Antibacterial Study of Peptide-Gold Nanostar Complex against Staphylococcus Aureus

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Abstract

Nowadays we are facing increasing serious clinical bacterial infections. With the emergence of drug-resistant bacteria, it was urgently need new antibacterial materials. Antibacterial peptides, metal nanoparticles and other new antibacterial agents have become current research hotspots. Here, we designed a nanomaterial coupled with antimicrobial peptides and gold nanostars. It has good stability in aqueous solution and acts effectively in inhibiting biofilms which were difficult to clear. At the same time, it can absorb near-infrared laser energy and convert it to heat energy, and use efficient photothermal effect for sterilization. The method reported in this article provides a valuable choice for future antibacterial research.

Keywords

Bacterial infection; Antimicrobial peptide; Gold nanostars; Photothermal effect.

1. INTRODUCTION

Nowadays, human beings are facing increasingly serious bacterial infections, especially the drug resistance of staphylococcus aureus (S. aureus), has brought huge threat to human health [1]. With the development of technology, antibiotics have been invented to deal with bacterial infections. However, bacterial resistance makes antibiotic treatment gradually decreasing [2], especially when bacteria form biofilm in pathological micro-rings, its resistance to antibiotics has increased thousands of times compared with before [3]. In this case, it was urgently needed to develop a strategy that can effectively inhibit the growth of S. aureus, and at the same time, destroy the biofilm.

Metal nanoparticles such as gold nanoparticles have attracted researchers’ attentions due to their special physical-chemical properties and good biocompatibility [4]. Gold nanoparticles can be divided into varies forms, including gold nanorods [5], gold nanocages [6, 7], gold nanoclusters [8] and gold nanostars [9, 10]. Gold nanoparticles which are not modified by any chemical functional group can produce a very strong photothermal effect in the visible field [11], and thus is widely concerned in light thermal antibacterial. In addition, gold nanoparticles especially gold nanostars are often used in drug delivery for they have high specific surface area, which theoretically can load more drugs [12].

Antibacterial peptide is also one of the focuses of research on bacterial resistance. Antimicrobial peptides inhibit or kill bacteria by destroying the integrity of their cell membranes [13]. At the same time, the relationship between the antibacterial peptide is not easily resistant to the bacteria due to the mechanism of action. However, peptide is not stable in the physiological environment, and the effect will also be decreased when faced with
bacterial biofilms [3]. Therefore, researchers attempt to couple antibacterial peptides with nanoparticles to increase its stability and sterilization [14].

In this article, a composite nanomaterial APAS which consists of three parts were designed. Gold nanostars is the basis of loading the drug, the antimicrobial peptide labeled with near-infrared fluorescent dye Cy7 was loaded on a gold nanostars, which functioned as killing S. aureus. SH-PEG is used as a stabilizer to improve the stability of APAs in aqueous solutions and help remove bacterial biofilms more effectively. In addition, the APAs nanomaterial can also produce a lot of heat under laser irradiation to achieve a better antibacterial effect.

2. RESULTS AND DISCUSSION

To prepare APAs, firstly, a one-step synthesis method was used to synthesize gold nanostars nanoparticles. Therefore, Fmoc solid-phase synthesis was used to synthesize the targeted antimicrobial peptide and the near-infrared fluorescent dye Cy7 was subsequently labeled to peptide. Finally, the SH-PEG and the antimicrobial peptide with cysteine are coupled to the gold nanostars nanoparticles through of gold-sulfur bonds. In order to confirm the successful coupling of gold nanostars with antimicrobial peptides, the changes of hydrated particle size and Zeta potential of APAs nanocomposites were measured. The experimental results were shown in Figure 1. The hydrodynamic particle size of the gold nanostars were 45 nm which obeyed a normal distribution (Figure 1-a). While, after coupling SH-PEG, the particle size increased slightly. Therefore, the particle size increased to about 200 nm after the antimicrobial peptide was bounded (Figure 1-b). For zeta potential, the gold nanostars themselves were negatively charged, while the potential became neutral after SH-PEG was coupled. The antimicrobial peptide was positively charged due to its bactericidal mechanism. When the antimicrobial peptide was successfully coupled with the gold nanostars, the APAs nanomaterials finally appeared positive charge. Such changes in Zeta potential indicate that we have successfully synthesized APAs nanomaterials (Figure 1-c). In addition, the particle size stability of APAs in aqueous solutions was also assayed. As shown in Figure 1-d, APAs nanomaterials remained uniform in particle size for seven consecutive days, indicating that APAs nanomaterials have good stability in aqueous solutions.

Figure 1. (a) Hydrodynamic size distribution of AuNS. (b) Hydrodynamic sizes of AuNS, AuNS-PEG and APAs. (c) Zeta potentials of AuNS, AuNS-PEG and APAs. (d) Hydrodynamic sizes of APAs during 7 days storage.
Gold nanostars can absorb near-infrared laser energy and convert it into heat energy. On the other hand, the near-infrared fluorescent dye Cy7 also has a good photothermal effect. The photothermal effect will be more significant when combining the two kinds of materials. Laser was used to irradiate nanomaterials to generate heat energy to ablate bacteria, so we conducted research on the photothermal effect of APAs nanomaterials. 808 nm laser was used to irradiate different concentrations of APAs, and at the same time their temperature changes were monitored with a thermal imager. As shown in Figure 2-a, the temperature of the PBS group under the monitoring of the thermal imager did not change before and after 6 minutes of laser irradiation. As the concentration of APAs increased, the photothermal effect was more significant, and the temperature increased by 25°C within 6 minutes at the highest concentration (Figure 2-b).

The growth and reproduction of bacteria can be divided into 4 periods: sluggish phase, logarithmic phase, stable phase and decay phase. Bacterial growth curve is also one of the important methods to detect the antibacterial properties of nanomaterials. As shown in Figure 3, the growth curve of the PBS group was completely the same regardless of whether the laser was irradiated or not, indicating that the 808 nm laser irradiation (808 nm, 1.8W/cm²) would not affect the growth of bacteria. APAs can affect S. aureus without laser irradiation, suppressing its growth during the logarithmic growth phase of the bacteria. However, the logarithmic growth phase of S. aureus was delayed again when was incubated with APAs after laser irradiation, proving that APAs nanomaterials can effectively inhibit the growth of S. aureus, and the effect is more obvious after 808 nm laser irradiation.

Bacteria will form a biofilm during the growth and reproduction process. Once the biofilm is formed, the drug resistance of the bacteria will suddenly increase, and the sterilization effect of the drug will be greatly decreased. Therefore, whether APAs nanomaterials can effectively inhibit the formation of S. aureus biofilm is the focus of our investigation. We incubated the APAs nanomaterials and S. aureus for two days, and then stained the biofilm formed with crystal violet to assay its inhibitory effect on S. aureus. As shown in Figure 4-a. The biofilm in the PBS group was visible after being stained with crystal violet, while the biofilm in the APA group was less, and there was almost no biofilm in the APA+IR group after laser irradiation. Ethanol was used to dissolve the biofilm and the absorbance at 590 nm was measured to quantify the biofilm. The quantitative results are shown in Figure 4-b, which proves that APAs nanomaterials can...
effectively inhibit the formation of S. aureus biofilm, and the inhibition effect is better after laser irradiation.

![Figure 3](image1.png)

**Figure 3.** Growth curves of S. aureus incubated with APAs (2 nM) before and after irradiation. The growth of S. aureus without APAs was used the control.

![Figure 4](image2.png)

**Figure 4.** (a) Inhibition effects of APAs nanomaterials (2 nM) on S. aureus bacterial biofilm (NIR laser 808 nm, 1.8 W/cm² was employed). (b) Corresponding quantitative analysis of crystal violet staining of biofilm in Figure 4-a.

3. **CONCLUSION**

In summary, we use Au-S bond coupling to combine AuNS, SH-PEG and Cy7 into composite nanomaterials named APAs, which are used to inhibit the growth of S. aureus and can also eliminate S. aureus biofilm. It is worth noting that APAs have good particle size stability in aqueous solutions and have excellent photothermal effects. After testing, it was found that APAs can generate a lot of heat to kill bacteria under 808 nm laser irradiation. It was believed that the strategy proposed in this article provide a valuable choice for the treatment of S. aureus infection.

4. **METHODS AND EXPERIMENTS**

**Materials.**

Chloroauric acid tetrahydrate (HAuCl₄·4H₂O AR), Sodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. 1-(3-dimethylaminoprop)-3-ethylcarbodiimide hydrochloride (EDC·HCl) was purchased from Adamas Beta, and SH-PEG (Sulphhydrly polyethylene glycol) (Mw = 4 000 Da) was purchased from Sigma-Aldrich. Near-infrared multiple methyl cyanine dye (Cy7) was synthesized in our laboratory. All experiments were performed with deionized (DI) water (Millipore Milli-Q grade, 18.2 MΩ).
Preparation of AuNS.

1350 μL of HAuCl4·4H2O (40 mM) was added to 90 mL of HEPES buffer solution (0.14 M, pH 7.4), and allowed to stand for 1 h in the dark to obtain AuNS solution. Centrifuge at 13,000 rpm, retain the pellet and resuspend in 10 mL of deionized water.

Preparation of APAs.

Using ChemMatrix resin and amino acids as raw materials, GLFVDK(Mtt)GKRWWKWWRRGC peptide was prepared by Fmoc solid-phase synthesis method. After the synthesis of the peptide is completed, the Mtt protecting group in Lys was removed. 5 times the molar amount of the peptide is weighed out of Cy7 and the equivalent molar ratio of EDC, DIEA and HOBT (molar ratio AMP: Cy7: EDC: DIEA: HOBT = 1:3:1:1:1), dissolve in DMF and react overnight in the dark.

The unattached dye was washed the next day, 20% piperidine (Volume ratio Py: DMF=1:4) was added to remove the Fmoc protecting group at the N-terminus of G amino acid. Then cleavage solution (volume ratio TFA: EDT: TIS: H2O=94:2.5:1:2.5) was used to cleave the peptide from the resin. After reacting at room temperature for 3 h, the peptides were separated from the resin, precipitated with ice ether, centrifuged at 4000 rpm to remove the supernatant, the precipitate was dried with nitrogen and dissolved, and purified by high performance liquid chromatography. The components with peaks at 220 nm and 749 nm were collected. After freeze-drying, the final sample was obtained.

Then, 200 μL of the SH-PEG (2 mg/mL) was stirred with 10 mL of AuNS (0.2 nM) for 30 min at room temperature, then reacted with 1 mL of the peptide solution (24 μM) for 20 h at room temperature. After the reaction was completed, it was centrifuged three times at 13,000 rpm, the precipitate was retained, and resuspended in 1 mL of deionized water obtained APAs.

Morphology and potential characterization of APAs.

Take 20 μL of the prepared composite nanomaterial APAs solution, dilute it to 1 mL with DI water, characterize the hydrodynamic size and Zeta-potential of APAs with a dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern).

In order to explore the stability of APAs in deionized water, take 10 μL of APAs nanomaterials for seven consecutive days, dilute to 1 mL with deionized water, and then measure the hydrodynamic sizes of the material with a Malvern particle size analyzer. Measure multiple times and take the average value, record the data.

Experiment of APAs nanomaterials inhibiting biofilm.

Select a flat-bottom 96-well plate, add 200 μL of TSB culture solution to the selected 9 wells, and then add 2 μL of S. aureus bacterial solution which in the logarithmic growth phase, and divide them into three groups (PBS control group, APAs group, APAs+IR (808 nm laser irradiation group), each group of 3 holes. Add 20 μL of PBS to the first group, add 20 μL of APAs to the second and third groups, and use 808 nm laser (power density in the third group) 1.8 W/cm2) irradiate for 6 min. Place the 96-well plate in a biochemical incubator at 37°C and incubate for 48 h. After the culture is completed, take it out, aspirate the upper culture medium with a syringe, wash the wells with PBS 2-3 times, air-dry for 10 minutes, and then add 100 μL to each well. The crystal violet solution with a concentration of 1% was dyed for 20 minutes. Gently aspirate the upper crystal violet solution, wash with sterile PBS for 3 times, and then air-dry for 10 minutes, add 200 μL of 80% ethanol solution to each well, place the 96-well plate on a constant temperature shaker and shake for 2 hours. Wait The ethanol solution completely dissolves the crystal violet. Use a microplate reader to detect the absorbance of each well at 590 nm, take the average value and record the data.
ACKNOWLEDGMENTS

This work was supported by the Research and Innovation Program for Graduate Students in Colleges and Universities of Jiangsu Province (SJCX20_1007, KYCX20_2572).

REFERENCES


