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Research Article

Improving Antimicrobial Activity of Carbon/Gelatin Composite by Ce(III)

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Carbon/gelatin/Ce (CGCe) composite was prepared with high antimicrobial activity. And the structure, thermal property, and antimicrobial activity of the composite were investigated. The research results showed that CGCe has a higher thermal property and antimicrobial activity against *S. aureus* and *E. coli* compared with CG. The influences of molecular weight of gelatin, pH, and concentration of Ce (III) on the antimicrobial activity were discussed, and the IC50, MIC, and MBC against *S. aureus* of CGCe are 185 μm·mL^{-1}, 525 μm·mLl^{-1}, and 700 μm·mL^{-1}, respectively, and the IC50, MIC, and MBC against *E. coli* of CGCe are 255 μm·mL^{-1}, 700 μm·mL^{-1}, and 1050 μm·mL^{-1}, respectively.

1. Introduction

Gelatin, which is mainly derived from land animals and fish-processing by-products, including skin, bone, tendon, and scale, has many excellent physical and chemical properties, such as good adhesion, dispersibility, and biocompatibility [1–4]. Thus, gelatin is widely used in food, health care, medicine, and other fields [5–7]. The applications of gelatin in the electrode and ink are hot research areas [3, 8–10]. However, due to the high nutrition of gelatin, it is easy to breed bacteria in the humid environment. So the application of CG has been restricted.

In recent years, rare earth cerium element and its complexes have attracted the worldwide attention because of their high medical value [11, 12]. Rare earth composite materials can play the role in antibacterial, anti-inflammatory, anticoagulant, prevention and treatment of cancer, and arterial hardening [13–15].

Besides, rare earth elements also have the characteristics of low toxicity, weak accumulation, no teratogenic changes, and no smell [16]. The related reports studied the preparation of complexes with gelatin and rare earth cerium, confirming that the antibacterial properties of gelatin solution were improved by using cerium, and the gelatin cerium complex had good antibacterial properties [17, 18]. However, the CG compounded with rare earth, which can improve the antibacterial property, is rarely reported. In this paper, we used gelatin and cerium nitrate as raw materials to prepare gelatin/cerium complex solution and then combined gelatin, cerium, and carbon materials to prepare CGCe. We discussed the antibacterial properties of CGCe and tested the properties of CGCe used as Chinese ink.

2. Materials and Methods

2.1. Materials. Provided gelatin was purchased from Rousselot Co. (Guangdong, China). Cerium(III) nitrate hexahydrate was supplied by QinXi Chemical Co. (Shanghai, China). The carbon ink was supplied by Yidege Ink Industry Co. (Beijing, China); nutrient agar was supplied by Luqiao, Beijing; *Staphylococcus aureus* and *Escherichia coli* were supplied by Tianjin Centers for Disaster Control and Prevention. Other reagents were all used for purity analysis.

2.2. Preparation of CGCe. 0.217 g $Ce(NO_3)_3$ was dissolved in 5 mL deionized water and the different pH was adjusted. Then, the $Ce(NO_3)_3$ solution was added to the gelatin solution (2 wt.%, 45 mL), and the mixed solution was placed

in a water bath at 60°C for 4 h to achieve a homogeneous system. Then, 3.6 g of the gelatin cerium complex solution was added with 0.1 g carbon ink and a certain amount of Turkey red oil and glycerol and placed in a ball mill for several hours to prepare the CGCe composite solution. And the CGCe composite solution was further dried to prepare CGCe.

2.3. Structural Characterization of CGCe. The CGCe composite solution was dried to form a film. Then, the CGCe film was cut into strips as CGCe film samples. The infrared absorption spectrum of the CGCe film was tested by Fourier-transform infrared spectrometer (Nexus 670, Nicolet, USA), and the wave number range was 4000-400 cm^{-1}. The CGCe composite solution was diluted to a fixed multiple and measured by UV-vis spectrophotometer (TU-1810, UNICO, USA) at a range of wavelength from 200 to 400 nm. The thermal property of CGCe was measured by a thermogravimetric analyser (HCT-1, China) from 25°C-800°C.

2.4. Antibacterial Properties of CGCe. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CGCe were tested by the colony counting method. The bacterial solutions separately inoculated with *S. aureus* and *E. coli* were incubated at 37° C for 24 h and diluted to 1.0×10^6 CFU/mL. The bacterial solutions were inoculated to the nutrient agar in the Petri dish, and CGCe solutions with different concentrations were added. After incubation at 37°C for 24 h, the growth of colonies in the culture dish was observed. The lowest concentration of the CGCe solution that completely inhibited the growth of colonies was determined as MIC. The highest concentration of the CGCe solution that exhibited no bacterial growth on agar plates after incubation at 37°C for another 18 h was identified as the MBC. Lasting antibacterial properties of CGCe were determined by the agar digging method, and the inhibition zone diameters were measured in a time range of 12-96 h. The half-maximal inhibitory concentration (IC50) of CGCe was evaluated by the serial dilution method, and the optical density measured at 600 nm using pan-wavelength microplate spectrophotometer (Molecular Devices, America).

2.5. Influence Factors of Antibacterial Properties of CGCe. We changed the molecular weight of gelatin, pH of system, and the concentration of Ce(III), then tested the antimicrobial activity of CGCe.

2.6. Application Test of CGCe Ink. CGCe was applied as an antibacterial ink, and its properties were tested as follows and compared with commercial ink (commercial 1 and 2): the blackness by reflection densitometer (MN-B2, China), the particle size by DLS (Omni, USA), the writing to observe carbon distribution by biologic photomicroscope (SG-1100, China), the water resistance activity by water bath method, the wettability by water contact angle method; the antimicrobial properties by the colony counting method and agar digging method, and the writing properties were tested.

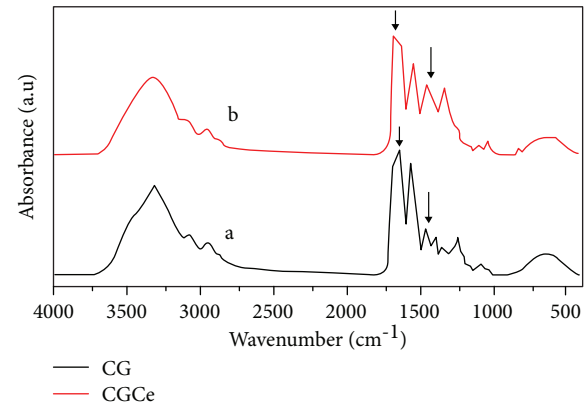


Figure 1: FTIR spectra of CG and CGCe.

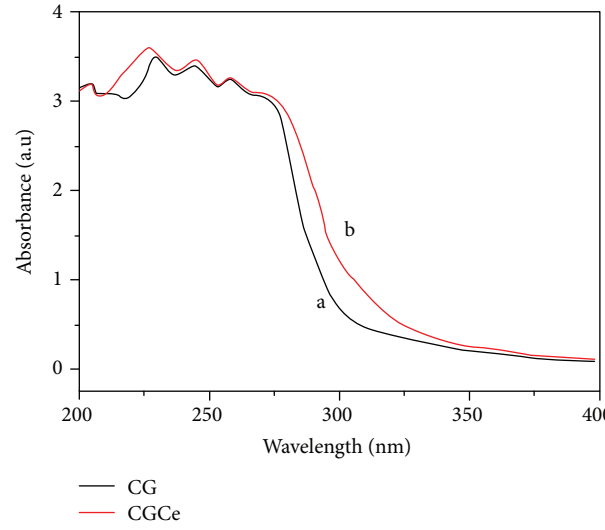


Figure 2: UV-vis spectra of CG and CGCe.

3. Results and Discussion

3.1. Characterization of CGCe. Previous researches demonstrated that there are three coordinate sides in gelatin which may react: oxygen in -COOH in side chains, nitrogen in $-NH_2$, and nitrogen in $-CONH_2$. When the pH is at about 4.6, the carboxyl group could react with Ce(III). It can be obviously seen that the FTIR spectra (Figure 1) of the CGCe are different from those of the CG: the amide I band shifted from 1454.34 cm^{-1} to 1450.16 cm^{-1}; the amide II band shifted from 1639.28 cm^{-1} to 1673.46 cm^{-1}. $\Delta\nu$ (difference of symmetric carboxyl and antisymmetric carboxyl) changed to 223.30 cm^{-1}, which proved that Ce(III) reacted with the carboxyl group in side chains of gelatin.

The UV-vis spectra (Figure 2) show that the gelatin/C composite displayed a distinct absorption peak at 230 nm and a weak absorption peak at 250 nm. In contrast, curve B shows that CGCe displayed a broad and sharper absorption peak at 230 nm and a sharper absorption peak at 250 nm, which might be attributed to the reaction between gelatin and Ce(III).

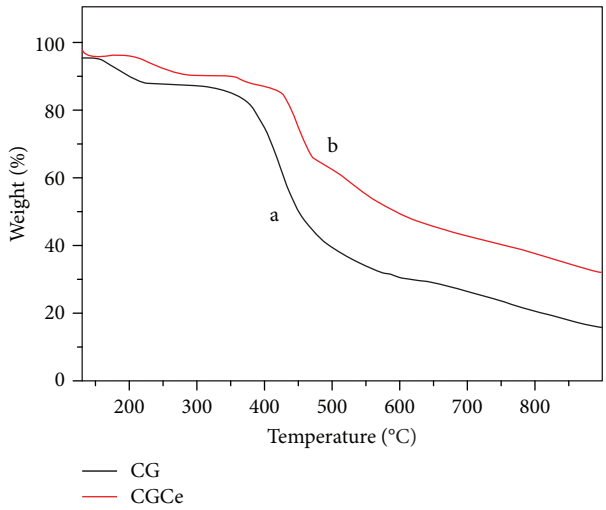


Figure 3: TGA test of CG and CGCe.

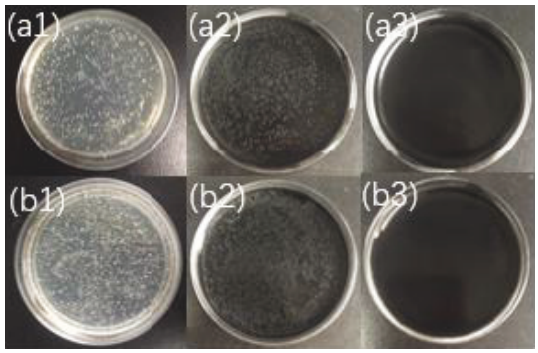


Figure 4: Antimicrobial qualitative test: ((1) gelatin; (2) CG; (3) CGCe; (a) *S. aureus*; (b) *E. coli*).

The TGA curves (Figure 3) revealed that the onset thermal-decomposed temperature between CG and CGCe has obviously improved by 69.4°C (from 272.1°C to 341.5°C), which demonstrated that the thermal stability had been improved due to the electrostatic crosslinking effect of Ce(III).

3.2. Antibacterial Qualitative Analysis of CGCe. The result of antibacterial activity qualitative experiment against *S. aureus* and *E. coli* of gelatin, CG, and CGCe tested by the colony counting method (Figure 4) showed that the Petri dishes of gelatin and CG had obvious growth of bacteria, while the Petri dishes of CGCe had no bacteria, which can indicate that Ce(III) can improve the antibacterial activity of CGCe.

3.3. Antibacterial Property of CGCe. The antibacterial properties of CGCe obtained from different gelatin molecular weights are shown in Figure 5. It displays that with the increase of gelatin molecular weight, the inhibition zones of CGCe decreased. It is because CGCe that is prepared with low-Mw gelatin has a low molecular weight, which can help the Ce-gelatin/C composite penetrate the lipid layer in cell membranes of bacteria more easily to retard bacterial growth.

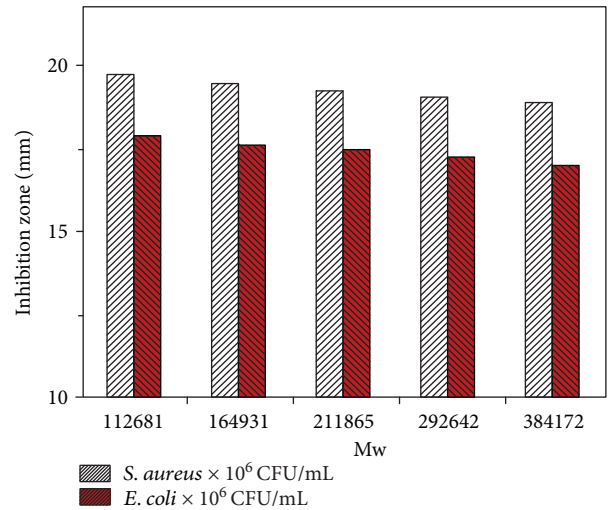


Figure 5: Influence of Mw of gelatin on antimicrobial activity.

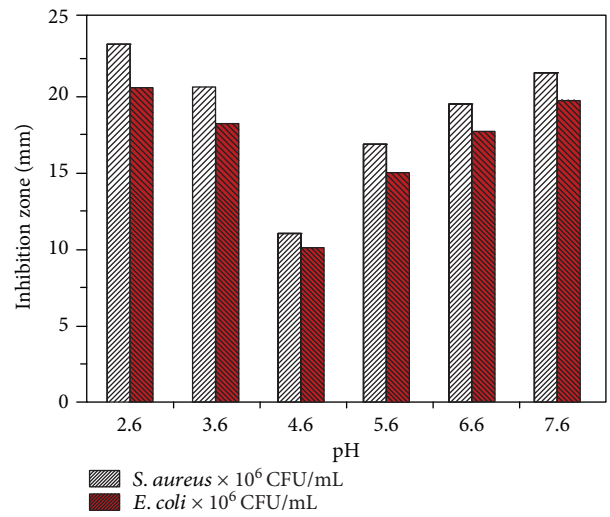


Figure 6: Influence of pH on antimicrobial activity.

The effect of CGCe prepared by different pH systems on the antibacterial property is shown in Figure 6. With the increase of pH, the diameters of the inhibitory zone of CGCe against *S. aureus* and *E. coli* decreased first when the pH value is in the range of 2.6-4.6. The inhibition zone got lowest when pH = 4.6. After that, the diameters of the inhibition zone on the two bacteria began to increase with the further increase of the pH value. The reason is that pH = 4.6 is the isoelectric point of gelatin and CGCe has the lowest solubility. So CGCe can hardly penetrate the glycerophospholipid bilayer in cell membranes of bacteria, which lead to a lower antibacterial property.

Figure 7 shows the antibacterial activity of CGCe at different Ce(III) concentrations. It displays that with the increase of concentration of Ce(III), the inhibition zones against *S. aureus* and *E. coli* of CGCe were higher. It is indicated that with the addition of effective inhibitory substance Ce(III), the inhibitory effect of CGCe on bacteria is more obvious.

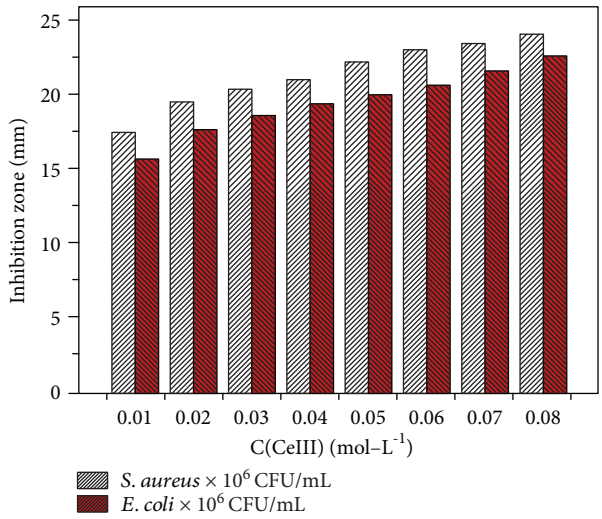


Figure 7: Influence of concentration of Ce(III) on antimicrobial activity.

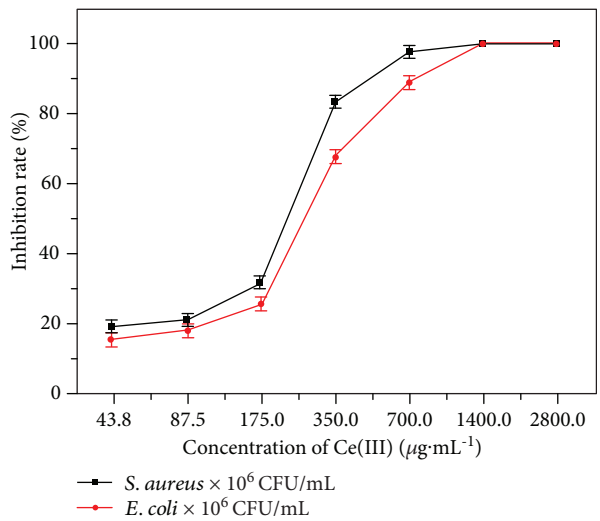


Figure 8: Inhibition rate of CGCe against *S. aureus* and *E. coli*.

3.4. IC50, MIC, and MBC. Figure 8 and Table 1 show that the IC50, MIC, and MBC against *S. aureus* of CGCe are 185 $\mu g \cdot mL^{-1}$, 525 $\mu g \cdot mL^{-1}$, and 700 $\mu g \cdot mL^{-1}$, respectively. And the IC50, MIC, and MBC against *E. coli* of CGCe are 255 $\mu g \cdot mL^{-1}$,700 $\mu g \cdot mL^{-1}$, and 1050 $\mu g \cdot mL^{-1}$, respectively. These results suggest that the antimicrobial activity of CGCe can be improved with the added small quantity of Ce(III).

3.5. Long-Lasting Antibacterial Property. The long-lasting antimicrobial properties of CGCe were tested. The result shows (see Figure 9) that 96 hours later, the inhibition zones against *S. aureus* and *E. coli* could still keep 89.3% and 89.5% of the 12-hour data, which indicated that CGCe had an excellent long-lasting antimicrobial activity.

From the above antibacterial test results, it can be seen that CGCe has better antibacterial activity against *S. aureus* than *E. coli*. It may be because the cell wall of the Gram-positive bacteria consists of 90% peptidoglycan (a complex polysaccharide network) whereas that of the Gram-negative bacteria contains only 10% of this polysaccharide and *S. aureus* retains a greater amount of CGCe in the cell wall, helping to make the zone of inhibition wider.

Table 1: IC50, MIC, and MBC of CGCe.

Bacteria	$\mu g \cdot mL^{-1}$		
	IC50	MIC	MBC
S. aureus	185	525	700
E. coli	255	700	1050

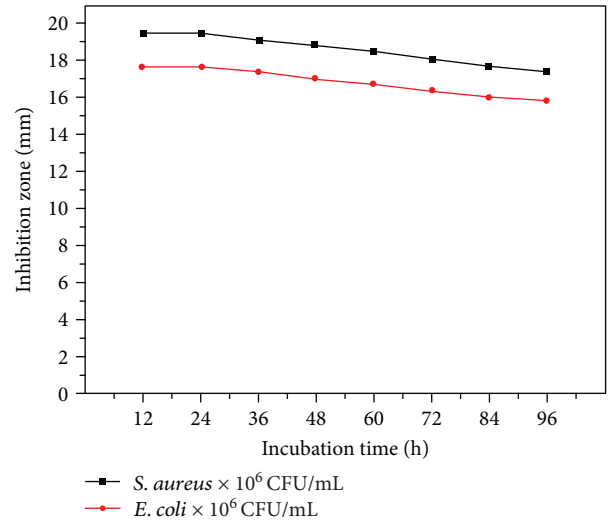


Figure 9: Long-lasting antibacterial property of CGCe.

Table 2: Color test results of the ink.

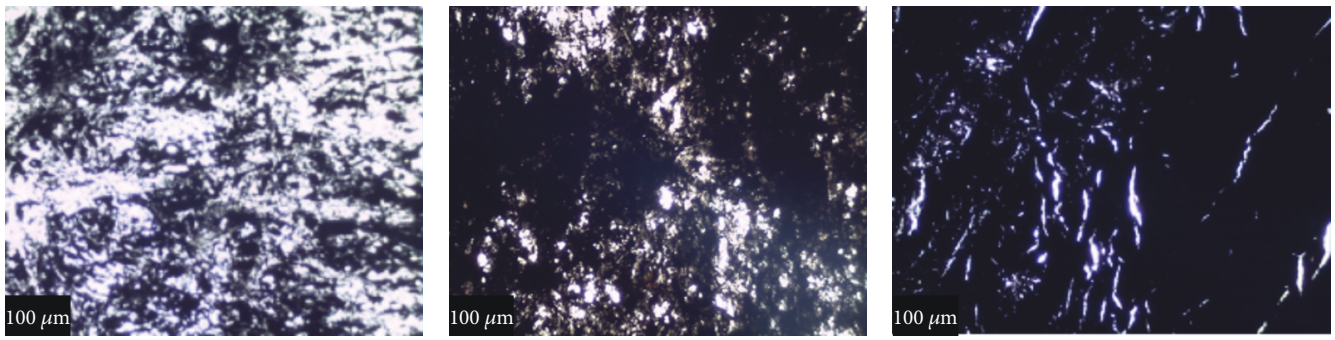
Samples	Average blackness
Commercial 1	1.357 ± 0.02
Commercial 2	1.510 ± 0.08
CGCe ink	1.452 ± 0.02

Table 3: Color test results of the ink after centrifugation.

Samples	Average blackness before centrifugation*0.9	Average blackness after centrifugation
Commercial 1	1.2231	1.353 ± 0.03
Commercial 2	1.3590	1.459 ± 0.07
CGCe ink	1.3068	1.358 ± 0.02

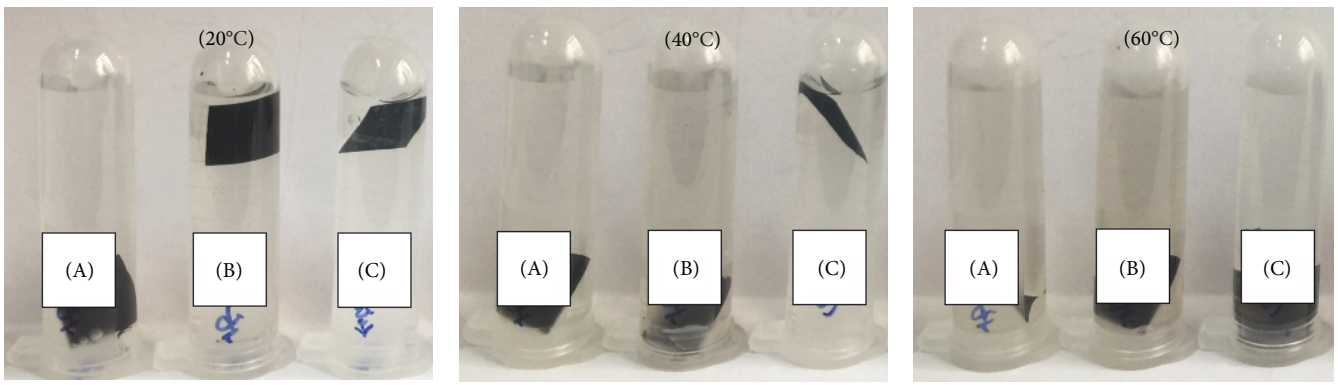
Table 4: Particle size and dispersity of different inks.

Samples	Particle size	Dispersity
Commercial 1	147.36 ± 6.874	0.312 ± 0.052
Commercial 2	167.84 ± 10.560	0.314 ± 0.081
CGCe ink	172.08 ± 2.557	0.152 ± 0.018



(a) (b) (c)

Figure 10: OM image of different ink ((a) commercial 1; (b) commercial 2; (c) CGCe ink).



(a) (b) (c)

Figure 11: Water resistance test of different inks ((A) commercial 1; (B) commercial 2; (C) CGCe ink).

3.6. Antibacterial Ink with CGCe. Tables 2 and 3 show that the blackness of commercial ink 1, commercial ink 2, and CGCe ink were 1.357, 1.510, and 1.452. The centrifugal blackness was 1.353, 1.459, and 1.358. The blackness of the CGCe ink was as good as that of the commercial ink and better than commercial 1. The standard for ink (QB/T 2860-2007) describes that the blackness of common ink, middling ink, and high-quality ink has to be equal or greater than 1.35, 1.40, and 1.45, respectively, and the centrifugal blackness of middling ink or high-quality ink has to be equal or greater than 90% of the original blackness. Therefore, the CGCe ink could satisfy the demand of standard for high-quality ink.

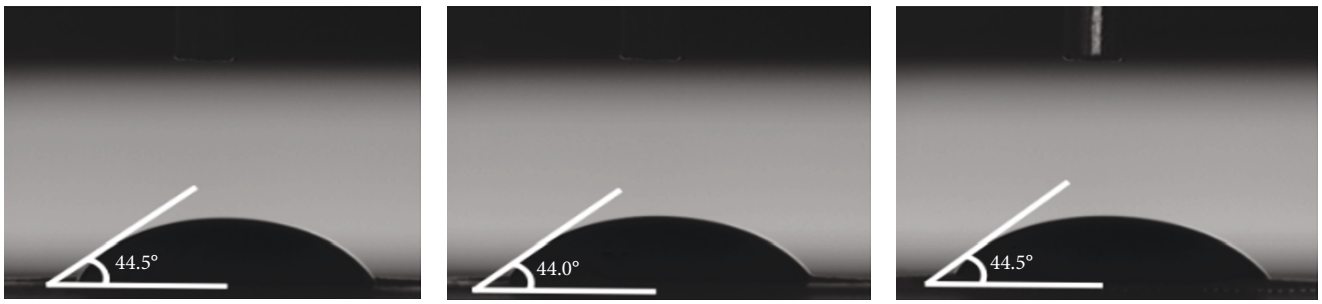
The particle size and dispersity of different inks (Table 4) showed that the particle size of the CGCe ink was larger than that of the commercial ink, which means the fineness needs to be refined. The dispersity of the CGCe ink was smaller than that of the commercial ink, indicating that the CGCe ink dispersed better.

The distribution of carbon in the different ink writings was observed by an optical microscope at 400 times (Figure 10). It can be seen that carbon in the commercial 1 ink was less, and the blank area was obvious, indicating that the carbon distribution was not uniform enough; that of commercial 2 was more, but the blank regional was still obvious; the adhesion of carbon in the CGCe ink writing was sufficient and uniform; also, the blank area was inconspicuous.

Table 5: Rate of losing of different inks.

Samples	Temperature (°C)				
	20	30	40	50	60
Commercial1	0.00	0.00	0.73	0.76	0.94
Commercial2	0.71	0.73	1.36	1.51	3.97
CGCe Ink	0.00	0.00	0.41	0.55	0.77

The standard for ink (QB/T 2860-2007) describes that after soaking in water (paper written with ink) for 6 hours, the solution can still keep clear at room temperature. The results show that the three inks can meet all the requirements (Figure 11). To compare the specific distinction among different inks in water-resistant activity, the temperature was improved from 40°C to 50°C. With the temperature increased, the rate of losing ink has increased (Figure 11 and Table 5). At the same temperature, the rate of losing ink of the CGCe ink was lower than that of the commercial ink, which explained that the modified antibacterial ink had a good water-resistant activity.



(a) (b) (c)

Figure 12: Water contact angle of different inks ((a) commercial 1; (b) commercial 2; (c) CGCe ink).

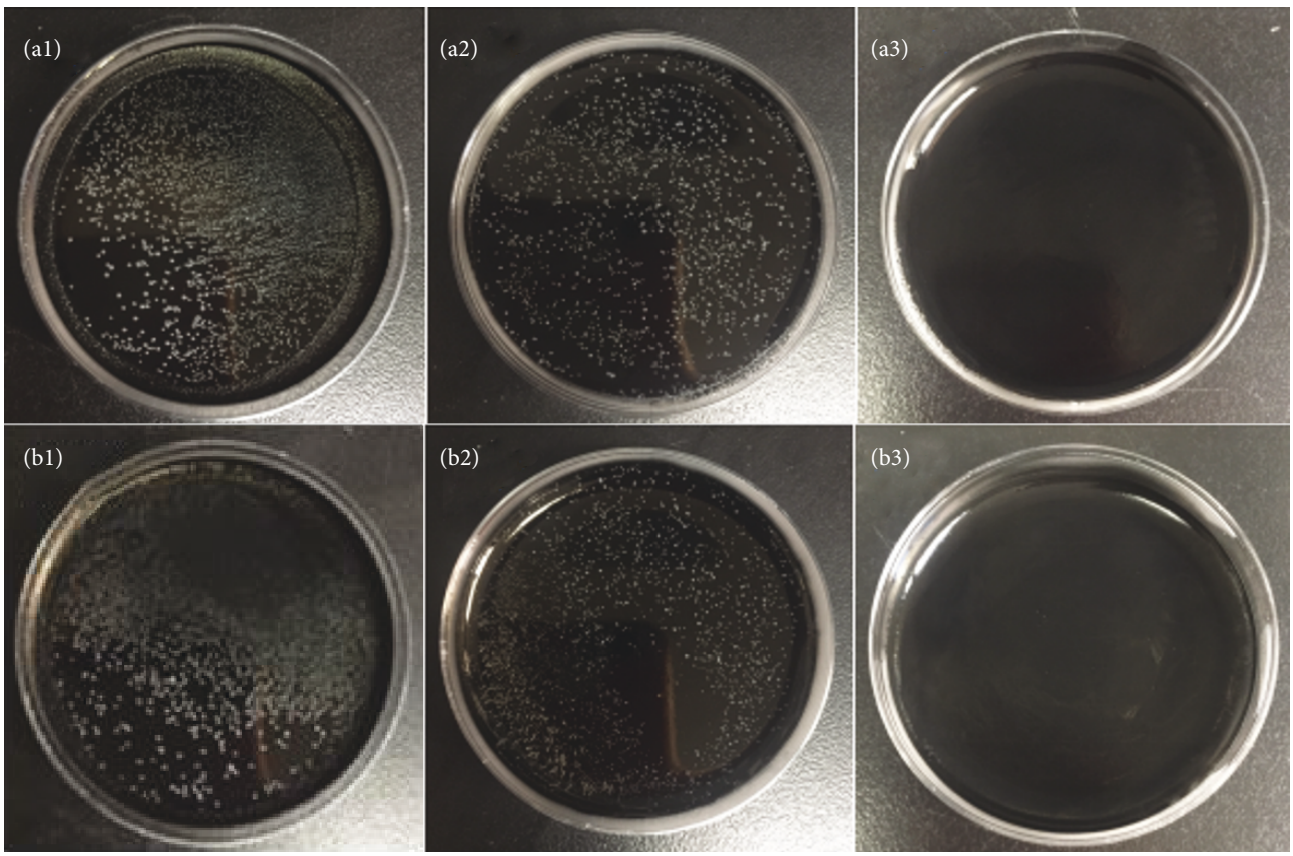


Figure 13: Antimicrobial qualitative test ((1) commercial 1; (2) commercial 2; (3) CGCe ink; (a) *S. aureus*; (b) *E. coli*).

The water contact angle of the commercial 1 ink, commercial 2 ink, and CGCe ink was 44.5°, 44.0°, and 44.5°(Figure 12) and showed that the wetting properties of the CGCe ink is as good as that of the commercial ink because glycerol and Turkey red oil are both surface-active agents and can improve the wetting property of the CGCe ink.

According to Figures 13 and 14, the Petri dishes of the commercial ink had obvious growth of bacteria and had no obvious inhibition zones, while the Petri dishes of the CGCe ink had no growth of bacteria and obvious inhibition zones, which indicated that the CGCe ink had a better antibacterial activity than the commercial ink against *S. aureus* and *E. coli*. Meanwhile, the CGCe ink used Ce(III) instead of phenol; therefore, it had higher biosecurity.

According to Table 6, the writing image of different inks showed that the CGCe ink was as good as the commercial ink. The CGCe ink had a good sense of depth and a good blackness and can be used to write smoothly; meanwhile, it can dry rapidly and the wring had no granular sensation and better than the commercial 2 ink. It can be concluded that CGCe ink can be used in practical application.

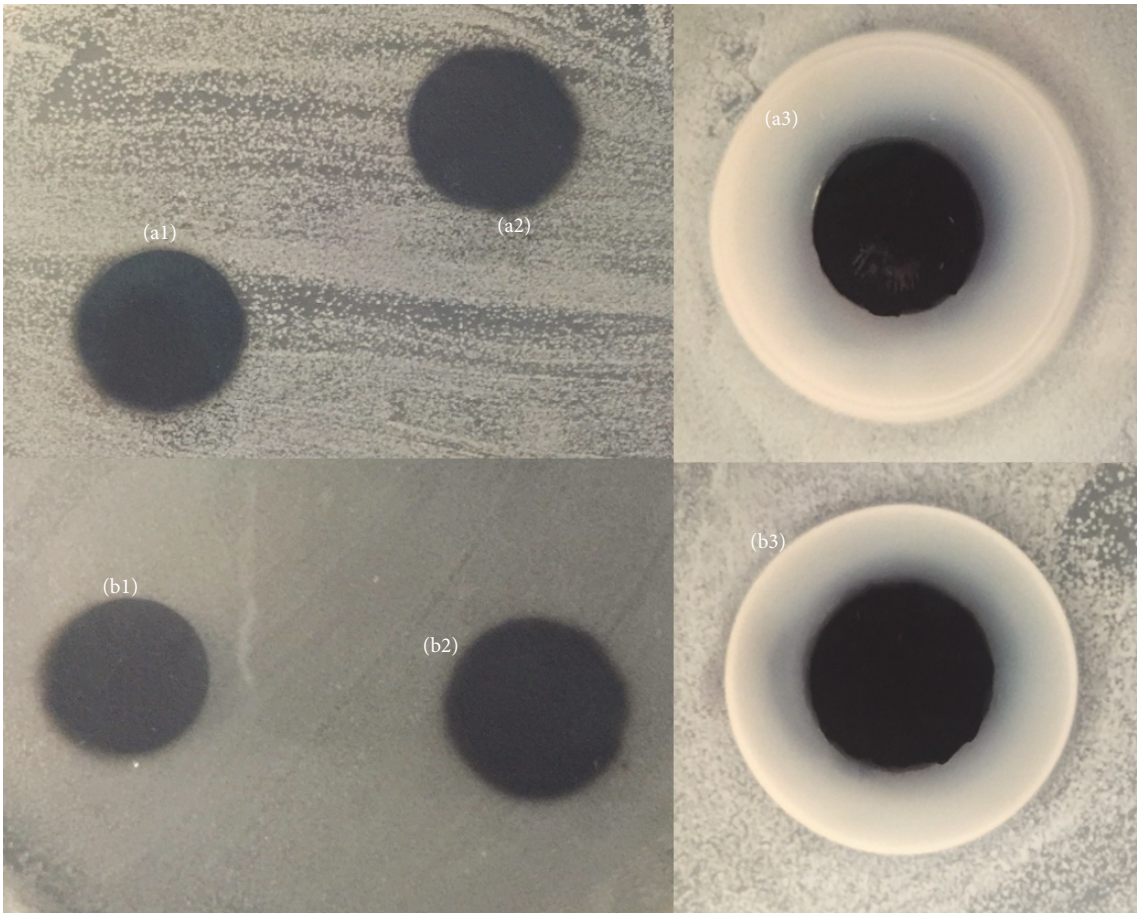


Figure 14: Inhibition zone test ((1) commercial 1; (2) commercial 2; (3) CGCe ink; (a) *S. aureus*; (b) *E. coli*).

Table 6: Writing activity test of different inks.

Samples	Writing ease	Drying rate	Granular sensation
Commercial 1	Good	Fast	No
Commercial 2	Good	Fast	A little
CGCe ink	Good	Fast	No

4. Conclusions

The antibacterial activity of CG has been improved using Ce(III) as an additive. The results of the FTIR spectra and UV-vis spectra tests showed that the O of -COOH on the side chains of gelatin reacted with Ce(III) to form a coordination structure.

CGCe has a relatively high thermal stability and antibacterial activity and a good antimicrobial durability against *S. aureus* and *E. coli*.

The results showed that the IC50, MIC, and MBC against *S. aureus* of CGCe are 185 $\mu g \cdot mL^{-1}$,525 $\mu g \cdot mL^{-1}$, and 700 $\mu g \cdot mL^{-1}$, respectively, and the IC50, MIC, and MBC against *E. coli* of CGCe are 255 $\mu g \cdot mL^{-1}$,700 $\mu g \cdot mL^{-1}$, and 1050 $\mu g \cdot mL^{-1}$, respectively.

The CGCe ink was as good as the commercial ink and shows better antibacterial activity and biosecurity than the commercial ink.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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