Hindawi
Advances in Polymer Technology
Volume 2022, Article ID 6213187, 8 pages
https://doi.org/10.1155/2022/6213187

*Research Article*

# Synthesis and Characterization of a Coumarin Antimicrobial Polymer Fluorescent Coating

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Received 23 April 2022; Accepted 19 October 2022; Published 3 November 2022

Academic Editor: Filippo Rossi

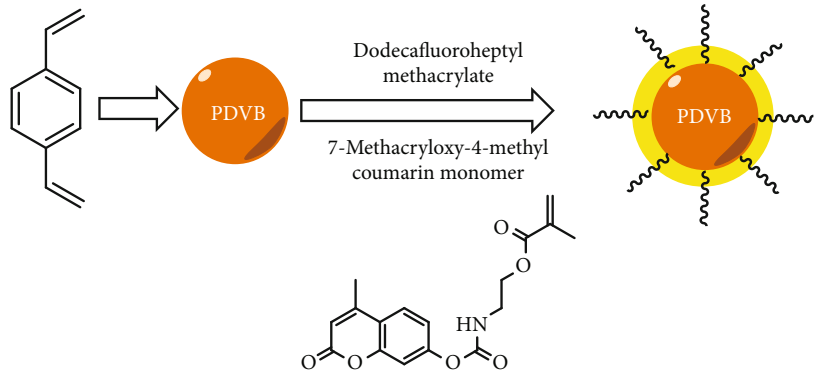


Polymer materials are widely used in medical materials, food packaging, coatings, and other fields. However, the surface of the materials is easily contaminated by microorganisms, resulting in serious problems. To solve this issue, a new type of antibacterial polymer fluorescent coating was successfully synthesized by copolymerization of divinylbenzene with 7-methacryloxy-4-methyl coumarin, dodecafluoroheptyl methacrylate, and other monomers. The surface structure and thermal stability of the coating were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy, and thermogravimetric analysis. Fluorine was polymerized into the polymer, improving the thermal stability compared to polystyrene and polydivinylbenzene. The bactericidal and antibacterial adhesion properties of the coating materials were studied by a contact germicidal test and antibacterial adhesion test. The polymer had a strong inhibitory effect on *Staphylococcus aureus*. After immersion in room temperature water, the film maintained its strong inhibitory effect on *S. aureus* fluorescence intensity and had high fluorescence stability.

## 1. Introduction

Polymer materials are widely used in medical materials, food packaging, coatings, and other fields; however, microbial contamination on the surface of materials is a very serious problem because it may lead to microbial infection and deterioration of the material properties [1–4]. Introducing an antibacterial function on the surface of polymer materials may solve this problem [1, 2]. Materials with antibacterial function are traditionally created by introducing bactericidal components such as direct physical blending of fungicides, grafting or modification methods to introduce antibacterial functional groups, or preparing composites with metal oxides, silver, copper, and other materials. However, these methods do not have strong sterilization persistence. In addition, over time, bacteria will form a biofilm after adhesion to the surface, thus greatly reducing the sterilization efficiency [5].

To obtain an ideal antibacterial surface, it is necessary to achieve the following functions: prevent the initial attachment of bacteria, kill all bacteria that overcome this antiadhesion barrier, and remove the dead bacteria. To achieve these functions and better address the problems of antimicrobial durability and antimicrobial adhesion, a system of antimicrobial surfaces containing two antimicrobial strategies was developed. Antibacterial adhesion can be achieved by repelling or killing adjacent bacteria using highly negatively charged polymers (electrostatic repulsion) and hydrogel-like polymers (spatial repulsion), or special polymers with low surface energy (superhydrophobic repulsion) [6, 7]. Bacteria can be killed by releasing antibacterial parts of the substrate or constructing a contact sterilization surface [8–10]. Therefore, the combination of bactericidal and superhydrophobicity can prevent bacteria attaching to the surface and can more easily kill the bacteria. Additionally, the strategy can maintain the cleaning effect of the material surface [11]. Unfortunately, there have been few studies on the function of superhydrophobic antibacterial materials. In particular, there is little research on multifunctionalization combined with fluorescence.




Scheme 1: Synthesis of the polymer microsphere.

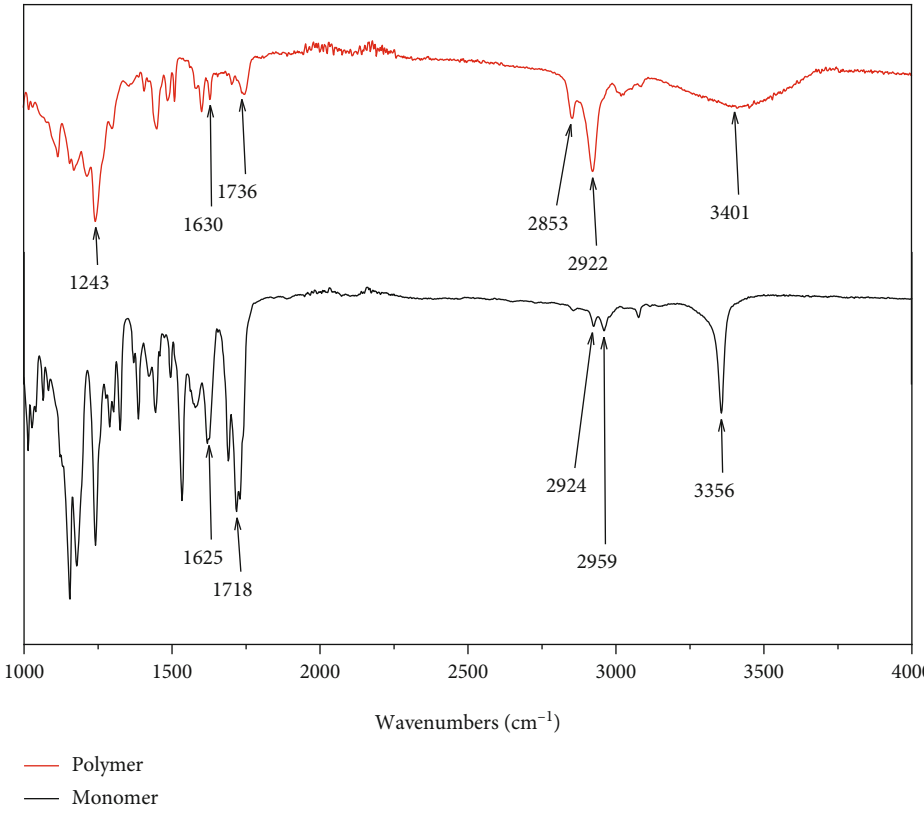



Figure 1: FTIR spectra of the umbelliferone derivative monomer and polymer.

Coumarin is a natural antibacterial compound with fluorescence, and its derivatives have a variety of biological and physicochemical properties such as antibacterial, antifungal, and insecticidal [12–15]. In addition, coumarin diffraction materials have been widely studied and applied in many fields, such as fluorescence labels, fluorescence images, nonlinear optical chromophores, fluorescence probes, and laser dyes, because of their high emissivity, wide spectral range, and excellent optical stability [16–20]. Traditional fluorescent substances, such as rare earth complexes, have hydrophilicity, and their fluorescence properties tend to deteriorate when they are replaced by water molecules in aqueous environments [21]. In addition, rare earth materials are generally toxic [22]. Therefore, coumarin polymers are more stable in water and have more stable fluorescence than traditional fluorescent substances. Coumarin polymers also have excellent antibacterial properties. Brahmbhatt et al. [23] prepared poly3-phenoxycoumarin ethylene and measured its toxicity to a variety of fungi and bacteria using a spectrophotometric method. Venkatesan et al. synthesized the copolymer of 7-methacryloxy-4-methyl coumarin and butyl methacrylate using free radical solution polymerization and studied its thermal properties and antibacterial activity against a variety of bacteria [24]. Overall, coumarin and its derivatives have excellent antibacterial performance and good potential as fluorescent materials, which are ideal for multifunctional antibacterial fluorescent coating polymers.

In this study, a new antibacterial polymer fluorescent coating was prepared by copolymerizing divinylbenzene

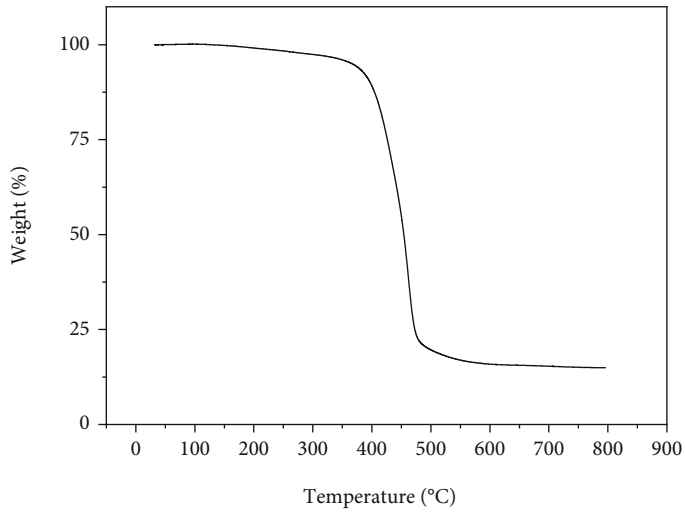



Figure 2: Thermogravimetric analysis of the polymer.

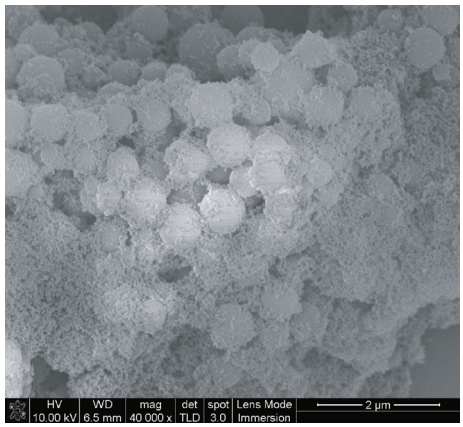



Figure 3: SEM image of the thin film.

with 7-methacryloxy-4-methyl coumarin and dodecafluoroheptyl methacrylate (Scheme 1). The surface structure and thermal stability of the coating were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). The fluorescence properties of the coating were investigated by fluorescence microscopy, and the static contact angle of the coating surface was measured. The sterilization and antibacterial adhesion properties of the coating materials were studied using contact sterilization and antibacterial adhesion experiments. The fluorescent coating material overcomes the shortcomings of rare earth fluorescent coatings that are unstable in water and potentially toxic, resulting in excellent safety, antibacterial, and antiadhesion properties with broad application potential as a new multifunctional fluorescent coating.

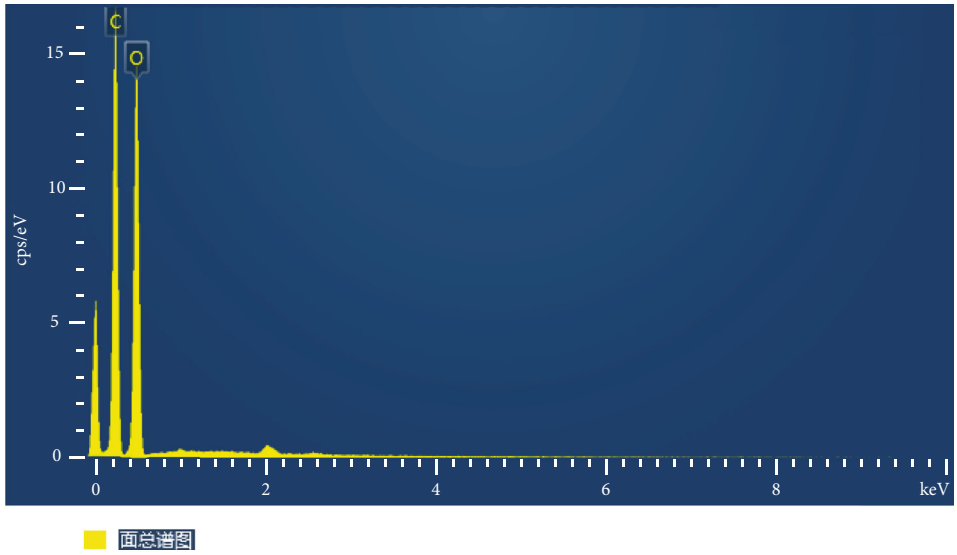
## 2. Experimental

*2.1. Materials.* 4-Methylumbelliferone, dodecafluoroheptyl methacrylate, 2-isocyanatoethyl methacrylate, dibutyltin dilaurate, styrene, sodium dodecyl sulfate, potassium persulfate, 1-hexadecanol, petroleum ether, divinylbenzene, ethyl acetate, acetone, and ethyl alcohol were all purchased from Shanghai Macklin Biochemical Co., Ltd. *Staphylococcus aureus* (ATCC25923) was obtained from Wenzhou Lanbo Biotechnology Co., Ltd.

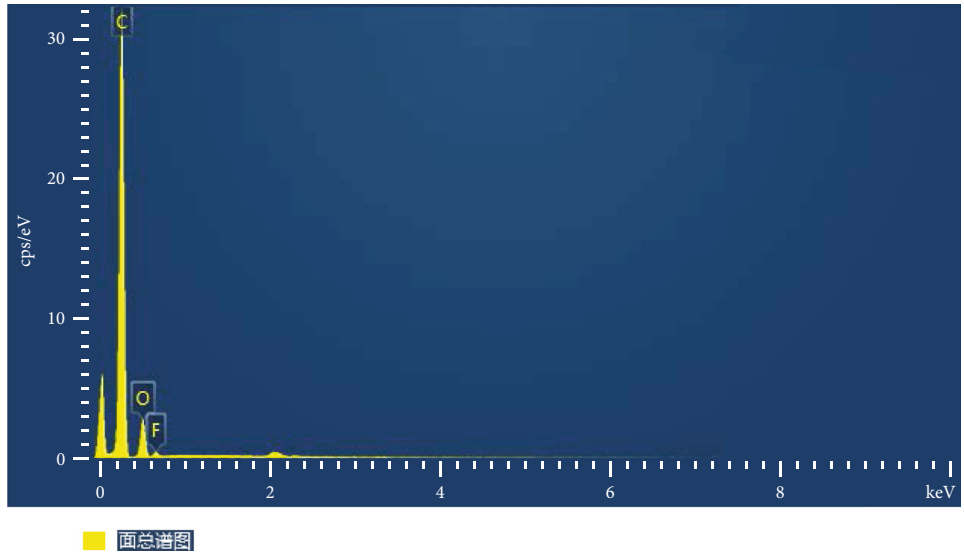
*2.2. Synthesis of 7-Methacryloxy-4-Methyl Coumarin Monomer.* 7-Hydroxy-4-methyl coumarin and acetone were added to a single-neck bottle. After coumarin was dissolved, ethyl isocyanate methacrylate and dibutyltin dilaurate were added; the solution was cooled to room temperature and stirred for 24 h. After the reaction, petroleum ether was added to the reaction solution until a large amount of white crystals precipitated, and then, a Buchner funnel was used for filtration. The resulting product was washed and crystallized with petroleum ether: ethyl acetate (1 : 1) three times, drained dry, and placed in the shade to dry naturally. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.59 (d, $J = 8.4$ Hz, 1H), 7.12-7.15 (m, 2H), 6.26 (s, 1H), 6.17 (s, 1H), 5.65 (s, 1H), 5.42 (s, 1H), 4.33 (t, $J = 5.2$ Hz, 2H), 3.62 (q, $J = 5.5$ Hz, 2H), 2.43 (s, 3H), 1.98 (s, 3H).

*2.3. Preparation of the Superhydrophobic Antibacterial Film.* A solution of 0.25 g sodium dodecyl sulfate, 0.835 g hexadecyl alcohol, and 85 g water in a 250 ml four-necked flask was refluxed for 30 minutes in an oil bath at 70°C under $N_2$ conditions. The reaction system was cooled to room temperature, 13 g divinylbenzene was added, the bottle was placed in an ice bath for ultrasonic oscillation for 2–3 min, and NaCl was added to the ice water bath. The initiator (0.05 g KPS/5 ml water) was added to the solution using a constant pressure dropping funnel, and the solution was heated to 70°C and stirred for 8 h to obtain product A.

A solution of 20 ml water, 0.35 g sodium dodecyl sulfate, 4 g styrene, 5.5 g dodecafluoroheptyl methacrylate, and 1.5 g 7-methacryloxy-4-methyl coumarin monomer was stirred for 30 min to generate solution B. Using two constant pressure dropping funnels, solution B and an initiator (0.45 g




(a)




(b)

Figure 4: (a) EDS plot of the monomer. (b) EDS plot of the polymer.

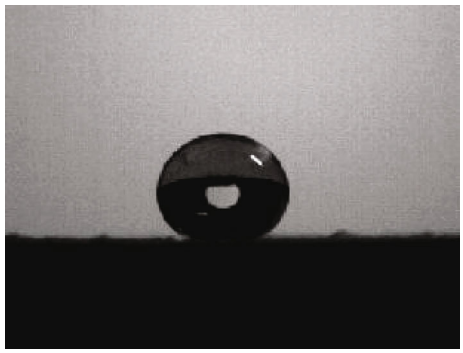


Figure 5: Static contact angle of the polymer film.

KPS/15 ml water) were simultaneously added to Product A. The reaction was then incubated at 80°C for 2 h. The product was cooled to room temperature and centrifuged for 10 min at 3000 rpm. Finally, the product was recovered and dispersed in ethanol. After that, the polymer nanoparticle films were prepared with polymer nanoparticles by directly casting on the clean slide glass, which was coated with the silica gel adhesive. Finally, the films were dried under conditions (room temperature of 25°C and relative humidity of 40%) and then transferred to the vacuum drying oven and dried for additional 24 h. The thickness of the film was 0.365 mm.

*2.4. Characterization.* The film samples were analyzed by FTIR using an EQUINOX55 infrared spectrometer (Bruker, Rheinstetten, Germany). SEM (JEOL JSM-7800F) was used to observe the surface morphology of the film sample. TGA measurements were performed on a NETZSCH TG 209 thermal analyzer. The static contact angle of water was measured at room temperature with a contact angle goniometer (DataPhysics, Germany). At room temperature, the

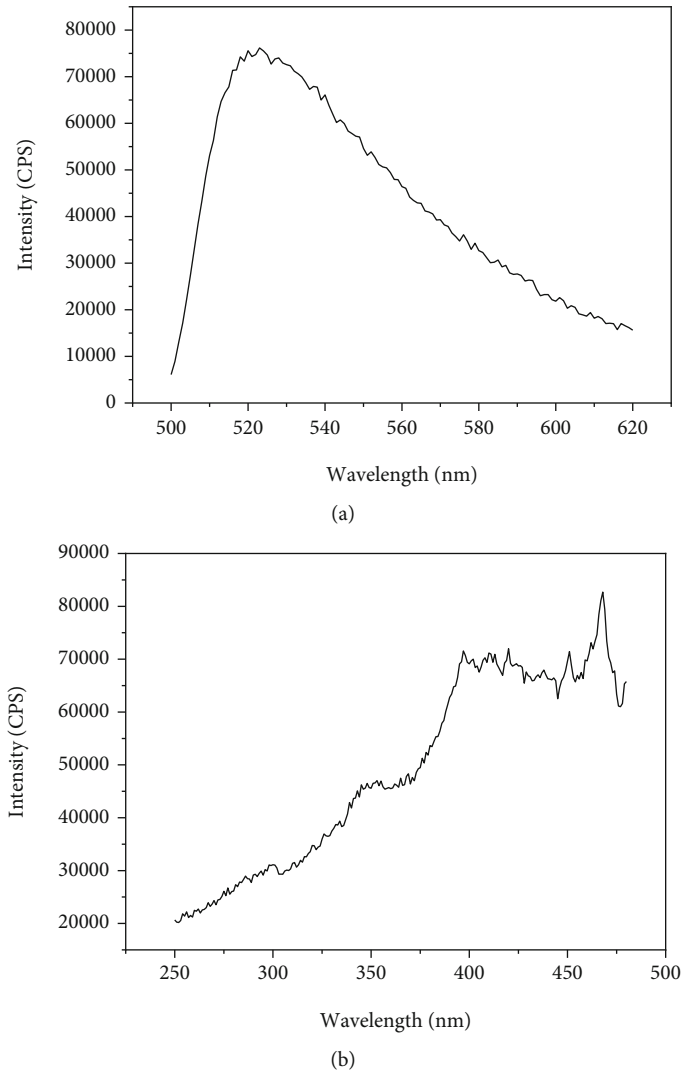



Figure 6: (a) Emission spectra and (b) excitation spectra of the polymer.

fluorescence emission spectra were measured with a PTI-700 fluorescence spectrophotometer at a scanning rate of 600 nm/min. The samples were imaged using an OLYMPUS IX71 inverted fluorescence microscope equipped with a CCD camera.

*2.5. Antibacterial Activity Test.* *Staphylococcus aureus* was cultured overnight in an incubator at 37°C, and the bacterial concentration was adjusted to $1 \times 10^5$ CFU/ml. To test the antibacterial performance of the film-coated samples against *S. aureus*, the samples were completely immersed in a diluted bacterial solution of $10^4$ CFU/ml after UV irradiation for a period of time and then placed in a shaker. After 4 h of culture, 100 $\mu$l of the solution was evenly spread on the solid medium, flipped upside down, and incubated at 37°C for 24 h. A sterile plastic sheet under the same conditions was used as the control. After 24 h, the number of bacteria on the agar plate was calculated using the plate counting method. All bacterial tests were repeated three times.

The antiadhesion ability of the film sample against *S. aureus* was further tested. The sterile plastic sheet and film samples were tilted and immersed in a diluted bacterial solution of $10^4$ CFU/ml at 37°C for 4 hours. The samples were removed and gently rinsed with phosphate buffer solution (PBS) ten times to remove unadhered bacteria. The samples were then placed in a new PBS solution tube, and the bacteria were removed using 5 min of ultrasound at 37°C. The tube was shaken to homogenize the bacteria in the PBS solution, and then, the bacteria solution was removed by continuous dilution and smeared on an agar plate. After 24 h of culture, the number of bacteria on the agar plate was calculated by the plate counting method.

## 3. Results and Discussion

*3.1. FTIR Analysis.* The structure of the product was determined by FTIR analysis. The FTIR spectra of the umbelliferone derivative monomer and polymer are shown in Figure 1.

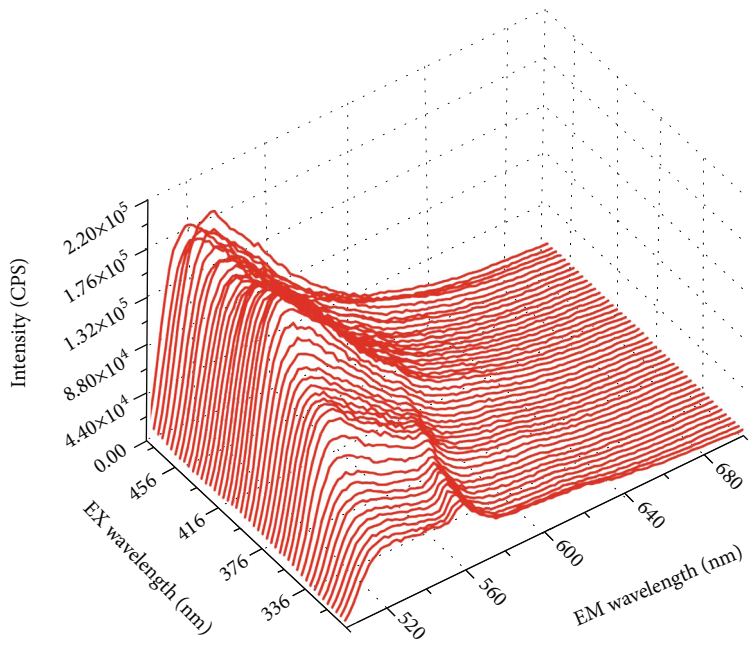



Figure 7: 3D spectrogram of the polymer.

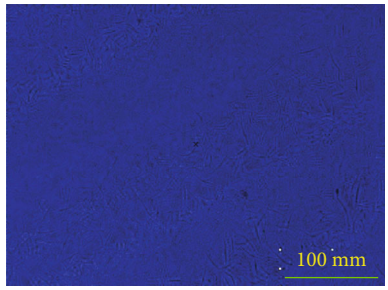



Figure 8: Fluorescence microscopy image of the polymer.

In the monomer, the peaks at 2959 $cm^{-1}$ and 2924 $cm^{-1}$ correspond to C-H bond asymmetric and symmetric stretching vibration frequencies in the $CH_3$ group, respectively. The two peaks at 1718 $cm^{-1}$ and 3356 $cm^{-1}$ were attributed to the C=O bond stretching vibration of ester group and N-H bond stretching vibration of the amide, respectively. The absorption band at 1625 $cm^{-1}$ is caused by the stretching vibration of the amide I band C=O bond. Moreover, in the polymer, absorption bands at 2853 $cm^{-1}$ and 2922 $cm^{-1}$ are caused by C-H asymmetric and symmetric stretching vibration in the $CH_3$ group and at 3401 $cm^{-1}$ are due to the stretching vibration of the N-H bond of the amide. The absorption band at 1630 $cm^{-1}$ is caused by the stretching vibration of the amide I band C=O bond. The absorption band at 1736 $cm^{-1}$ was attributed to the C=O stretching vibration of ester group. For the polymer curve, a characteristic peak at 1243 $cm^{-1}$ is caused by the C-F stretching vibration frequency in the $CF_2$ group, and the presence of this peak proves the successful doping of fluorine in the polymer.

*3.2. Thermal Stability and Mechanical Behavior Analysis.* The TGA curve (Figure 2) clearly shows that the copolymer underwent a single-step degradation, indicating purity. The copolymer had excellent thermal stability from 90°C to 300°C. When the mass loss of the polymer samples reached 5%, the thermal degradation was at 366°C, and the maximum degradation rate corresponded to 460°C. The presence of crosslinking agents and fluorine-containing functional groups resulted in better thermal stability than polystyrene (a maximum degradation rate of approximately 400°C) and polyvinyl benzene (maximum degradation rate of approximately 430°C). The mechanical behavior of the film was evaluated by testing the tensile strength of the film. It was found that the tensile strength of the membrane was 8.3 MPa, while the elongation at break was 220%. This shows that it has excellent physical and mechanical properties. The biggest defect of superhydrophobic membrane materials is poor physical and mechanical properties. Therefore, overcoming this defect is conducive to the application of food packaging.

*3.3. SEM Analysis.* For SEM analysis, the umbelliferone derivative polymer was evenly spread on the glass slide using silica gel and the film was allowed to dry. The SEM image shows that the surface of the film maintained a microsphere shape (Figure 3). These results confirm that the adhesive effectively held the nanoparticles, maintaining suitable adhesion and the original surface morphology of the film material. Figure 4(a) is the EDS plot of the monomer and Figure 4(b) is the EDS plot of polymer. Notably, fluorine was fully polymerized into polymer microspheres.

*3.4. Contact Angle Analysis.* The static contact angle is a method to evaluate the hydrophobicity of the material surface. The static contact angle of the film surface increased with the increase in the wetting resistance. The static contact angle was affected by the chemical composition of the polymer film surface, with the fluorine component improving

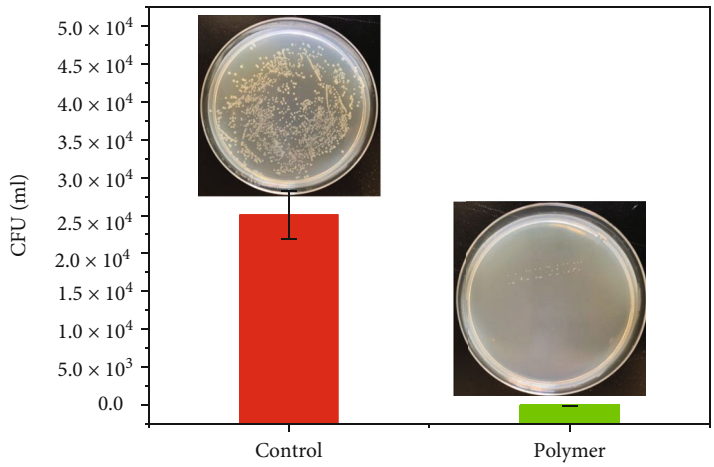



Figure 9: *S. aureus* adhered to the control and polymer groups.

the hydrophobicity of the polymer. Figure 5 shows the 152° static contact angle of the polymer film, indicating that the static contact angle of the polymer film increased when fluorine groups were introduced into the polymer chain, achieving superhydrophobic properties. Fluorine tends to migrate towards the surface and it is easier to locate, thus reducing the inner surface energy, while the static contact angle increased with the decrease in the surface energy. Therefore, by increasing the monomers containing fluorine, the surface hydrophobicity of the polymer nanoparticle coating can be greatly improved and superhydrophobicity can be achieved.

*3.5. Fluorescence Analysis.* The emission spectrum and excitation spectrum of the solid state umbel ketone polymer are shown in Figure 6. The emission spectrum in Figure 6(a) was recorded at an excitation wavelength of 420 nm, indicating a fluorescence effect, and the fluorescence intensity was the highest at 525 nm. Moreover, the excitation spectrum in Figure 6(b) was recorded at 565 nm, indicating fluorescence. Figure 7 shows the 3D spectrum of the umbel ketone polymer. The peak intensity at 525 nm gradually increased with the increase in excitation wavelength, while the peak intensity at 565 nm gradually decreased and disappeared.

In contrast, rare earth particles are easily quenched by fluorescence in water, and the prepared film has excellent waterproof performance. The water resistance and fluorescence stability of the polymer films were investigated by immersion for 5 h, 24 h, and 48 h at room temperature. Surprisingly, their fluorescence intensity changed a little. As shown in Figure 8, polymer films also had high fluorescence stability in the aqueous environments. Water or aqueous solutions are common environments for fluorescence imaging applications; as a result, the polymer film prepared in this study can be used in both dry and wet environments.

*3.6. The Antibacterial Activity of the Material.* The antiadhesion of the polymer film to Gram-positive *S. aureus* was further studied. As shown in Figure 9, compared with the amount in the control group, the amount of *S. aureus* on the surface of the polymer was significantly reduced. The number of *S. aureus* in the control group was $2.51 \times 10^4$ CFU, and the number of viable bacteria adhered to the polymer was less than the detection limit (<10 CFU). The number of *S. aureus* adhered to the surface of the polymer sample decreased (<99.9%). Additionally, the surface of the film sample had superhydrophobicity, which effectively reduced the bacteria adhesion. When the superhydrophobic surface was immersed in water, most of the surface was covered by tiny bubbles, greatly reducing the contact between bacteria and the surface, and the bacteria did not cross the air–water interface because of surface tension. Therefore, the hydrophobic surface effectively prevented bacterial adhesion, the ability to inhibit adhesion was enhanced, and the formation of biofilm was significantly delayed. In addition, because of the presence of 7-methacryloxy-4-methyl coumarin, the surface of the film sample had an antibacterial effect, which kills the adhered bacteria that was on the superhydrophobic surface, and thus had a stronger bactericidal and antiadhesion effect.

## 4. Conclusion

A new antibacterial polymer fluorescent coating was prepared by copolymerizing divinylbenzene with 7-methacryloxy-4-methyl coumarin and dodecafluoroheptyl methacrylate. The bactericidal adhesion properties of the coating materials were studied by contact sterilization and antibacterial adhesion experiments. The prepared film material had excellent antibacterial properties. Moreover, with the increase in hydrophobicity, the ability to inhibit bacterial adhesion was enhanced and the formation of biofilm was significantly delayed. In addition, the fluorescence intensity of the films was almost unchanged when immersed in water at room temperature for up to 48 h, and the polymer films also had high fluorescence stability in the water environment. The static contact angle of the polymer film was 152° after the introduction of fluorine groups into the polymer chain, which achieved a hydrophobic effect. The film had high thermal stability because of the crosslinking agents and fluorine-containing functional groups. Overall, the polymer has broad application prospects as a novel multifunctional fluorescent coating.

## Data Availability

The data used to support the findings of this study are included within the article. Further data or information is available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Wen-Qiang Shi and Jin Zhao contributed equally to this work.

## Acknowledgments

This work was funded by the Zhejiang Provincial Natural Science Foundation (LY16B020006), the Science Foundation of Zhejiang Sci-Tech University (18062144-Y), and the Science and Technology Research Projects of Lishui Sci-Tech Bureau (Grant No. 2020GYX02).

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