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Construction and Antibacterial Properties of Photo Thermal Sterilization and Antifouling Surface

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***Abstract*—The adhesion of bacteria on the surface of materials is one of the biggest limitations for the long-term use of many medical implant devices. The adhesion of bacteria and other microorganisms on the surface of implanted biomaterials will form biofilm, which will cause infection to patients and bring great pain to patients. Therefore, it is of great significance to design coatings with dual functions of antibacterial and antifouling. In this paper, the photothermal sterilization effect of gold nanorods (AuNRs) and the antifouling characteristics of fluorine compounds are combined to prepare a surface material with sterilization antifouling at the same time, which solves the problem that it is difficult to remove dead bacteria on the surface of sterilization materials, resulting in the decline of sterilization performance.**

***Index Terms*- AuNRs, Fluorine compounds, photothermal therapy, Antifouling, Antibacterial**

I INTRODUCTION

In the medical field, medical implant devices are widely used in the diagnosis and treatment of diseases. Commonly used medical implant devices, such as artificial bone, vascular catheter, urinary catheter and endotracheal intubation, but while these implant devices bring convenience to clinical treatment, there are also some problems[1,2]. For example, for medical implants in contact with blood, even a very small amount of adsorbed fibrinogen may cause platelet adhesion, resulting in thrombosis[3]. This will lead to local infection, bring great pain to patients, and cause a lot of economic losses. The formation of bacterial biofilm on the surface of biomaterials after implantation is the main reason for the periodic infection of biomaterials. This biofilm is formed by the adhesion and growth of bacteria and other biological macromolecules on the surface of implants[4,5]. Therefore, in order to avoid infection in vivo, the surface of biomaterials is required to have certain antibacterial ability. However, the traditional antibacterial coating only considers sterilization and does not consider the adhesion of bacteria to corpses[6].

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This leads to the formation of biofilm on the surface of the material and increases the tolerance of bacteria to antibiotics[7]. Tens of billions of dollars are lost every year due to iatrogenic infection[8]. Therefore, it is of great significance to design coatings with dual functions of antibacterial and antifouling.

In recent years, the "antifouling-bactericidal" coating technology is regarded as one of the most promising antibacterial strategies. Compared with single function coatings, this technology has the ability to resist pollution first and then kill the adhered bacteria. It has unique advantages in maintaining the long-term sterilization and surface self-cleaning function of materials[9-11].

Compared with traditional chemotherapy, photothermal therapy is a type of treatment method that induces local heat generation by light-induced photothermal agents to ablate bacteria, and is widely used in antibacterial infections[12,13]. A variety of photothermal agents have emerged in recent years, among which AuNRs have become the first choice for photothermal therapy due to their localized surface plasmon resonance (LSPR) in the near-infrared region and good biocompatibility[14-16]. Near-infrared light-triggered photothermal therapy can penetrate deep into tissue with minimal damage to surrounding areas, and can also fight pathogenic cells by disrupting cell membrane permeability and metabolic signaling, denaturing proteins/enzymes, and inducing bacterial death[17,18]. Therefore, AuNRs have a wide range of applications in many fields such as biological probes, biochemical markers, biomolecule detection, medical imaging analysis and tumor treatment.

The fluorine atom has a small radius, a large electronegativity, and a high C-F bond energy (about 485.58KJ/mol)[19]. At the same time, the fluorine atoms repel each other, forming a fluorine atomic stack with a symmetrical distribution surrounding the carbon chain[20]. The special structure makes the whole molecule a non-polar organization, and the polarizability of the fluorine atom is small, so it has excellent chemical stability, heat resistance, pollution resistance, aging resistance and low surface energy and other properties[21-23]. Hydrophobic materials with low surface energy can not provide enough surface energy to adhere proteins, bacteria and other microorganisms, but can release pollutants to achieve anti-fouling effect[24]. These characteristics endow fluorine-containing coatings with a wide range of applications in anti-fouling, anti-corrosion, and self-cleaning.

Firstly, the AuNRs were evenly coated on the surface of

the glass slide connected with -SH, and then connect the fluorine compound to the gold nanorods through the thiol group to obtain a sterilization-antifouling coating surface with a relatively uniform morphology. The surface contains gold nanorods and fluorine compounds at the same time, which has both good bactericidal activity and antifouling performance, and solves the problem that the dead bacteria on the surface of the bactericidal material are difficult to remove, resulting in a decline in bactericidal performance.

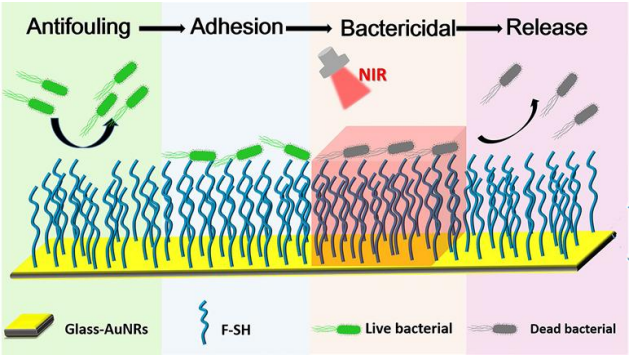

Figure 1. The schematic illustration of antifouling-bacter icidal of G-Au-F

II. EXPERIMENTAL AND METHODS

A Materials

Hexadecyl trimethyl ammonium bromide (CTAB) , Sodium oleate (NaOL), $HAuCl_4 \cdot 3H_2O$, $AgNO_3$, Sodium borohydride ($NaBH_4$), R-mercaptopropyltrimethoxysilane (MPTES), HCl, 1H,1H,2H,2H-Perfluorodecanethiol (F-SH), L-Ascorbic acid (L-AA), Concentrated sulfuric acid (H_2SO_4), Hydrogen peroxide (H_2O_2).

B Synthesis and characterization of AuNRs

AuNRs were synthesized by seed mediated growth method[25]. Specifically, preparation of seed solution: Add $HAuCl_4$ $3H_2O$ (5 mL, 0.5 mM) drop by drop into CTAB (5 mL, 200 mM), stir at low speed for 15 min, add the newly prepared $NaBH_4$ (1mL, 10 mM) solution to the above solution, stir at high speed for 2 min, at this time, the solution changes from yellow to brown yellow, and age at 30℃ for 30 min before standby.

The growth method is to dissolve 7.0 g CTAB and 1.234 g NaOL in 250 mL of warm water, add $AgNO_3$ (24.0 mL, 4 mM) when the temperature drops to 30℃, add $HAuCl_4$ $3H_2O$ (250 mL 1 mM) after 15 min, gently stir for 30 min, then add 2.1 mL of concentrated hydrochloric acid to adjust the pH, and then add L-AA (1.25 mL 0.064 M) after 15 min. At this time, the solution becomes colorless. Stir vigorously for 30 s, add 0.8 mL of seed solution, stop stirring after 30 s, and stand at 30℃ for 12 h. The resulting AuNRs were centrifuged at 8000 rpm to remove excess CTAB and dispersed in ultrapure water.

Absorption spectrum of AuNRs were measured with a UV-vis spectrophotometer (UnicamUA500, Thermo electronics corporation).The morphology of AuNRs was investigated on a TEM (JEM-2010, Japan JEOL).

C. Preparation of G-Au and G-Au-F

Firstly, the glass surface was hydroxylated with piranha etching solution (H_2SO_4: H_2O_2 = 7:3). Specifically, a glass slide with a diameter of 1 cm is placed in a beaker, ultrasonic washed with an appropriate amount of deionized water, ethanol and acetone, and then dried with nitrogen. Add 35 mL concentrated sulfuric acid to the dried glass slide, then add 15 mL 30% hydrogen peroxide dropwise under continuous stirring, and then move it into 90℃ oil bath for heating and stirring for 2 h. Pour the above liquid, cool it to room temperature, wash it with deionized water for three times to neutral, and then seal it in pure water.

The surface sulfhydrylation of glass slide was carried out by vapor deposition. Lay the dried glass slide with hydroxylated surface in a 24 well plate, and add 100% into the blank hole 100 μL MPTES. Place the orifice plate horizontally in a vacuum dryer, pump for 15 min with a vacuum pump, and then stand at room temperature for 12 h for vapor deposition. Take out the glass slide, wash it with water and ethanol for seveal times, and dry it with nitrogen at room temperature to obtain the glass slide with amino surface for standby.

Add 100 μL AuNRs dropwise into the perforated plate with sulfhydryl glass slide, and let it stand at room temperature until it is dried by natural air. Take out the slide, wash it with pure water and ethanol for several times, and dry it with nitrogen. The obtained AuNRs modified surface is recorded as G-Au. The F-SH is connected to the G-Au surface by vapor deposition, which is recorded as G-Au-F.

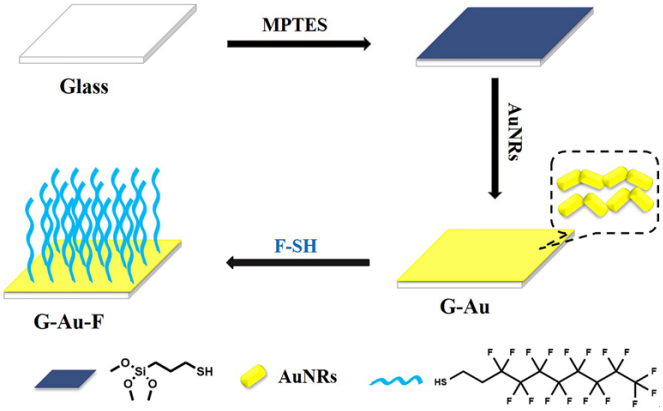

Figure 2. The preparation process of G-Au-F

D Characterization of static contact angle and photothermal properties of G-Au-F

Static contact angle test: Select solvent deionized water, test three points for each sample and take the average value; The test was carried out at room temperature with a relative humidity of 65%. Contact angle tester model: jc2000d1 (powerreach, Shanghai).

Photothermal properties: In order to study the photothermal conversion ability of the material surface, the prepared surfaces G-Au and G-Au-F were placed in aqueous solution, and the temperature change under 808 nm laser

irradiation was measured and imaged by infrared thermal imager (FLIR A300, USA). Unmodified blank slides were used as the control group.

E antifouling and Antibacterial test

The Antifouling ability of the material surface was tested by plate counting method. The gram negative bacterium *E. coli* (ATCC 35218) was used as the bacterial model. Firstly, *E.coli* was introduced into liquid medium after two subcultures, and the final concentration was 1×10^6CFU/mL bacterial solution. Then place the sample in a 24 well plate, soak it in 75% ethanol for several minutes, remove the ethanol and dry it, kill it under the UV lamp for half an hour, and set aside. Add 1 mL of bacterial solution to each well plate containing samples, and then culture at 37℃ for 12 h, and wash away the planktonic bacteria with normal saline. Take out the slide and put it into a centrifuge tube containing 2 mL of sterile PBS, ultrasonic for 8 s, vortex and mix evenly, and take 100 μL suspension was evenly coated on solid medium, and the number of colonies was counted after incubation at 37℃ for 16 h.

The activity of bacterial cells under fluorescence microscope was observed by living/dead bacterial staining. Specifically, after co culture according to the above method, wash the planktonic bacteria with normal saline, irradiate them with 808 nm (1.0 w/cm^2) laser for 10 min, and then add 10 μL（3 μM) SYTO-9 and PI were incubated at 4℃ in the dark for 30 min and observed by fluorescence microscope.

III RESULTS AND DISCUSSION

A. characterization of AuNRs

The AuNRs modified by CTAB were synthesized in aqueous solution by seed mediated growth method. As shown in Figure 3(B), the obtained AuNRs have a unified regular structure. According to the statistics of gold nanorods in different TEM images, the average length is 90 ± 10 nm, the average width is 22 ± 5 nm, and the aspect ratio is 4.1 ± 0.6. As shown in Figure 3(A), we characterized the UV-Vis absorption of AuNRs. It can be seen that they have a strong absorption peak at about 808 nm, which provides a favorable basis for their photothermal conversion under near-infrared laser irradiation.

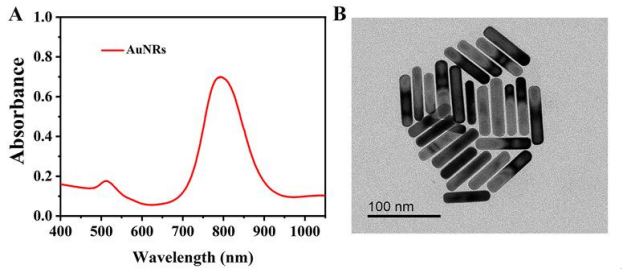

Figure 3. (A) UV-visible absorption spectrum and TEM of AuNRs

B. characterization of G-Au-F

The measured static contact angle data are sorted into a histogram as shown in Figure 4. From the graph, it can be seen intuitively that the static contact angle of G-Au surface is increased compared with the blank slide, but it still shows hydrophilicity. The static contact angle of the material surface (G-Au-F) grafted with fluorine-containing compounds increased significantly. The difference between the static contact angle and the blank glass surface is obvious, indicating that the surface of the material grafted with AuNRs and fluorinated compounds is hydrophobic. It can also be inferred that fluorinated compound (F-SH) was successfully grafted on the surface of the material.

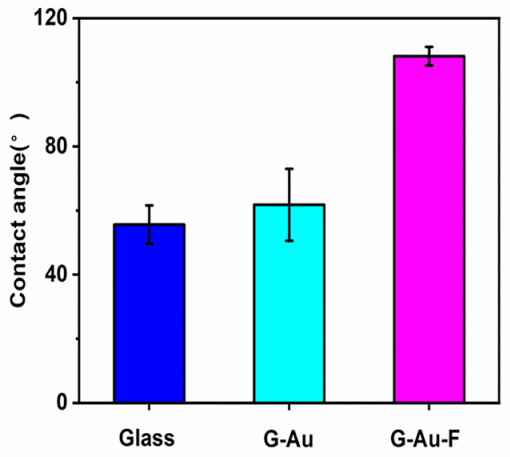

Figure 4. Water contact angles of Glass, G-Au, and G-Au-F

C. Characterization of surface photothermal properties

AuNRs show strong absorption in the near-infrared absorption band of 700~800 nm due to its surface plasmon resonance (SPR), suggesting its great potential in photothermal therapy. Photothermal therapy is a physical therapy method that uses near-infrared laser to induce local temperature rise and kill bacteria, which can effectively avoid the emergence of bacterial drug resistance. As shown in Figure 5-7, the image and temperature change of the nanocomposite under 808 nm laser irradiation were recorded by infrared thermal imager. As shown in Figure 6, with the increase of laser irradiation time, the temperature of water suspension increases continuously. After laser irradiation for a certain time, the temperature increases rapidly and tends to be flat. After the surface of glass slide is modified with AuNRs, the temperature of water suspension can also reach more than 80 ℃ after laser irradiation for 10 min.

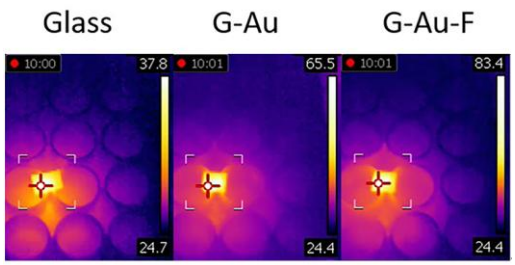

Figure 5. Infrared thermal images of Glass, G-Au, and G-Au-F before and after irradiation by 808 nm NIR for 10min

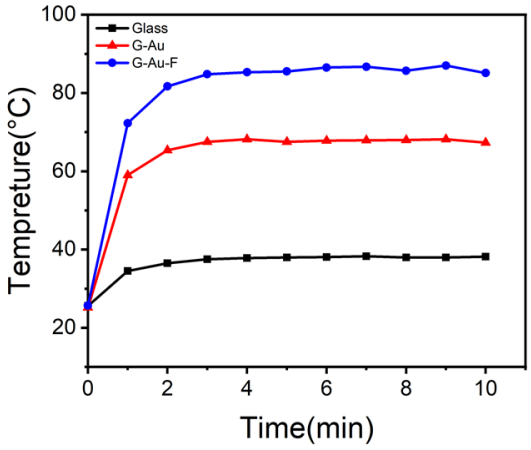

Figure 6. Heating curves of Glass, G-Au and G-Au-F

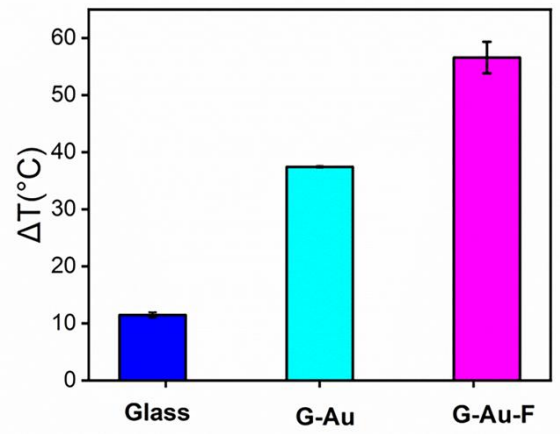

Figure 7. Increased temperatures (ΔT) of the samples after irradiation by 808 nm NIR for 10 min

D. antifouling and Antibacterial test

Taking *E. coli* as the model strain, the antifouling and antibacterial activities of G-Au-F were evaluated by plate counting method and live/dead bacteria staining method. As shown in Figure 8, after the material was co-cultured with *E. coli* for 12 h, a large number of bacteria adhered to the surface of the blank slide, and the antifouling effect of G-Au-F was significant. From the results of live/dead staining (Figure 9), it can be seen that G-Au-F material has good bactericidal effect on *E. coli*, while G-Au material has no better bactericidal effect on *E. coli* than G-Au-F. This may be because the fluorine mixture on the surface of G-Au-F has good antifouling properties, which makes it difficult for bacteria to adhere to the material surface. Even if there is a small amount of bacterial adhesion, the heat generated by AuNRs is enough to kill bacteria. The surface G-Au-F has bactericidal effect and reduces the adhesion of bacteria on the material surface.

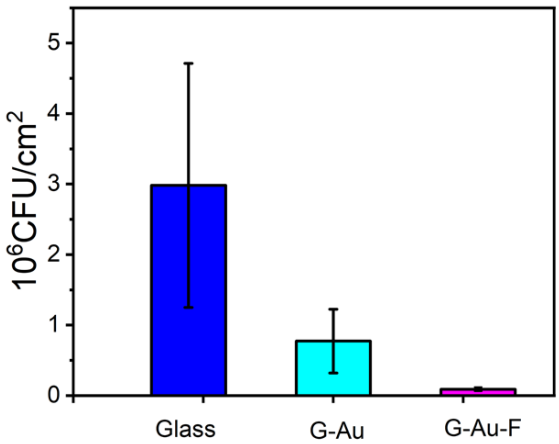

Figure 8. Antifouling effect of Glass, G-Au, and G-Au-F in cultured *E. coli* for 12 h

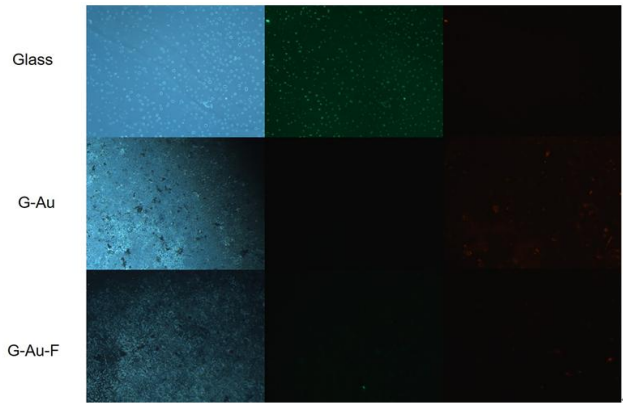

Figure 9. After the Glass, G-Au, and G-Au-F were cultured in *E. coli* for 12 h and irradiated with near-infrared light for 10 min, the bacteria on the surface of the membrane were stained

IV CONCLUSION

In this study, bactericidal and antifouling surfaces containing both AuNRs and fluorine compounds (F-SH) were prepared. The AuNRs were characterized by UV spectrophotometer and TEM. The surface properties were characterized by static contact angle and photothermal properties. *E. coli* was used as a model bacterium to study the bactericidal properties of the surface.

AuNRs have strong UV absorption at 808nm. The average length of AuNRs is 90 ± 10nm, the average width is 22 ± 5nm and the aspect ratio is 4.1 ± 0.6. The change of static contact angle proves that the surface has been successfully prepared. After irradiation with 808nm near-infrared light for 10min, the temperature can reach 85℃, which proves that the surface has good photothermal properties. Standard plate counting method and live/dead staining method can show that G-Au-F has excellent antifouling and antibacterial properties. It can be shown that G-Au-F has excellent antifouling-antibacterial properties. The project provides a new way to solve the problem of bacterial infection on the surface of materials, but there are still many problems to be solved.

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