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Preparation and Performance of Chitosan-gallic acid/Methacrylic Anhydride Composite Antibacterial Hydrogel

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Abstract: Firstly, chitosan-gallic acid derivative (GA-CS) was prepared by reacting chitosan (CS) with gallic acid (GA), which was combined with chitosan (CM) modified by methacrylic anhydride (MA) acylation as an antibacterial modifier, and the composite hydrogel was prepared by light curing. The mechanical properties, swelling rate, surface contact angle, water retention and micromorphology of the hydrogel were tested to explore the effect of GA-CS on the antibacterial effect of the composite hydrogel. The results showed that the compressive strength of the composite hydrogel was 225kPa, and with the addition of the antimicrobial monomer GA-CS, the antibacterial effect and water retention rate of the composite hydrogel increased. However, the surface contact angle with water increased compared with that without GA-CS, and the swelling rate decreased. When the mass ratio of GA-CS to CM was 2:98, the diameter of the inhibition zone increased from 9.85mm to 13.96mm of the pure chitosan sample, the surface contact angle with water was 17.6°, the swelling rate of the sample was 79.32% in 24 hours, and the water content of more than 50% could be maintained for about 7 hours.

Keywords: Chitosan; Gallic Acid; Methacrylic Anhydride; Hydrogels; Antibacterial.

1. Introduction

Hydrogels are composed of a three-dimensional network of polymers, which have good biocompatibility, high water content, and hydrophilicity, which can provide a good healing environment for wounds, and their porous structure also provides the possibility of adding antimicrobial monomers to improve the antibacterial effect, and are often widely used as wound dressing substrates [1,2]. Chitosan (CS) is a natural cationic polysaccharide polymer with good antibacterial, hemostatic, and wound healing biological properties, and has been widely used in the field of wound dressings [3]. However, the many amino groups on the chitosan backbone make it appear weakly alkaline, insoluble in water or organic solvents, and only in acidic media, the amino groups can be protonated to promote their dissolution. In addition, strong intermolecular and intramolecular hydrogen bonds also limit its solubility, leading to a decrease in antimicrobial efficacy. Some researchers [4] have combined copper ions with chitosan hydrogels to improve their antibacterial effect, but the presence of a large number of copper ions will cause harm to the human body. Some researchers have also compounded cerium oxide nanoparticles into chitosan hydrogel coatings [5], which show good antibacterial effects, but their cytotoxicity is high.

Gallic acid (GA) is a small phenolic molecule of natural plant origin with antioxidant and antimicrobial effects [6]. Gallic acid has received extensive attention in applications such as food, medicine, biological sciences, and cosmetics [7]. Gallic acid is a polyphenolic organic compound, the structure contains three phenolic hydroxyl groups and one carboxyl group, which can be prepared by grafting reaction with the amino group in chitosan with the help of Schiff base reaction, and a large number of phenolic hydroxyl groups in the structure can show good antibacterial effect.

Based on the above ideas, in this paper, chitosan-methacrylamide modified chitosan was first prepared, and the acidity of methacrylic anhydride was used to exert the antibacterial properties of chitosan, and carbon-carbon double bonds were introduced to make it cross-linked and solidified to form polymer three-dimensional network hydrogels under appropriate conditions. Then, chitosan-gallic acid derivatives (GA-CS) were prepared by grafting method, and GA-CS and CM were mixed and solidified with different mass ratios to prepare different composite hydrogel antibacterial dressings. To investigate the effect of GA-CS on the antibacterial and adhesion properties of composite hydrogels.

2. Experimental Part

2.1. Reagents & Instruments

Chitosan (deacetylation degree ≥ 95%, viscosity 100-200mPa.s), gallic acid, methacrylic anhydride, photoinitiator 2959, chemically pure; Tris-hydrochloric acid buffer (1mol/L, pH 8.0), the above drugs are purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., absolute ethanol, glacial acetic acid, analytical pure, Tianjin Chenguang Chemical Co., Ltd.; Nutrient agar, Shanghai Kramar Reagent Co., Ltd.

Fourier transform infrared spectrometer (FT-IR, Nicolet-380), Thermo Fisher, United States; Contact Angle Tester (DY-5010A), Dongguan Dongyang Machinery Co., Ltd.; Drawer UV curing machine (INTELLI-RAY 400), Shenzhen Huishuo Electromechanical Co., Ltd.; Electric Pressure Steam Sterilizer (XFH-30CA Type), Zhejiang Xinfeng Medical Equipment Co., Ltd.; Constant temperature culture shaker (YHZ-300C type), Shanghai-Heng Scientific Instrument Co., Ltd.

2.2. Preparation of CM/GA-CS Composite Hydrogel

2.2.1. Preparation of Chitosan-gallic Acid Derivatives

According to Ref. [8], 3.0 g of gallic acid was added to an appropriate amount of Tris-HCl buffer, stirred at 60°C until completely dissolved, and 1.0 g of chitosan powder was added and stirred at constant temperature for 12 h. After the reaction was complete, 25mL of absolute ethanol was added to precipitate for 24 hours, and the precipitate was washed with PBS buffer (pH=7.45) and distilled water for 3 times, and chitosan-gallic acid derivative (GA-CS) was obtained by drying. The reaction mechanism is shown in Figure 1.

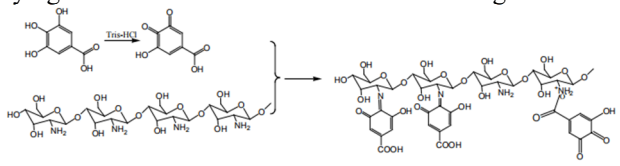

Figure 1. Reaction mechanism of chitosan with gallic acid

2.2.2. Preparation of Chitosan-methacrylamide

0.4g chitosan powder was dissolved in 20mL of dilute acetic acid with a concentration of 1%, stirred to 60 °C, then 5.0mL of methacrylic anhydride was added drop by drop, stirred at constant temperature for 1.5h, $NaHCO_3$ solution was added, and then the mixed solution was dialysized in deionized water until the internal and external pH values reached equilibrium, and the chitosan-methacrylamide (CM) prepolymerization solution was obtained by evaporation and concentration. The reaction mechanism is shown in Figure 2.

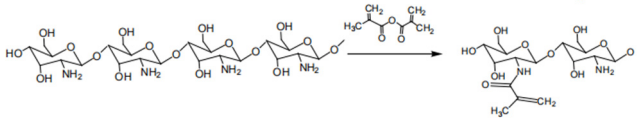

Figure 2. Reaction mechanism of chitosan with methacrylic anhydride

2.2.3. Preparation of Chitosan-gallic Acid/Methacrylic Anhydride Composite Hydrogel

The GA-CS and CM prepolymerization solutions were stirred and mixed with different mass ratios (0:100, 1:99, 2:98), photoinitiator 2959 with a mass fraction of 1% was added, mixed evenly and transferred to the mold, and placed in the ultraviolet curing chamber for 120s to cure the CM/GA-CS composite hydrogel, and the samples were labeled as CM/GA-CS0, CM/GA-CS1 and CM/GA-CS2 respectively.

2.3. Testing and Characterization

Infrared spectroscopy of samples CS, GA-CS, and CM was performed in a scanning range of 4000-500 cm^{-1}. The hydrogel samples were freeze-dried and gold-sprayed, and the topography of the hydrogel surface and cross-section was observed by SEM. The mechanical properties of hydrogels were evaluated by compressive stress-strain curves using an electronic universal testing machine, and CM/GA-CS0 samples were selected to prepare hydrogel cylinders with a radius of 12.5mm and a height of 10mm. The contact angle of the hydrogel surface to the water was measured by the contact angle tester, and each sample was measured 5 times to take the average value.

Swelling rate test: Under the condition of 25 °C, the composite hydrogel sample with initial mass of m_0 was put into the PBS buffer solution with pH = 7.45, soaked for 24 hours, the sample was taken out and the surface moisture was dried with filter paper, and the mass was weighed as m_1. The swelling rate of the hydrogel is calculated according to equation (1).

$$\text{Swelling rate} = [(m_1-m_0)/m_0] \times 100\% \quad (1)$$

Water retention test: Under the condition of 37°C, the composite hydrogel sample with an initial weight of m_t is placed in the air, and its mass m_d is measured at certain intervals during the process of water loss. The water holding capacity of hydrogels is calculated according to equation (2).

$$\text{Water retention rate} = m_t/m_d \times 100\% \quad (2)$$

Antibacterial test: Referring to GB/T20944.1-2007, the antibacterial performance of the sample was tested [9], The test strain was Escherichia coli, and the antimicrobial performance of the sample was characterized by the diameter of the inhibition zone. The hydrogels were made into round samples with a diameter of 4mm, the liquid sterile agar medium was poured into the Petri dish, and after it cooled and solidified, an appropriate amount of bacterial solution was taken and evenly coated on the plate, and the samples were placed in different areas of the Petri dish and sealed at 37°C. After 24 hours, the diameter of the inhibition zone was determined by vernier caliper.

3. Results and Discussion

3.1. FTIR Analysis of Chitosan before and after Grafting

The FTIR spectra of GA-CS are shown in Figure 3, with a wide absorption peak at 3425 cm^{-1} for CS and GA-CS, corresponding to the expansion and contraction vibration of -OH in the chitosan structure. The 1413cm^{-1} area corresponds to the characteristic absorption peak of $-NH_2$ in the chitosan structure. The intensity of the absorption peak in the corresponding GA-CS decreased, indicating that the amino group in chitosan was involved in the reaction. At 1597cm^{-1}, it represents an increase in the absorption peak of C=N stretching vibration, demonstrating the formation of Schiff base bonds, resulting in a decrease in the intensity of the absorption peak for NH3+. These results show that the amino group in CS undergoes a Schiff base reaction with the oxidized GA, and gallic acid is successfully grafted in the chitosan structure.

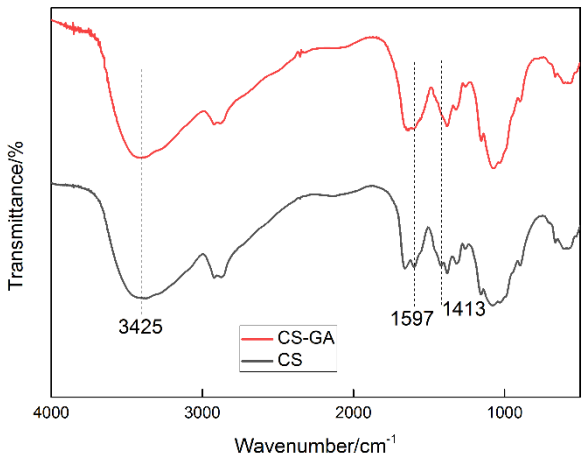

Figure 3. FTIR spectrogram of CS and GA-CS

The FTIR spectrum of chitosan after methacrylic anhydride acylation is shown in Figure 4(Curve CM), where the absorption peak of the amino group corresponding to the chitosan structure at 3200-3400cm^{-1} coincides with the hydroxyl absorption peak, but it can also be seen that the intensity of the absorption peak here decreases after the

chitosan is reacted with methacrylic anhydride. The absorption peak of the primary amine N-H bond in chitosan structure at 1413 cm^{-1} significantly decreases after methacrylic anhydride acylation, while the characteristic absorption peak of the amide bond appears at 1520 cm^{-1}. In the CM absorption curve, a new absorption peak appeared at 1625cm^{-1}, which was caused by the introduction of C=C stretching vibration in the structure, indicating the reaction between -NH_2 in chitosan and methacrylic anhydride. These results indicate that methacrylic anhydride was successfully grafted onto chitosan molecules through amide reaction.

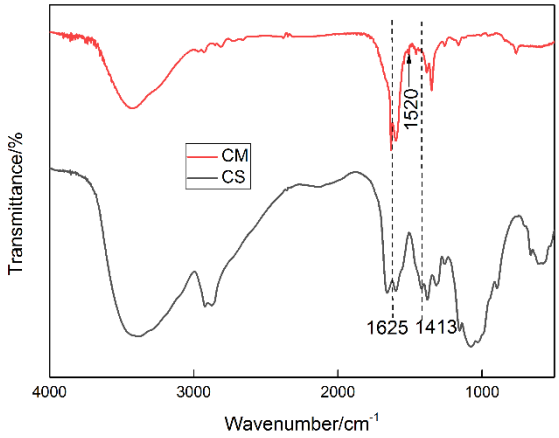

Figure 4. FTIR spectrogram of CS and CM

3.2. Compression Resistance and Micromorphology Analysis of Composite Hydrogels

The stress-displacement curves of the composite hydrogel are shown in Figure 5. When the compressive displacement is increased to 5mm, the maximum compressive stress occurs at 225KPa. At this point, the compressive strain is calculated to be 40%. This indicates that the carbon-carbon double bond in CM is cross-linked by a light-curing agent under ultraviolet light, forming a stable three-dimensional network structure with certain mechanical strength and elastic behavior.

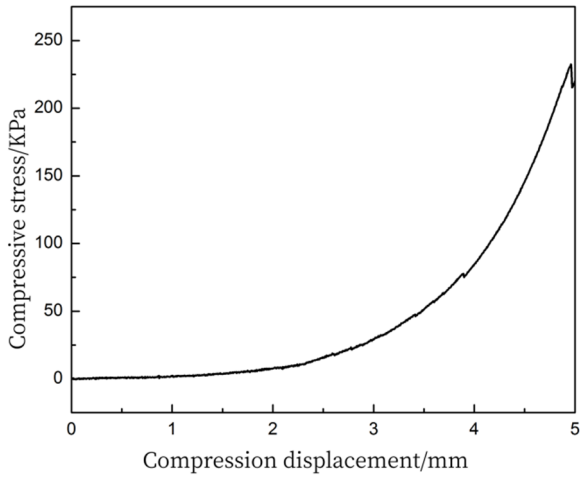

Figure 5. Stress-displacement curves of CM/GA-CS0 samples of hydrogels

The SEM topography of the three different composite hydrogels is shown in Figure 6. As can be seen from Figure 6, when the addition of GA-CS is 1%, the pores of the hydrogel gradually increase, the inner wall tends to be smooth, and the performance becomes better. When the addition of GA-CS was 2%, a small number of porous structures appeared in the hydrogel, indicating that the increase of GA-CS may have some adverse effects on the cross-linking network, but the appropriate amount of GA-CS addition could increase the density of the pore wall, and then increase the mechanical strength of the hydrogel.

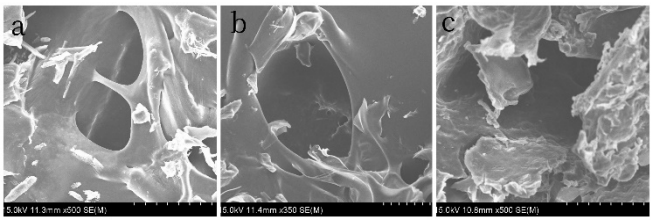

Figure 6. SEM images of different hydrogel samples
a: CM/GA-CS0, b: CM/GA-CS1, c: CM/GA-CS2

3.3. Swelling Rate Test Analysis

The results of the composite hydrogel swelling rate test are shown in Figure 7, and the swelling rate of the three hydrogel samples is maintained at 79%-95%. The swelling rate was relatively high, indicating that the composite hydrogel had a low degree of crosslinking. The high swelling rate allows the hydrogel to absorb wound tissue exudate, and the low degree of cross-linking makes it easy for the hydrogel to cover the wound infection, increasing the possibility of its application in the field of wound dressings. The swelling rate of hydrogels decreased with the increase of GA-CS content. This is because the carboxyl group on the GA-CS structure may react with the amino group in the CM structure, increasing the degree of crosslinking of the matrix and thus reducing the swelling rate.

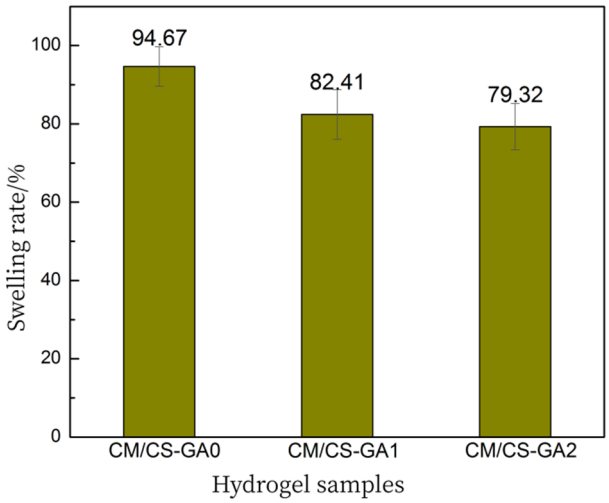

Figure 7. Swelling rates of different hydrogel samples

3.4. Water Retention Test Analysis

The water retention of the composite hydrogel is shown in Figure 8. The water retention rate can be maintained for about 4h when it is above 75%, and about 7h when it is above 50%. With the addition of GA-CS, the water retention rate of the hydrogel was increased in the first 10 h, which should be the effect of the phenolic hydroxyl and carboxyl structures on gallic acid in the introduced GA-CS.

3.5. Contact Angle Test Analysis

The contact angle test results of the composite hydrogel surface to water are shown in Figure 9. The three composite hydrogels are hydrophilic surfaces. The hydrophilicity of the composite hydrogel is due to the existence of a large number

of hydrophilic groups such as - OH and - NH_2 in the structure of the hydrogel [10]. When water molecules contact the outer surface of the hydrogel, the hydrophilic groups combine with water molecules. With the addition of GA-CS, the -COOH in GA-CS may react with -NH2, consume the hydrophilic groups in the composite hydrogel, increase the crosslinking degree of the matrix, and lead to the decrease of the hydrophilicity of the matrix. However, the composite hydrogel still has a small contact angle, indicating that the composite hydrogel still has excellent hydrophilicity.

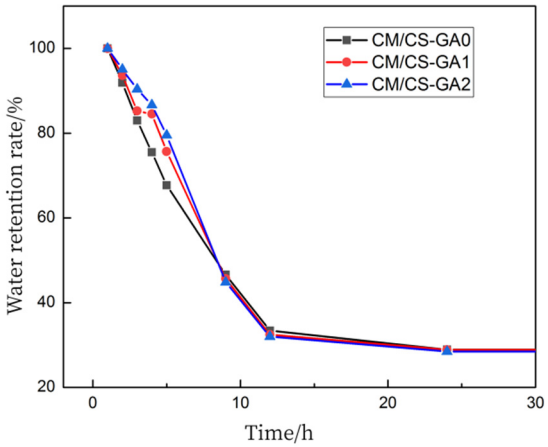

Figure 8. CM/GA-CS0, CM/GA-CS1, CM/GA-CS2 water retention of the sample

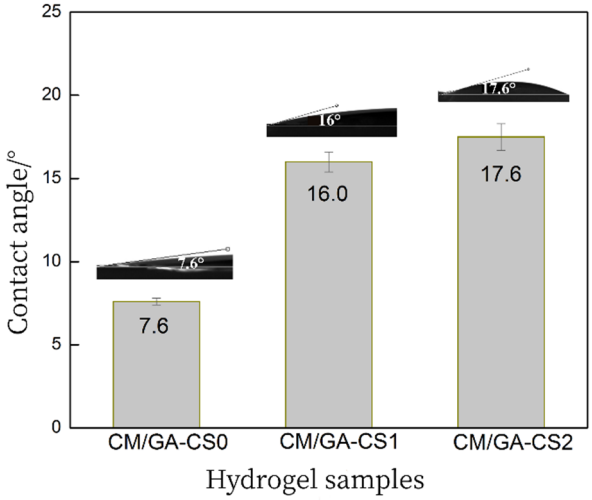

Figure 9. CM/GA-CS0, CM/GA-CS1, CM/GA-CS2 contact angle of the hydrogel sample

3.6. Antimicrobial Test Analysis

The test results of inhibition zone of composite hydrogel samples are shown in Figure 10. The diameters of inhibition zone of CM/GA-CS0, CM/GA-CS1, and CM/GA-CS2 against E. coli are (9.85 ± 0.12) mm, (12.15 ± 0.19) mm, and (13.96 ± 0.15) mm, respectively. The antibacterial activity of chitosan mainly comes from its positive charge on the surface, which interacts with the negative charge on the surface of microbial cell membranes, reducing biological activity and inhibiting bacterial growth and reproduction [11,12]. Due to the phenolic hydroxyl structure in GA-CS, it can interact with bacterial cell membranes and inhibit bacterial growth. Under the synergetic action with chitosan matrix, the antibacterial activity of the composite hydrogel was increased.

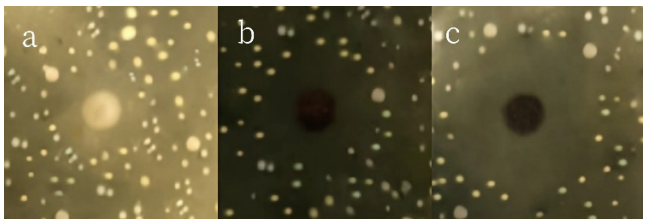

Figure 10. Inhibition of different hydrogel samples against E. coli a.CM/GA-CS0, b.CM/GA-CS1, c.CM/GA-CS2

4. Conclusion

In this paper, gallic acid grafted chitosan anti-bacterial agent GA-CS and methacrylic anhydride grafted chitosan hydrogel matrix CM were prepared respectively. The composite hydrogels of CM/GA-CS0, CM/GA-CS1, and CM/GA-CS2 were prepared by UV curing technology with different mass ratios of GA-CS and CM. When the compression deformation of the composite hydrogel is 40%, the compression stress is 225KPa, the swelling rate of the hydrogel is 70% -95% in 24h, and the water content above 50% can be maintained for about 7h. With the increase of GA-CS content, the water contact angle of the composite hydrogel surface gradually increases, but it still has excellent hydrophilicity and enhanced antibacterial effect.

Acknowledgments

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