an activatable probe for detecting bacterial infections *in vivo*. Their approach was to have a fluorescent group in the probe which cleaves when the probe interacts with bacterial endogenous enzymes. During this process, a fluorescent signal was emitted which could be used to achieve the diagnostic effect [27]. Thus, fluorescence imaging is a promising novel optical imaging diagnostic technique that can target the site of bacterial infection for detection purposes [28]. Ruthenium (Ru) complexes have been widely used in the detection and imaging of tumors and other diseases due to their high luminous efficiencies and intensities which make them good fluorescent probes [29–31]. In this paper, we screen the antibacterial activity of several Ru complexes (previously synthesized by our research group) and identify those with good antimicrobial activity. The Ru complexes used as the fluorescent imaging probe *in vivo* in this study not only had excellent fluorescence performance but also a bactericidal effect. To the best of our knowledge, this is the first example of Ru complexes acting as antimicrobial and bacterial imaging agents.

However, most fluorescent agents lack targeting properties and will not accumulate at lesion sites after entering an organism, so it is not sufficient to use a fluorescent imaging agent on its own for the detection of disease. Rather, it is necessary to increase targeting to enhance the detection capability of the fluorescent agent. In this regard, antibacterial peptides are a good choice. In many studies, it has been found that antimicrobial peptides [32–34] have the effect of targeting bacteria and bacterial-induced infection. Unfortunately, these peptides are usually toxic to human cells and easily decompose in the body, thus limiting their application [35,36]. $UBI_{29\text{-}41}$ (TGRAKRRMQYNRR, 1693Da) is a synthetic antimicrobial peptide fragment that has the ability to target bacterial infection and specifically accumulate in bacteria [37–39]. Thus, it is a good candidate to use as part of a targeted fluorescent probe with antimicrobial effect by combining it with a Ru complex. In order to solve the problems associated with instability and toxicity, it may be then conjugated with Se NPs in order to prepare a functional composite nanoprobe. The resulting nanoprobe has good biocompatibility and stability. Moreover, the antibacterial activity is enhanced via a synergistic effect.

In this study, we use a simple method to prepare nanoprobes with excellent fluorescence properties and great potential for biomedical imaging theranostic applications. First of all, Se NPs containing bovine serum albumin was prepared. This not only reduces the size of the composite NPs but also increases their stability and biocompatibility. Then, antibacterial peptides PEP and Ru complexes were modified on the surface of the Se NPs. Such modifications make full use of the characteristics of the three parts. Thus, rapid and accurate diagnosis and treatment of bacterial infections can be achieved. The results of our experiments show that Se@PEP-Ru2 NPs is able to identify and distinguish bacterial infection from inflammation and tumor-induced tissue infection with high specificity. It also has excellent antibacterial activity and can promote the ability of an infected wound to heal. Thus, we have successfully developed a new type of nanoprobe capable of selectively imaging bacteria and with high antimicrobial properties. This expands upon previous applications of Se NPs, and reduces the use of antimicrobial agents to reduce drug toxicity. It thus has the potential to be used as a substitute for antibiotics in clinical applications.

2. Materials and methods

2.1. Materials and reagents

Sodium selenites (Na_2SeO_3), Bovine serum albumin (BSA), Glutathione (GSH), Sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Chemical Co. $UBI_{29\text{-}41}$ (MW: 1.69 KDa) was obtained from Nanjing Leon Biological Technology Co., LTD (Nanjing, China). Ru complexes were Prepared in our laboratory. *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and *Escherichia coli* ATCC 8739 (*E. coli*) were obtained from Guangdong Microbiology Culture Center. Nude mice were obtained from the Guangdong Medical Experimental Animal Center. Ultrapure MilliQ water (18.2 MW) was used in all experiments and all solutions were stored in the refrigerator at 4 °C.

2.2. Synthesis and characterization

Synthesis of Se NPs. 1 mL 25 mM Na_2SeO_3 was mixed with 4 mL 25 mM GSH containing 20 mg BSA. The mixture was stirred at room temperature and adjusted to pH 7.2 with 1.0 M NaOH, and then a red solution was formed instantly. The solution was centrifuged at 12000 rpm for 10 min and the red precipitate was collected [12]. Finally, the red precipitates were washed three times with phosphate buffer saline (PBS) (0.01 mol/L, pH 7.4) to obtain the conjugates Se@BSA NPs, abbreviated as Se NPs.

Synthesis of Se@PEP NPs. The aqueous solution of Se NPs activated by adding 1-ethyl-3-[3-diMethylaminopropyl] carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) (the molar ratio of Se NPs: NHS: EDC is 1: 1.5: 1.5) at room temperature for 4 h. 28.17 μL $UBI_{29\text{-}41}$(5 mM)was added into the activated Se NPs solution, and the new solution was stirred at room temperature for 12 h. Then, the solution was centrifuged at 12000 rpm for 10 min and the red precipitate was collected [40]. The precipitates were washed three times with PBS (0.01 mol/L, pH 7.4). The NPs were named as Se@PEP NPs.

Synthesis of Se@PEP-Ru NPs. The antimicrobial effect of the Ru complexes was screened by the plate antibacterial test. The results showed that the antimicrobial effect of chiral Ru complexes was better than other Ru complexes (Fig.S1), Δ/Λ-[Ru (phen) $_2$ (*p*-HPIP)]$(ClO_4)_2 \cdot 2H_2O$ (Δ/Λ-OH), which were abbreviated as Ru1 and Ru2, respectively, and their molecular structures were also shown in the supporting information. 12.5 μL of the two chiral Ru complexes solution (Ru1 and Ru2, 2 mM) were added dropwise to the aqueous solution of Se@PEP NPs with constant stirring, respectively, and the pH value was maintained at 9–11 during the process. Stirring was continued for 2–5 h to obtain two kinds of wine red nano-sol (Se@PEP-Ru1 NPs/Se@PEP-Ru2 NPs). 4 °C storage liquid sol, centrifuged supernatant, washing, drying, red ruthenium complex functional nano-selenium particles was obtained [41]. The prepared Se@PEP-Ru NPs was characterized using a variety of analytical techniques. The results of Se@PEP-Ru2 NPs are shown below as representative examples. The morphologies of three types Se NPs were observed with transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and Ultraviolet–visible spectroscopy (UV–vis).

2.3. Bacterial uptake of Se@PEP-Ru2 NPs

The uptake of Se@PEP-Ru2 NPs by *S. aureus* and *E. coli* was studied. All bacteria in the Luriae-Bertani (LB) medium were grown overnight at 37 °C in a 5% CO_2 incubator, and the bacteria (150 mL overnight culture) were resuspended in 50 mL of fresh LB medium and incubated at constant temperature bed. Steady-growth bacteria (10.0 mL) were incubated with Se@PEP-Ru2 NPs (200 μL), or