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Application of Photothermal Diagnosis and Treatment Nanosystems in *Staphylococcus aureus* Infection

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Abstract

Nowadays there are more and more detection and treatment methods for bacterial infections. However, achieving sensitive detection and effective treatment remains a huge challenge. Here, we report a strategy for designing an integrated nanomaterial for the diagnosis and treatment of *S. aureus* infection. We use the covalent coupling method to form Au Colloid, PAH and Cypate dyes into a complete nanocomposite (Au-PAH-Cypate). It is worth noting that the nanomaterial has good stability and biocompatibility in an aqueous environment. In addition, Au-PAH-Cypate can realize the NIR fluorescence detection of S. aureus, and based on this ability, through the loaded Cypate, convert the absorbed NIR laser energy into heat energy, destroy the cell structure of *S. aureus*, make *S. aureus* die, and achieve the effect of photothermal antibacterial. The method reported in this work hopes to provide reference value for the design of integrated nanomaterials for bacterial diagnosis and treatment.

Keywords

Bacterial infection, Nanocomposite materials, NIR fluorescence imaging, Photothermal antibacterial.

1. INTRODUCTION

As we all know, *S. aureus* bacterial infections pose a huge threat to human health and bring enormous economic and medical pressure to families and society. Although more and more technologies can be used to detect and eliminate bacteria as society progresses, how to obtain sensitive and accurate detection of bacterial infections and effective treatment in real time is still a huge challenge. In this situation, it is an urgent requirement to develop a strategy for sensitive detection and timely treatment of *S. aureus* bacterial infections.

Nowadays, people are increasingly interested in precious metal nanoparticles,1 and researchers 'attention is gradually focused on gold nanoparticles (AuNPs). Due to its special physical and chemical properties, it is often used in drug delivery.2 Gold nanoparticles are chemically stable, with particle sizes ranging from a few nanometers to hundreds of nanometers.3,4 With the in-depth research of researchers, the morphology of gold nanoparticles has become increasingly rich, including gold nanorods,5 gold nanopyramids,6 gold nanospheres,7,8 gold nanocage9 and gold nanostars,10,11 etc. Because of the special properties such as the surface plasmon effect of gold nanoparticles,12 gold nanoparticles are widely used in different fields of biochemistry.13,14

Cypate is a carboxyl derivative of indocyanine green (ICG), which has strong absorption in the near-infrared light region. Researchers usually combine Cypate with other nanomaterials to enhance its stability in an aqueous environment. We can use the properties of Cypate to achieve the purpose of photothermal antibacterial.

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In this work, we report a composite nanomaterial design strategy that combines gold nanoparticles with Cypate dyes as a whole for the detection and treatment of *S. aureus* infection. The nanomaterials play a guiding role for treatment because of the sensitive detection of *S. aureus*' near-infrared fluorescence imaging. Using the photothermal properties of Cyapte loaded on it, it can effectively kill *S. aureus* at the infected site, achieving the integration of detection and treatment.

2. RESULTS AND DISCUSSION

In the process of preparing Au-PAH-Cypate nanomaterials, first we synthesized Au Colloid by a simple method, then applied the principle of positive and negative charges to attract each other, coated PAH on the surface of Au Colloid, finally, using the method of EDC/NHS covalent coupling, the Cypate dyes were covalently conjugated to Au-PAH to prepare Au-PAH-Cypate nanocomposites. By testing the hydrodynamic size of Au Colloid, Au-PAH and Au-PAH-Cypate, the results are shown in Figure 1a. The hydrodynamic size of Au-PAH-Cypate nanomaterials is the largest, followed by Au-PAH, Au Colloid is the smallest, indicateing that we successfully coated PAH on the surface of Au Colloid, and Cypate dyes were also successfully coupled with Au-PAH, and finally Au-PAH-Cypate nanomaterials were synthesized. Au Colloid is negatively charged. After being coated with PAH, Au-PAH finally shows positive charge due to the electrical positiveness of PAH. The final electropositivity of Au-PAH-Cypate is weaker than that of Au-PAH, indicating that Cyapte dyes were successfully coupled with Au-PAH. The change of Zeta-potential also shows that we successfully synthesized Au-PAH-Cypate nanomaterials (Figure 1b).

Due to the maximum emission wavelength of the Cypate dyes is at 820 nm, in order to investigate the fluorescence performance of Au-PAH-Cypate nanomaterials, we use a fluorescence spectrometer to detect the fluorescence of Au-PAH-Cypate and other materials at 820 nm. As shown in Figure 2a. Compared to Au Colloid and Au-PAH, Au-PAH-Cypate has a high fluorescence intensity at 820 nm. On the contrary, Au Colloid and Au-PAH show almost no fluorescence, indicating that the Cypate dyes were successfully covalently coupled with Au-PAH to prepare the Au-PAH-Cypate nanomaterials, and the materials have good fluorescence properties. In order to determine the concentration of Cypate on Au-PAH-Cypate nanomaterials, we used a standard solution of Cypate dyes in the concentration range of 0 μM - 4 μM to create a standard curve, the equation of the curve is $y = 0.22762x + 0.01306$ (Figure 2b).

In order to verify the fluorescence detection ability of Au-PAH-Cypate nanomaterials for *S. aureus*, Au-PAH-Cypate nanomaterials (20 μg/mL) and *S. aureus* (107 CFU) were co-cultured for 30 min, and then confocal laser scanning microscope images the treated bacteria. As shown in Figure 3, the bacteria displayed in the fluorescent field completely overlap the bacteria observed in the bright field, and the fluorescence performance is obvious, indicating that Au-PAH-Cypate nanomaterials have good fluorescence detection ability for *S. aureus*.

Bacterial growth curve is also one of the important methods to detect the antibacterial properties of nanomaterials. As shown in Figure 4a, after being treated with Au-PAH-Cypate nanomaterials (20 μg/mL), *S. aureus* without NIR laser irradiation (808 nm, 1.5w/cm2) has a poor initial growth state, but after 12 h, its growth state is good and tends to be stable. On the contrary, although the growth state of *S. aureus* after NIR laser irradiation was slightly restored after 12 h, it was still sluggish, indicating the low bacterial activity. The growth curve of *S. aureus* once again shows that Au-PAH-Cypate nanomaterials have good photothermal antibacterial properties. Figure 4b are the representative photos of the coated plate before and after NIR laser irradiation of *S. aureus* co-cultured with Au-PAH-Cypate nanomaterials (20 μg/mL) for 30 min. It can be seen that *S. aureus* without NIR laser irradiation has a high survival rate and good growth status. After NIR laser irradiation, *S. aureus* was killed in large numbers, and only a small

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amount of *S. aureus* survived. By quantifying *S. aureus* on the two groups of agar plates, the survival rate of the two groups of *S. aureus* can be seen more clearly. In summary, Au-PAH-Cypate nanomaterials have a good photothermal elimination effect on *S. aureus*.

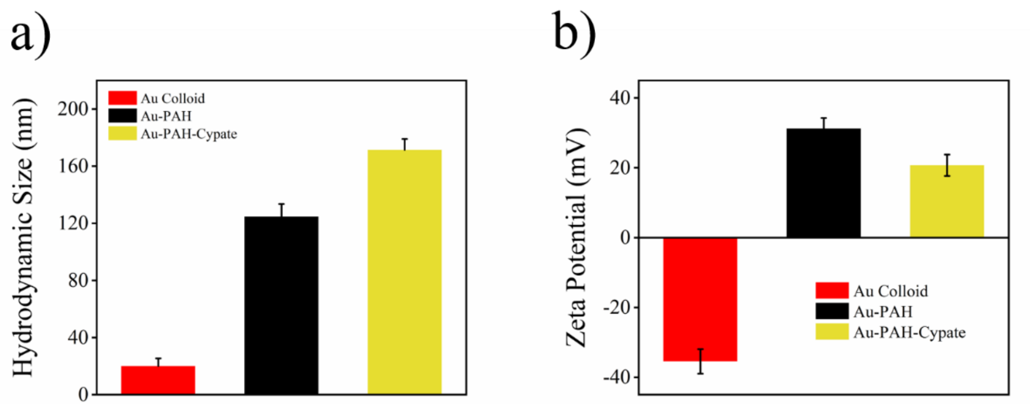

Figure 1. (a) Hydrodynamic sizes of Au Colloid, Au-PAH, and Au-PAH-Cypate. (b) Zeta potentials of Au Colloid, Au-PAH, and Au-PAH-Cypate.

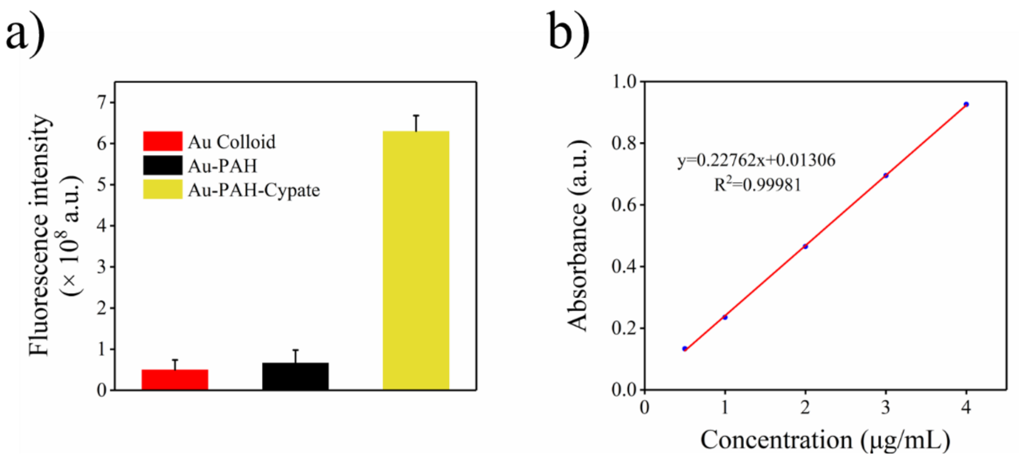

Figure 2. (a) The fluorescence intensities of Au Colloid, Au-PAH, and Au-PAH-Cypate at 820 nm. (b) Standard curve of Cyapte dyes, the equation of the curve is y = 0.22762x + 0.01306.

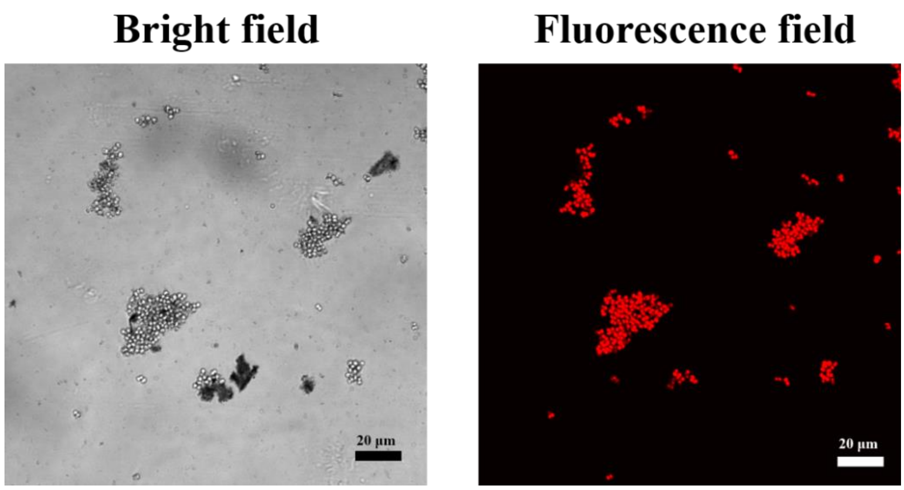

Figure 3. Representative CLSM images of S. aureus (107 CFU/mL) after treated with Au-PAH-Cypate (20 μg/mL).

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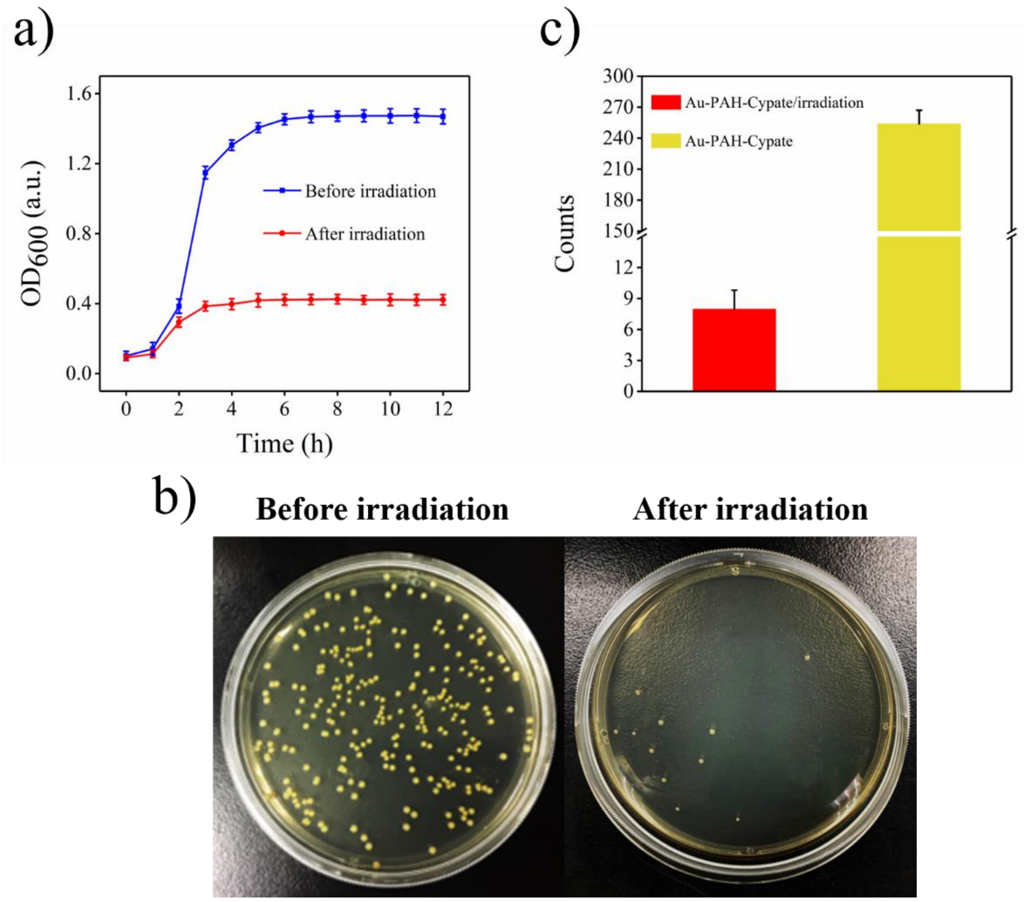

Figure 4. (a) Treated with Au-PAH-Cypate (20 μg/mL), the growth curve of S. aureus before and after irradiation (808 nm, 1.5W/cm2). (b) Treated with Au-PAH-Cypate (20 μg/mL), the representative photos of S. aureus culture before and after irradiation. (c) S. aureus quantification on two groups of agar plates before and after irradiation.

3. CONCLUSION

In summary, we use the covalent coupling method to form Au Colloid, PAH and Cypate dyes into a composite nanomaterial for the detection and treatment of *S. aureus*. It is worth noting that Au-PAH-Cypate nanomaterials have good hydaodynamic size stability and biocompatibility in an aqueous environment. In addition, the nanomaterial has a sensitive detection capability for *S. aureus*, showing accurate and strong fluorescence. Moreover, because of the loading of Cypate dyes, Au-PAH-Cypate can fully convert the absorbed NIR laser energy into heat energy to kill bacteria. We hope that the strategy reported in this work will provide reference value for the design of nanomaterials used to detect and treat bacterial infections.

4. METHODS AND EXPERIMENTS

Materials. Chloroauric acid tetrahydrate (HAuCl4.4H2O AR), Sodium citrate were purchased from Sinopharm Chemical Reagent Co., Ltd. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), and Poly (allylamine hydrochloride) (PAH; Mw = 15 000 Da) were purchased from Sigma-Aldrich Trading Co., Ltd. Cypate dyes were synthesized in our own laboratory. All experiments were performed with deionized (DI) water (Millipore Milli-Q grade, 18.2 MΩ).

Preparation of Au Colloid. Synthesize Au Colloid according to the method in the reported literature.16 Briefly, 500 mL of HAuCl4 (1 mM) was stirred and heated to make it boil, and then

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quickly added 50 mL of 38.8 mM Sodium citrate solution to the boiling solution. During the process, it was found that the color of the mixed solution changed from light yellow to purple red, indicating that the reaction was successful. After continuing to heat for 10 min, stop heating and continue stirring for 15 min. Cool the resulting solution to room temperature and filter with a 0.22 μm filter membrane to obtain Au Colloid with a particle size of 13 nm.

Preparation of Au-PAH. PAH coating of Au Colloid according to the method in the reported literature.17 In brief, 0.1 g PAH and 10 mL NaCl solution (1 mM) were mixed evenly, then 1 mL Au Colloid solution was added, stirred at room temperature for 3 h, the supernatant was removed by centrifugation, and the precipitate was dispersed in 5 mL DI water.

Preparation of Au-PAH-Cypate. Based on the previously prepared Au-PAH solution, the Cypate dyes were covalently conjugated to PAH by the EDC/NHS covalent coupling method,18,19 and then the composite nanomaterials Au-PAH-Cypate were obtained. In brief, added 300 μL of EDC and NHS mixed solution to 1 mL Cypate solution, and stirred at room temperature for 30 min. 5 mL of Au-PAH-Cypate solution was added to the above solution, stirred for 2 h, centrifuged, the supernatant was removed, and the precipitate was dispersed in 5 mL of DI water for use.

Morphology and potential characterization of Au-PAH-Cypate. Take 1 mL of the prepared composite nanomaterial Au-PAH-Cypate solution, dilute it twice with DI water, characterize the hydrodynamic size and Zeta-potential of Au-PAH-Cypate with a dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern).

Fluorescence determination of Au-PAH-Cypate. Three materials (Au Colloid, Au-PAH and Au-PAH-Cypate) were detected by fluorescence spectrometer (FLS980, Edinburgh). In brief, take 1 mL of each of the above three materials, use a fluorescence spectrometer to determine whether the three materials have characteristic peaks at 820 nm (the excitation wavelength is 780 nm), and take their peaks for quantification.

Establishment of Cypate standard curve. Prepare 1 mL of Cyapte dyes standard solution with a concentration of 10 μg/mL, dilute it to 0.5, 1, 2, 3, 4 μg/mL, and measure its absorbance at 780 nm with a microplate reader (Synergy NEO, BioTek), perform linear regression on the obtained data to obtain the standard curve equation of Cypate.

Bacterial fluorescence imaging. A mixed solution of 400 μL of Au-PAH-Cypate (20 μg/mL) and *S. aureus* (107 CFU) was incubated for 30 min in a constant temperature shaker. After the completion of the cultivation, centrifuge and resuspend the pellet in sterile PBS. Take 30 μL of the resuspended solution on a glass slide and observe whether *S. aureus* shows fluorescence with a confocal laser scanning microscope (FV1200, Olympus).

Detection of bacterial growth curve. Add 2 μL of *S. aureus* in the exponential growth phase to the mixed solution of 100 μL TSB and 100 μL Au-PAH-Cypate (20 μg/mL). The above samples were divided into two groups, one group was irradiated with NIR laser for 5 min, and the other group was not illuminated. The two groups of samples were put into a biochemical incubator for incubation. The absorbance of the samples at 600 nm was measured per 1 h and continuously measured for 12 h. The obtained data was processed with software to obtain the bacterial growth curve.

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Notes

The authors declare no competing financial interest.

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