

Article

Quaternary Ammonium Salt Carboxymethyl Chitosan/Sodium Oxidized Alginate/Tannic Acid/Sodium Tetraborate Hydrogel Dressings with Self-Healing, Antioxidant and Responsive Release of Tannic Acid

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Abstract

Infected wound repair remains a global healthcare challenge, primarily due to bacterial infection and a pathological microenvironment characterized by elevated glucose levels and oxidative stress. In this work, a quaternized carboxymethyl chitosan (QCMCS)/oxidized sodium alginate (OSA)/tannic acid (TA)/sodium tetraborate (STB) hydrogel was developed for controlled TA release and diabetic wound repair. The QCMCS/OSA/TA/STB hydrogel exhibited potent antibacterial activity, with inhibition rates exceeding 99% against *S. aureus* and MRSA and 86% against *E. coli*, arising from the synergistic action of QCMCS and TA. Meanwhile, the introduction of TA enhanced antioxidant performance (radical scavenging rates of 66.72% and 93.16% against DPPH and ABTS, respectively), and STB reinforced mechanical strength with a compressive resistance of 140.78 kPa through a dual cross-linking network. In vitro biocompatibility evaluations demonstrated that the hemolysis ratios of all hydrogels were below 5%, and the survival rate of Human umbilical vein endothelial cells (HUVECs) was over 93%. Reversible borate ester linkages between STB and the catechol groups of TA protect TA from oxidative degradation and allow stimulus-responsive release under elevated glucose and oxidative conditions. This responsive hydrogel represents a promising multifunctional platform for diabetic wound management.

Keywords: dynamic covalent hydrogel; borate ester crosslinking; tannic acid release; self-healing properties; antibacterial activity



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1. Introduction

Bacterial infection can disrupt wound tissues, trigger excessive inflammatory responses, and ultimately impede the healing process. The accumulation of reactive oxygen species (ROS) further exacerbates inflammation, while uncontrolled inflammatory cascades accelerate wound deterioration [1,2]. Polysaccharide-based hydrogels, owing to their excellent biocompatibility and extracellular matrix-like microstructure, are capable of absorbing wound exudates and serving as physical barriers against bacterial invasion, making them promising candidates for wound dressing applications [2,3]. However, conventional polysaccharide hydrogels generally suffer from insufficient antibacterial and antioxidant activities, a lack of stimulus-responsive bioactive substance release, and inadequate mechanical strength. Therefore, the development of multifunctional polysaccharide hydrogels for

the effective treatment of infected wounds is highly desirable. Infected wounds are difficult to heal because of a highly oxidative microenvironment, recurrent bacterial infections, and inadequate responsiveness to pathological wound microenvironments [4]. Consequently, the development of multifunctional, stimulus-responsive polysaccharide hydrogels capable of maintaining structural integrity while dynamically adapting to oxidative and infectious conditions is highly desirable for effective infected wound management [5,6].

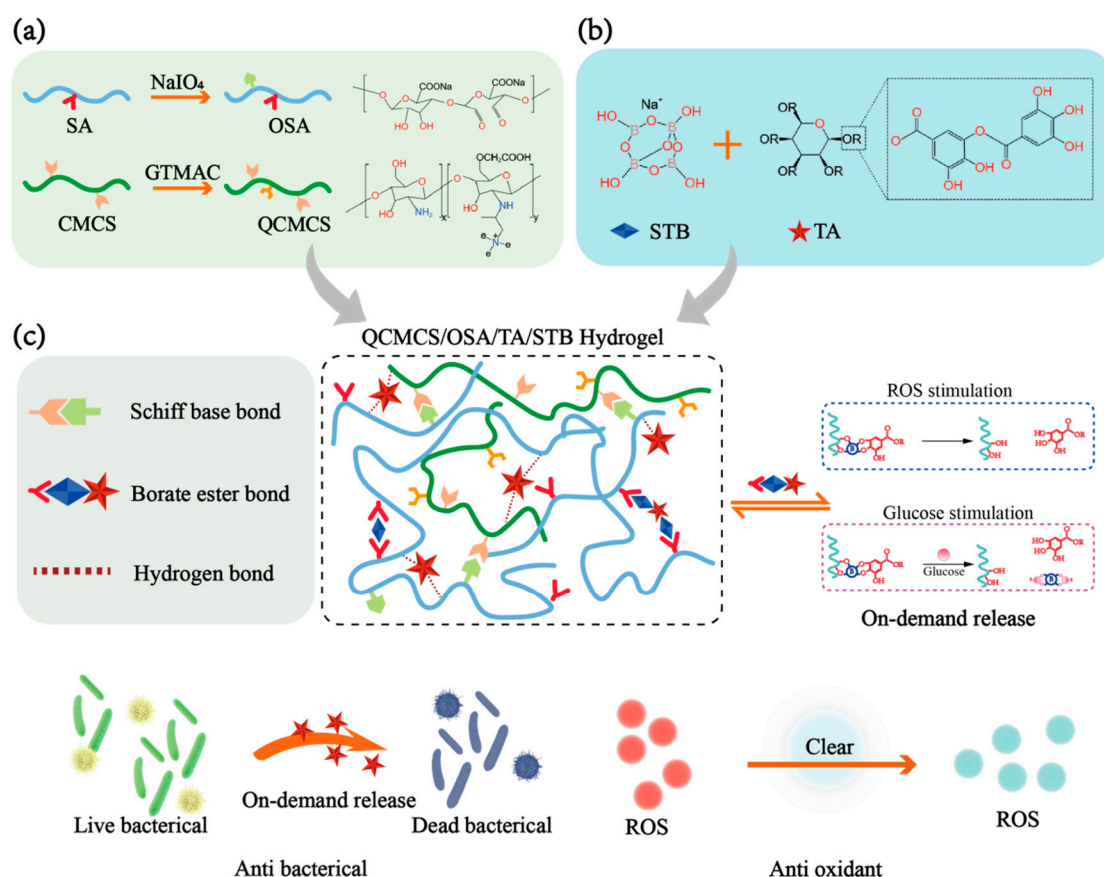
Carboxymethyl chitosan (CMCS), with excellent biocompatibility and biodegradability, is widely used in the fields of hydrogel wound dressings [7]. However, its inherent antibacterial activity remains limited, often requiring further modification and functionalization to enhance efficacy [8]. As a derivative of carboxymethyl chitosan (CMCS), quaternized carboxymethyl chitosan (QCMCS) exhibits improved solubility, enhanced biocompatibility, and stronger antibacterial activity due to the introduction of stable, pH-independent cationic groups [9,10]. Sodium alginate (SA), a natural polysaccharide widely used in hydrogel systems, offers excellent biocompatibility and abundant carboxyl groups suitable for chemical modification [11]. Upon periodate oxidation, SA is converted into oxidized sodium alginate (OSA), whose molecular chains contain a large number of hemiacetal/aldehyde groups [12]. These aldehyde groups of OSA can readily react with the amino groups of QCMCS to form dynamic Schiff-base crosslinked QCMCS/OSA hydrogels. Nevertheless, hydrogels constructed solely based on a single type of reversible dynamic bond generally suffer from poor resilience and limited fatigue resistance [5].

Plant polyphenols, such as tannic acid (TA), are widely recognized for their potent antioxidant and antibacterial properties and have shown considerable promise in skin wound healing applications [13]. The abundant phenolic hydroxyl groups in TA endow it with strong free radical scavenging ability and broad-spectrum antibacterial activity, enabling effective suppression of oxidative stress and bacterial infection at wound sites [14,15]. However, the direct incorporation of TA into hydrogel systems still faces several challenges. Owing to its small molecular nature and high water solubility, TA is prone to rapid diffusion and burst release from hydrogel matrices, which limits its sustained therapeutic efficacy [7]. In addition, TA-based hydrogels relying primarily on hydrogen-bond interactions often suffer from insufficient mechanical strength and structural instability, restricting their practical application in dynamic wound environments [16].

To overcome these limitations, sodium tetraborate (STB) has emerged as an attractive dynamic cross-linking agent for hydrogel design [9,17]. STB can form reversible borate ester bonds with cis-diol-containing moieties, such as the catechol groups in TA and the hydroxyl groups in oxidized polysaccharides, enabling the construction of dynamically cross-linked networks [18]. Compared with weak hydrogen-bond interactions, borate ester linkages possess substantially higher bonding strength, which provides effective mechanical reinforcement while maintaining network adaptability [13]. For instance, Liu et al. developed a sodium hyaluronate/tannic acid hydrogel crosslinked through dynamic borate ester bonds [19], while Cao et al. incorporated borax into guar gum to produce a hydrogel with remarkable mechanical and self-healing capabilities [20]. These studies collectively demonstrate that borate ester-based dynamic crosslinking is an effective strategy for reinforcing hydrogel networks. In addition, borate ester bonds are also known to be sensitive to glucose and oxidative stimuli, under which they can undergo dynamic cleavage in infected wound microenvironments [18]. This feature not only improves hydrogel stability before application but also enables controlled, stimulus-responsive release of TA in response to pathological cues, including elevated glucose concentrations and elevated ROS levels [21,22].

Unlike most previously reported borate-TA hydrogels that rely on a single dynamic interaction, the present system integrates reversible borate ester-mediated TA

protection-deprotection with dual dynamic crosslinking, thereby simultaneously improving storage stability and enabling wound-responsive activation. In this work, a quaternized carboxymethyl chitosan/oxidized sodium alginate/tannic acid/sodium tetraborate (QCMCS/OSA/TA/STB) hydrogel is constructed based on synergistic Schiff base and borate ester interactions to address key limitations of multifunctional wound dressings (Scheme 1). The hydrogel integrates mechanical reinforcement with antibacterial and antioxidant activities, while enabling controlled, glucose- and H_2O_2 -responsive release of TA without burst release. Its therapeutic efficacy in promoting infected wound healing is further investigated, underscoring its promise for biomedical wound management applications.



Scheme 1. Schematic illustration of the preparation process and functional applications of QCMCS/OSA/TA/STB hydrogels. (a) Preparation scheme of oxidized sodium alginate (OSA) and quaternized carboxymethyl chitosan (QCMCS). (b) The synthesis process of TA/STB structures. (c) Preparation and application of QCMCS/OSA/TA/STB hydrogels.

2. Materials and Methods

2.1. Materials

Chitosan (CS, degree of deacetylation: 95%, M_w : 2×10^5 Da) and carboxymethyl chitosan (CMCS, degree of carboxylation: 90%) were supplied by Shanghai Maclean Biochemical Technology Co., Ltd. (Shanghai, China). Tannic acid (TA), ammonia, glycidyltrimethylammonium chloride (GTMAC), sodium periodate (NaIO_4), and sodium tetraborate (STB) were also obtained from the same supplier. Sodium alginate (SA, M_w : 19.3×10^3 Da, viscosity: 200 ± 20 mPa·s, mannuronate/guluronate ratio (M/G): 0.8), ferrous sulfate, potassium sodium tartrate, and ethylene glycol were purchased from Aladdin Bio-Chem Technology Corporation (Shanghai, China).

2.2. Synthesis of Quaternized Carboxymethyl Chitosan (QCMCS)

QCMCS was fabricated based on a method described in the literature, with slight adjustments [23]. Briefly, CMCS (3.5 g) was dissolved in 150 mL of distilled water, followed by the addition of GTMAC (0.8 g). The reaction was carried out at 55 °C for 48 h under continuous stirring. The resulting mixture was then dialyzed using a dialysis membrane (MWCO: 14 kDa) against ultrapure water for 3 days, with water changed regularly. Finally, the purified product was freeze-dried to obtain QCMCS as a solid.

2.3. Synthesis of Oxidized Sodium Alginate (OSA)

OSA was fabricated based on a method described in the literature, incorporating slight adjustments [24]. In the synthesis, 10 g of SA was dissolved in 50 mL of ethanol, followed by the addition of 3.21 g of NaIO₄. The mixture was stirred at 37 °C in the dark for 12 h. Oxidation was terminated by introducing 10 mL of ethylene glycol, with stirring maintained for 1 h. The resultant product underwent dialysis against ultrapure water (MWCO: 3.5 kDa) for 3 days, with frequent water replacement, and was then freeze-dried to yield OSA. The aldehyde content was measured via hydroxylamine hydrochloride potentiometric titration: 1 g of OSA was dissolved in 250 mL of hydroxylamine hydrochloride solution containing 0.25 mol/L methyl orange. Released HCl was titrated using 0.1 mol/L NaOH, and the aldehyde concentration was calculated using the formula below [25]:

$$\text{The degree of oxidation} = \frac{V \times 0.001 \times C_{\text{NaOH}} \times 198.11}{2 \times W} \times 100\% \quad (1)$$

In this equation, V corresponds to the volume of NaOH consumed, C_{NaOH} indicates the NaOH concentration, and W represents the mass of the OSA sample.

Analysis revealed that the OSA exhibited an oxidation degree of 41%.

2.4. Preparation of QCMCS/OSA/TA/STB Hydrogels

To synthesize the QCMCS/OSA/TA/STB hydrogel, 0.18 g of QCMCS and 0.14 g of OSA were separately dissolved in 2 mL of phosphate-buffered saline (PBS) to obtain 9% (w/v) QCMCS and 7% (w/v) OSA solutions, respectively. For hydrogel formation, 0.6 mL of the OSA solution was mixed with 0.15 mL of a 1 wt% TA aqueous solution under uniform stirring. Then, 0.25 mL of a 0.5 wt% STB solution was added, and the mixture was stirred thoroughly. Finally, 0.9 mL of the QCMCS solution was introduced, and the resulting mixture was incubated at 37 °C for 5 min to form the QCMCS/OSA/TA/STB hydrogel [24,25]. The resulting hydrogel was visually transparent and homogeneous (Figure S1).

2.5. Characterization of QCMCS and OSA

The chemical compositions of QCMCS and OSA were examined via Fourier transform infrared spectroscopy (FTIR, INVENIO-S, attenuated total reflectance (ATR) mode), while their molecular structures were further probed using ¹H NMR spectroscopy (INOVA 600). For NMR measurements, CMCS, QCMCS, SA, and OSA were dissolved in D₂O. The degree of substitution (DS) of amino groups in QCMCS was calculated from the NMR signal intensities at $\delta = 1.98$ ppm (assigned to CMCS acetyl protons, g) and $\delta = 3.13$ ppm (corresponding to the methyl protons of the quaternary ammonium group, 4), as defined by the following formula [26]:

$$DS = \frac{I_4/9 \times DD\%}{I_g/3} \times 100\% \quad (2)$$

where I_4 and I_g were the integral regions of proton 4 and g in the ^1H NMR spectra, respectively; $DD\%$ was the degree of deacetylation of CS.

2.6. Characterization of QCMCS/OSA/TA/STB Hydrogels

Field emission scanning electron microscopy (SEM) (JSM-IT200) was employed to investigate the microstructure of the hydrogels. Before imaging, the samples were quickly frozen in liquid nitrogen and subsequently freeze-dried at $-50\text{ }^\circ\text{C}$ for 24 h. The chemical composition was probed through FTIR in attenuated total reflectance (ATR) mode (INVENIO-S). Cylindrical hydrogel specimens (10 mm in height, 15 mm in diameter) were prepared for compression tests, which were conducted at $37\text{ }^\circ\text{C}$ and 40% relative humidity using a CMT6503-5KN microcomputer-controlled electronic universal testing machine (Shanghai, China). Rheological evaluations were performed with a rotational rheometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with parallel-plate geometry at $37\text{ }^\circ\text{C}$, including oscillatory frequency sweeps (0.1–10 Hz), amplitude sweeps (1–1000% strain), and alternating step strain tests (1% and 500% strain). Through these measurements, the storage modulus (G'), loss modulus (G''), and self-healing behavior of the hydrogels were comprehensively assessed.

Energy dissipation of the hydrogel was quantified by calculating the area of stress–strain hysteresis loops derived from the aforementioned cyclic loading tests (100 cycles at 0.1% and 50% strain), and the relative fluctuation of energy dissipation values across cycles was analyzed to evaluate the stability of the dynamic cross-linking network.

2.7. Testing of Porosity

Hydrogel porosity was evaluated using the solvent displacement approach. Freeze-dried cylindrical samples were initially weighed (M_1), and their dimensions were measured to obtain the volume V (cm^3). The samples were placed in anhydrous ethanol until the pores were fully saturated. After careful surface wiping to remove excess ethanol, the samples were reweighed (M_2). The porosity (P) was then calculated according to the formula below [11]:

$$P = \frac{M_2 - M_1}{\rho \times V} \quad (3)$$

In the equation, ρ corresponds to the density of anhydrous ethanol, expressed in g/cm^3 .

2.8. Moisture Retention Performance Test

Hydrogel water retention was assessed by first measuring the initial weight (W_0) of samples with varying compositions. The samples were maintained at $37\text{ }^\circ\text{C}$ and 40% relative humidity, and their weights (W_t) were monitored at predefined intervals over a period of 120 h. All experiments were performed in triplicate, with the average values used for subsequent analysis. The water retention ratio was determined according to the formula below [27]:

$$\text{Water retention ratio} = \frac{W_t}{W_d} \times 100\% \quad (4)$$

2.9. Equilibrium of Swelling Rate

Hydrogel samples were initially weighed to determine the dry weight (W_d). The samples were subsequently immersed in 100 mL phosphate-buffered saline (PBS, $\text{pH} = 7.4$) and incubated at $37\text{ }^\circ\text{C}$ to simulate physiological wound conditions. After 12 h, which was sufficient to reach swelling equilibrium, the samples were removed, gently blotted with filter paper to eliminate excess surface liquid, and weighed to obtain the swollen weight

(W_w). All measurements were performed in triplicate, and average values were used for calculation. The swelling ratio was determined according to the following equation [3]:

$$\text{Swelling rate} = \frac{W_w - W_d}{W_d} \times 100\% \quad (5)$$

2.10. Antioxidant Properties of Hydrogels

Hydrogel antioxidant capacity was assessed using DPPH and ABTS radical scavenging assays. The DPPH solution (500 μM) was prepared in ethanol. For the assay, 100 μL of the hydrogel samples were added to sealed tubes, while the blank group received the same volume of ethanol, followed by 1 mL of DPPH solution. Samples were incubated in the dark at 37 $^{\circ}\text{C}$ for 30 min. Subsequently, the absorbance of the supernatant was measured at 517 nm with a microplate reader (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland). The scavenging rate of DPPH radicals was determined using the formula below [28]:

$$\text{DPPH scavenging} = \frac{A_b - A_s}{A_b} \times 100\% \quad (6)$$

In the equation, A_b corresponds to the absorbance of the blank (DPPH + ethanol), whereas A_s corresponds to the absorbance of the material sample (DPPH + hydrogels).

The ABTS experiment was carried out in a manner analogous to the DPPH assay. Each tube received 100 μL of hydrogel, and the blank group was supplemented with an equal volume of deionized water before adding 1 mL of ABTS solution. Samples were incubated in darkness at 37 $^{\circ}\text{C}$ for 10 min, and the absorbance of the supernatants at 734 nm was recorded with a microplate reader. The ABTS scavenging activity was determined according to the formula below:

$$\text{ABTS scavenging} = \frac{A_c - A_d}{A_c} \times 100\% \quad (7)$$

In the equation, A_c corresponds to the absorbance of the blank (ABTS + deionized water), whereas A_d corresponds to the absorbance of the material sample (ABTS + hydrogels).

2.11. Responsive Degradation Behavior of Hydrogels

Hydrogels based on borate ester were maintained in PBS solutions of pH = 7.8 and 6.0, representing non-healing and healing wound conditions, respectively [29]. To simulate the diabetic wound microenvironment, 16.6 mM glucose and 1 mM H_2O_2 were introduced, and the system was kept at 37 $^{\circ}\text{C}$. At predetermined intervals, the remaining hydrogels were retrieved, dried, and weighed. The fraction of residual hydrogel was calculated according to the formula below:

$$\text{Remaining hydrogel (\%)} = \frac{W_t}{W_0} \times 100\% \quad (8)$$

Among them, W_t and W_0 are, respectively, the dry weight of the remaining hydrogels and the initial dry weight of the hydrogels after degradation at different time points.

2.12. Responsive TA Release Behavior of Hydrogels

After hydrogel formation, the supernatant was separated by centrifugation (8000 rpm, 5 min). Residual TA in the supernatant was quantified via iron tartrate assay (536 nm, UV-Vis spectrophotometer) using a pre-established standard curve ($R^2 > 0.99$).

$$\text{EE (\%)} = \frac{M_t - M_s}{M_t} \times 100\% \quad (9)$$

where M_t is the initial TA mass and M_s is the residual TA mass. All tests were done in triplicate.

The in vitro release characteristics aim to study the responsive release characteristics based on pH, ROS and glucose [30]. Firstly, QCMCS/OSA/TA/STB hydrogels with a total volume of 600 μL were prepared. Then, the hydrogels were, respectively, immersed in 6 mL of PBS (pH = 7.4, pH = 7.4 + Glucose, pH = 7.4 + H_2O_2 , pH = 7.4 + H_2O_2 + Glucose, pH = 5.5, pH = 5.5 + Glucose) solution and incubated at 37 °C ($n = 3$). At a fixed time point, collect 0.5 mL of the extract and soak it in the corresponding fresh solution. In order to facilitate the subsequent determination of the concentration of TA in the extract, the collected extract was stored at a value of -20°C .

To prepare the ferrous tartrate reagent, 100 mg of ferrous sulfate and 500 mg of potassium tartrate were dissolved in 100 mL of water and reacted at room temperature for 10 min, yielding a pale yellowish-green solution. TA solutions at concentrations of 0, 5, 10, 20, 40, 60, 80, and 100 $\mu\text{g/mL}$ were prepared. In 96-well plates, 100 μL of either standard TA solution or hydrogel extract was added per well, followed by 50 μL of ferrous tartrate. The mixtures were incubated at 37 °C for 2 min, then 20 μL of PBS (0.1 M) was added. Absorbance at 540 nm was recorded, a standard curve was established, and the released TA concentration was calculated. The TA release rate was subsequently determined using the following equation:

$$\text{TA ReleaseRate (\%)} = \frac{C_t \times V_1 + \sum(C_t \times V_2)}{W_0} \times 100\% \quad (10)$$

In this equation, C_t denotes the TA concentration determined from the standard curve at each time point; V_1 is the volume of the extract (6 mL); $\sum C_t \times V_2$ represents the total TA released, where V_2 is 0.5 mL; and W_0 indicates the total TA content in QCMCS/OSA/TA/STB hydrogels.

2.13. Antibacterial Properties of Hydrogels

The antibacterial efficacy of the hydrogels was evaluated via the plate counting method. *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) were employed as Gram-positive models, whereas *Escherichia coli* (*E. coli*) served as the Gram-negative model. Bacterial suspensions (10^6 CFU/mL) were incubated with PBS (Control) or hydrogel samples for 24 h. After incubation, each bacterial-hydrogel mixture was divided into three equal portions. For colony enumeration, 100 μL of suitably diluted suspensions was plated on agar and incubated at 37 °C for 24 h. The agar plates were photographed for quantitative analysis.

2.14. Cell Activity of Hydrogels

To assess cytocompatibility, Human umbilical vein endothelial cells (HUVECs) were seeded into 24-well plates at a density of 2×10^4 cells per well and cultured at 37 °C with 5% CO_2 for 24 h. The culture medium was then replaced with hydrogel extracts, and cells were incubated for an additional 24, 48, and 72 h. At each time point, CCK-8 reagent was applied for 3 h, and absorbance at 450 nm was measured to determine cell viability. In parallel, cells were stained with propidium iodide (PI, red) and Calcein AM (green) for 30 min following each incubation period and examined via fluorescence microscopy.

HUVECs were originally obtained from a commercial supplier (ATCC, Manassas, VA, USA) and have been maintained and passaged in our laboratory for routine experimental use. Cells were cultured under standard conditions at 37 °C in a humidified atmosphere containing 5% CO_2 .

2.15. Blood Compatibility of Hydrogels

In this study, the blood compatibility of four hydrogels—QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB—was evaluated using rat red blood cells according to a previously established method [22]. Fresh red blood cells (RBCs) were collected from Sprague–Dawley (SD) rats, and each blood sample was treated as an independent experimental unit. For each hydrogel group and control group, three independent samples ($n = 3$) were used, with a total of 6 SD rats employed in the study. The RBCs were washed three times with PBS and diluted to obtain a 4% (v/v) suspension. Then, 100 μ L of each hydrogel was added to individual test tubes, followed by the addition of 1 mL of the diluted RBC suspension. Deionized water and PBS were used as the positive and negative controls, respectively. All tubes were incubated at 37 °C for 1 h. After incubation, the mixtures were centrifuged for 10 min, and the absorbance of the supernatant was measured at 545 nm. The hemolysis ratio was calculated using the following formula to evaluate the blood compatibility of the hydrogels.

$$\text{Hemolysis rate} = \frac{A_h - A_n}{A_p - A_n} \quad (11)$$

Here, A_h , A_n , and A_p correspond to the absorbance values of the supernatants in the hydrogel, PBS, and deionized water groups.

2.16. Ethical Statement

This study did not involve primary human tissues, human participants, or newly established human cell lines. All cell lines used were commercially available and obtained from the American Type Culture Collection (ATCC). All animal procedures followed the National Research Council's Guide for the Care and Use of Laboratory Animals, and testing was approved by the Animal Care and Use Committee of Wuhan University of Technology (No. WHUT2025–063).

2.17. Statistical Analysis

Experiments were performed in triplicate, and statistical analysis was conducted using IBM SPSS Statistics (version 27). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with significance levels indicated as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results and Discussion

3.1. Synthesis and Characterization of QCMCS/OSA/TA/STB Hydrogels

To enhance both solubility and antibacterial performance, QCMCS was synthesized by graft copolymerization of CMCS with GTMAC and characterized via ^1H NMR spectroscopy. A prominent proton resonance at 3.1 ppm was observed in Figure 1a, attributable to the methyl protons of the quaternary ammonium group, while the remaining signals were assigned to the CMCS backbone. These observations indicate that GTMAC was successfully grafted onto the CMCS chain. Integration of the ^1H NMR peaks yielded a substitution degree (DS) of 28.1%.

The molecular structure and functional groups of OSA were confirmed via FTIR and ^1H NMR spectroscopic analyses. As shown in Figure 1c, both SA and OSA exhibit broad absorption bands of hydroxyl stretching vibration within the range of 4000–3000 cm^{-1} [31]. The primary characteristic band of SA emerges at 2936 cm^{-1} , which arises from the combined effect of C–H stretching vibration and –COO– asymmetric and symmetric stretching vibration in the polysaccharide framework [32]. In contrast, a novel characteristic peak of OSA emerges at 1731 cm^{-1} , which is ascribed to the C–O absorption vibration of the aldehyde moiety [33],

indicating that the hydroxyl groups flanking C-2 and C-3 in the alginate-glucuronic acid monomer were oxidized to the aldehyde group by NaIO_4 [32]. Furthermore, as the periodate anion undergoes dihydrate formation exclusively via the oxidative scission of the C2-C3 bond, the FTIR spectra of OSA and SA exhibit notable similarity [30]. Figure 1b displays the ^1H NMR spectra of OSA and SA. A novel proton resonance emerges in the OSA spectrum at 5.6 ppm, corresponding to the hemiacetal proton formed by the aldehyde moiety in proximity to the hydroxyl group [33], thereby providing additional confirmation of the successful preparation of OSA.

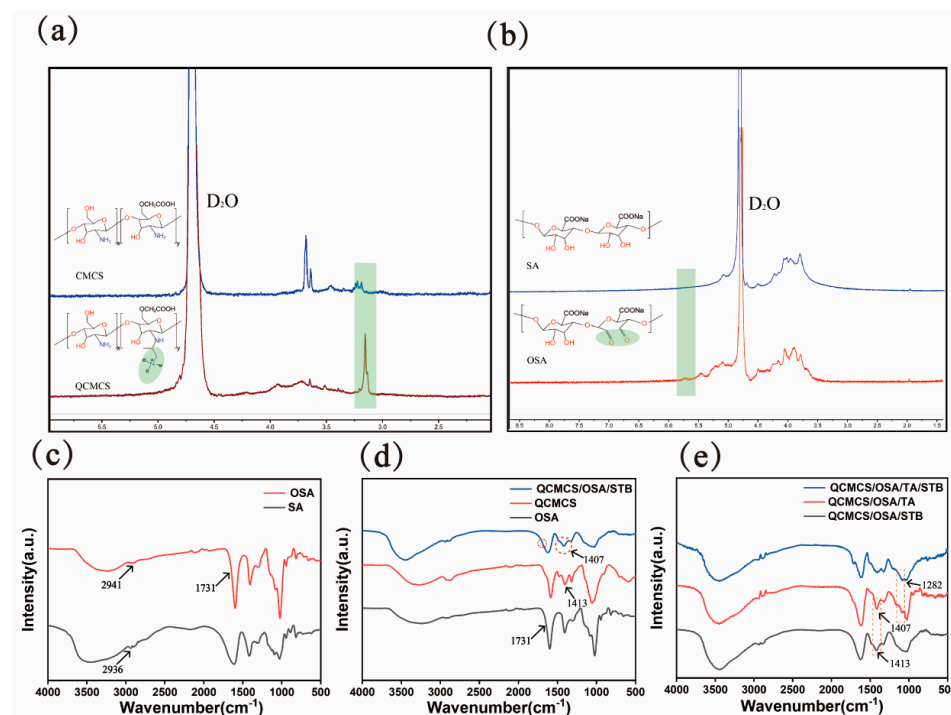


Figure 1. Characterization of the hydrogels, including QCMCS, OSA, QCMCS/OSA/STB, and QCMCS/OSA/TA/STB. (a,b) ^1H NMR spectra of CMCS, QCMCS, SA, and OSA; (c–e) FTIR spectra highlighting the chemical features of the corresponding hydrogels.

Figure 1d presents the FTIR spectra of QCMCS, OSA, and the QCMCS/OSA/STB hydrogel. The absorption band observed at 1413 cm^{-1} in the QCMCS spectrum is associated with the stretching vibration of the amino group ($-\text{NH}_2$) [26]. In the spectrum of the QCMCS/OSA/STB hydrogel, this vibration undergoes a slight shift due to the incorporation of OSA and STB [34]. Notably, the peak at 1726 cm^{-1} , corresponding to the aldehyde group of OSA, disappears completely, and the intensity of the peak at 1407 cm^{-1} decreases significantly. These changes result from the reaction between the aldehyde groups in OSA and the amino groups of QCMCS, leading to the formation of Schiff base bonds [24]. As shown in Figure 1e, the characteristic peaks of the QCMCS/OSA/STB hydrogel are also present in the FTIR spectrum of the QCMCS/OSA/TA/STB hydrogel. The vibrational band originally at 1407 cm^{-1} exhibits a red shift to 1413 cm^{-1} , along with a marked reduction in intensity, which can be attributed to hydrogen bonding interactions [23]. Additionally, a new stretching vibration band emerges at 1282 cm^{-1} , assigned to the B–O bond, providing clear evidence for the formation of borate ester linkages [28]. Together, these FTIR spectroscopic results confirm the successful synthesis of the QCMCS/OSA/TA/STB hydrogel.

3.2. Pore Size and Porosity of QCMCS/OSA/TA/STB Hydrogels

The polymerization and cross-linking reactions during the stirring process induce numerous bubbles to be entrapped within the gel matrix, thereby giving rise to a permeable

porous structure, which imparts the QCMCS/OSA/TA/STB porous conductive hydrogels with superior air permeability. Its permeability is evaluated by measuring its porosity, and the surface morphology is characterized by scanning electron microscopy.

SEM images reveal that the porous structure of the hydrogels can efficiently absorb the exudate from the wound and facilitate the exchange of nutrients and gases during the skin regeneration process. Scanning electron microscopy (SEM) images reveal that QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA and QCMCS/OSA/STB/TA hydrogels exhibit a unique three-dimensional network architecture (Figure 2a). It is worth noting that with the increment in the variety of modified components, the crosslinking density of hydrogels increases progressively. Meanwhile, the porosity measurements of the hydrogels (Figure 2b), porosity QCMCS/OSA pore rate of $75.3 \pm 0.6\%$, after TA and STB modification QCMCS/OSA/STB, QCMCS/OSA/TA and QCMCS/OSA/STB/TA porosities are $65.3 \pm 0.3\%$, $67.3 \pm 1.1\%$ and $56.6 \pm 0.2\%$. This observation is attributed to the ability of STB to form borate ester linkages with the residual hydroxyl groups on QCMCS and OSA. The hydroxyl moieties of TA can not only form hydrogen bonds with the amino groups in QCMCS/OSA, but also can undergo aldol condensation reactions with the residual aldehyde functionalities. The synergistic interplay of the two facilitates the formation of a high-density interconnected network in the hydrogel. This observation underscores that the microstructure of hydrogels can be optimized via the modification of TA and STB. Maintaining a moist environment helps promote wound healing.

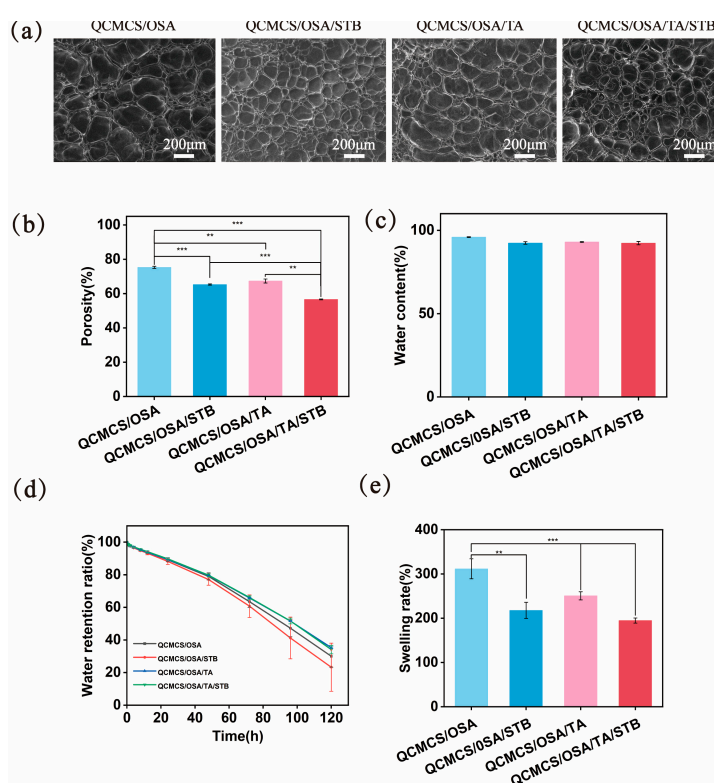


Figure 2. Hydrogel morphology and physicochemical properties. (a) Microstructural characteristics; (b) Porosity measurements of various hydrogels (mean $\pm$ SD, $n = 3$, ** $p < 0.01$, *** $p < 0.001$); (c) Water content evaluation (mean $\pm$ SD, $n = 3$); (d) Assessment of moisture retention; (e) Volume swelling ratios of the hydrogels (mean $\pm$ SD, $n = 3$, ** $p < 0.01$, *** $p < 0.001$).

3.3. Moisturizing Performance of QCMCS/OSA/TA/STB Hydrogels

By retaining appropriate moisture, hydrogels help create a favorable microenvironment for wound repair, thereby enhancing tissue regeneration while simultaneously absorbing surplus exudate to preserve optimal wound conditions [35]. As shown in Figure 2c, all hydrogels exhibited water retention capacities exceeding 91%, closely resembling the

natural extracellular matrix and highlighting their suitability for wound care applications. Figure 2d illustrates 120 h rates of the moisture retention behavior across different hydrogel compositions, indicating relatively slow water loss within the first 48 h with retention rates between 74% and 81%, followed by a more rapid decline beyond this period; notably, even after approximately 35% water content was retained, demonstrating satisfactory long-term performance. The prepared hydrogel maintained a water retention ratio of approximately 72% after 48 h, which was higher than that of commercial alginate wound dressings (approximately 65% under comparable conditions) [36] and comparable to clinically used hydrocolloid dressings, such as Duoderm, which typically exhibit retention rates of 70–75%. This level of water retention is sufficient to sustain a moist wound microenvironment over a prolonged period, meeting the practical requirements for effective clinical wound management [37]. However, the QCMCS/OSA/STB hydrogel showed suboptimal moisture retention, likely due to excessive STB addition compromising the inherent hydrophilicity and hydration capacity imparted by quaternization, ultimately leading to accelerated water loss.

3.4. Swelling Properties of QCMCS/OSA/TA/STB Hydrogels

Hydrogel swelling is critical for exudate absorption, infection prevention, and wound healing. As shown in Figure 2e, all hydrogels reached swelling equilibrium within 12 h at 37 °C in PBS. The QCMCS/OSA hydrogel exhibited the highest equilibrium swelling ratio (311.7%), followed by QCMCS/OSA/TA (250.6%), while STB-modified hydrogels (QCMCS/OSA/STB: 217.8%; QCMCS/OSA/TA/STB: 194.8%) showed reduced swelling, attributed to enhanced crosslinking density from STB restricting water diffusion [30]. The QCMCS/OSA/TA/STB hydrogel exhibited an appropriate swelling capacity that is comparable to the range reported for commercial wound dressings, including hydrocolloid dressings such as Duoderm (approximately 250–300%) [35] and alginate-based dressings (approximately 200–260%) [22]. This performance well matches the exudate absorption demand of moderate-exudate wounds, avoiding excessive swelling-induced detachment or insufficient absorption.

3.5. Antioxidant Properties of QCMCS/OSA/TA/STB Hydrogels

Oxidative stress, caused by an overabundance of reactive radicals in bacteria-infected wounds, not only disrupts cellular structures but also substantially interferes with the wound healing process [13]. Therefore, the development of hydrogel dressings with potent antioxidant activity has emerged as a key strategy to promote wound healing. In this study, the antioxidant capacities of four hydrogels—QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB—were systematically assessed using two established radical scavenging assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). As illustrated in Figure 3a,b, the DPPH radical scavenging rates were 3.93%, 6.83%, 72.53%, and 66.72%, respectively, while the ABTS radical scavenging rates reached 18.62%, 40.71%, 96.64%, and 93.16%, respectively. These results indicate that hydrogels containing TA (QCMCS/OSA/TA and QCMCS/OSA/TA/STB) exhibit significantly stronger antioxidant activity compared to those without TA. It is noteworthy that the incorporation of STB slightly reduced the radical scavenging performance relative to the TA-containing but STB-free hydrogel. This decrease results from the consumption of catechol groups in TA via borate ester bond formation with STB, limiting the availability of free hydroxyl groups for radical neutralization and delaying TA release [38], yet the hydrogel maintains considerable potential to mitigate oxidative stress during wound healing.

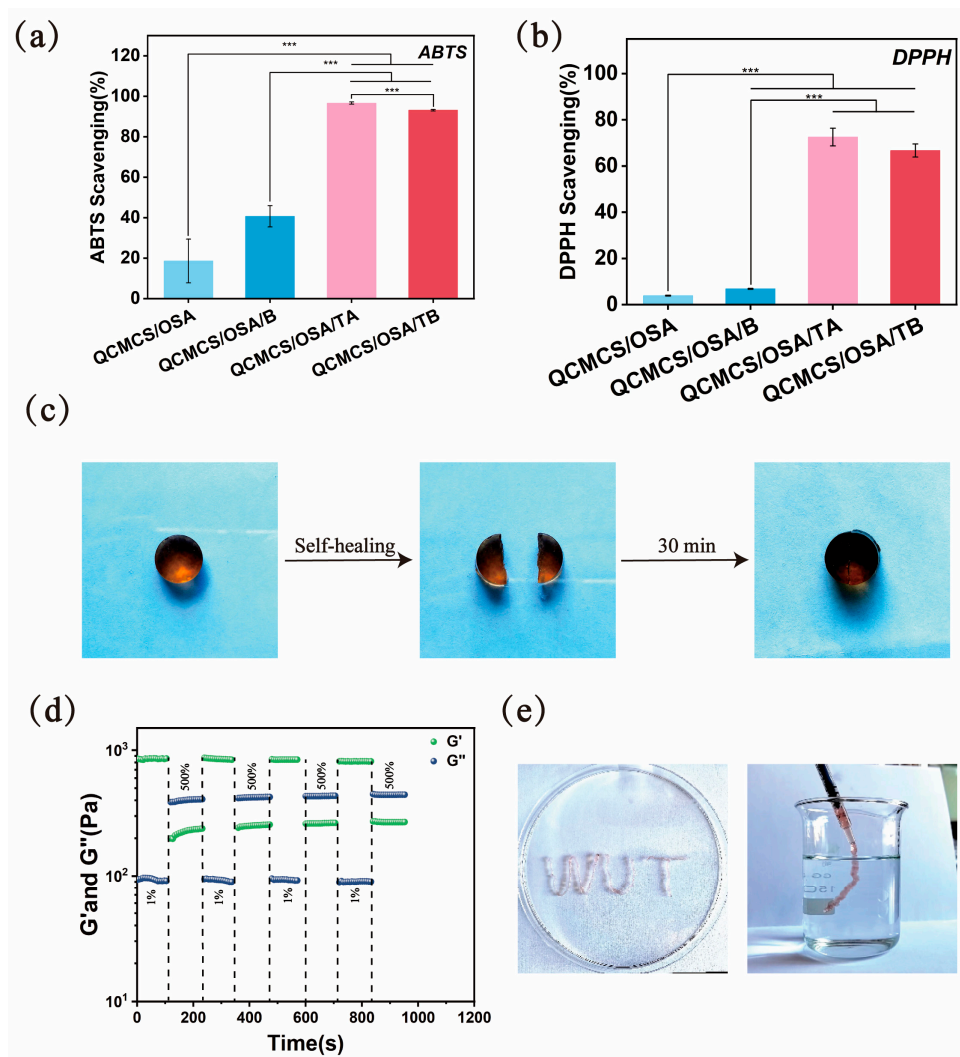


Figure 3. Evaluation of hydrogel performance: antioxidant activity, self-healing, and mechanical behavior. (a,b) Radical scavenging rates (ABTS and DPPH) of the hydrogels (mean $\pm$ SD, $n = 3$, *** $p < 0.001$); (c) Optical observation of QCMCS/OSA/TA/STB hydrogel self-repair; (d) Step-strain rheological measurement at 1% and 500% strains; (e) Optical images highlighting the injectability of the hydrogels.

The antioxidant functionality of hydrogel dressings offers multiple benefits for wound repair: it scavenges excess reactive oxygen species, alleviates oxidative damage, and protects crucial repair cells such as fibroblasts and endothelial cells. Furthermore, it helps modulate the inflammatory response, facilitating its timely resolution and promoting the transition to the proliferation phase. These dressings also support angiogenesis and collagen synthesis, improve wound perfusion and tissue regeneration, and reduce scar formation. Such properties are particularly valuable in the treatment of chronic wounds and other conditions characterized by elevated oxidative stress, ultimately enhancing both the rate and quality of healing.

3.6. Self-Healing Properties and Mechanical Properties of QCMCS/OSA/TA/STB Hydrogels

The self-healing capability and mechanical robustness of hydrogels are essential for maintaining structural integrity and fulfilling their protective role in wound dressing applications. As shown in Figure 3c, the QCMCS/OSA/TA/STB hydrogel was severed into two segments, which were then brought into contact at the cut surfaces. Under ambient conditions and without external intervention, the two sections reassembled into

a continuous hydrogel within 30 min, demonstrating excellent autonomous self-healing behavior. This capability was further quantitatively assessed using oscillatory step strain measurements (Figure 3d). At a low strain of 1%, the hydrogel remained structurally stable, while application of 500% strain induced structural disruption. Upon decreasing the strain, the hydrogel progressively regained its initial state, and the loss modulus (G'') was restored to its original value, suggesting that the dynamic Schiff base and borate ester crosslinks facilitated efficient self-healing. The injectability of the hydrogel is visually demonstrated in Figure 3e.

In practical scenarios, wound dressings are often subjected to mechanical stress due to patient movement. Hence, hydrogels with enhanced mechanical properties are critical for withstanding deformation and extending functional durability. The deformation of the hydrogel is shown in Figure S2. Chemically, the network is primarily stabilized via Schiff base bonds connecting OSA aldehyde groups with QCMCS amino groups, in combination with borate ester crosslinks formed by TA and STB. The structural integrity is further enhanced by additional borate ester bonds between STB and the hydroxyl groups of both OSA and QCMCS. As summarized in Figure 4a,b, the QCMCS/OSA hydrogel, crosslinked mainly through Schiff base bonds, exhibited a fracture stress of 36.50 kPa. Modification with TA and STB significantly improved mechanical strength: the fracture stresses for QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB hydrogels reached 40.78 kPa, 76.29 kPa, and 140.78 kPa, respectively. The superior mechanical performance of the QCMCS/OSA/TA/STB hydrogel is attributed to its dual dynamic crosslinking architecture, which effectively dissipates energy and enhances resistance to deformation.

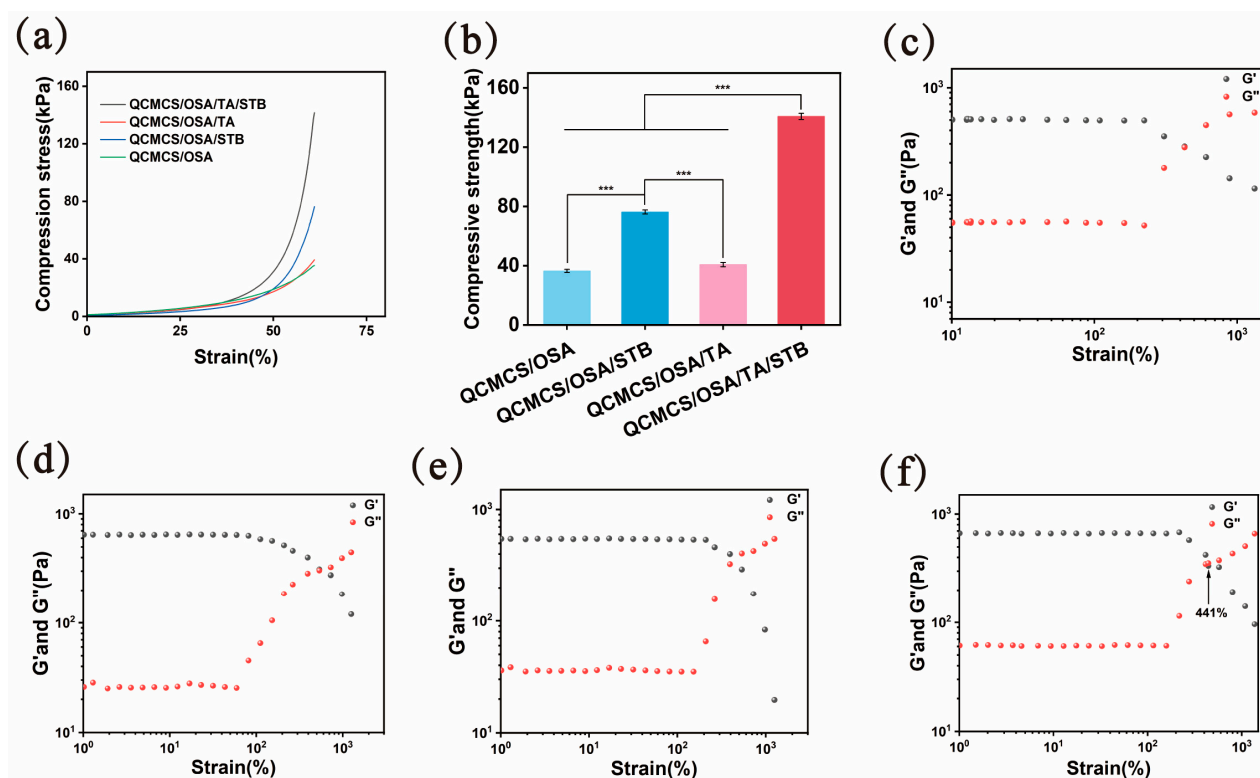


Figure 4. (a) Compressive stress–strain curves of the hydrogels; (b) Compressive strength of the hydrogels at 60% strain (mean $\pm$ SD, $n = 3$, *** $p < 0.001$); (c–f) Strain sweep analyses of the hydrogel samples performed at a fixed angular frequency of 6.28 rad/s.

Additionally, hydrogen bonds can be established between the hydroxyl groups of TA and the amino groups in QCMCS/OSA hydrogels via chemical interactions. In mechanical property assays (Figure 4c–f), under low-strain regimes, the energy storage modulus (G')

of QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB hydrogels consistently exceeds the loss modulus (G''). As the strain rises, the storage modulus (G') gradually drops. When the applied strain surpasses 441%, the G' and G'' curves cross, reflecting the total breakdown of the hydrogel's interpenetrating network. Furthermore, within the frequency scanning range of 0.1–10 Hz, G' of QCMCS/OSA/TA/STB hydrogels consistently exceeded G'' (Figure S3), demonstrating their structural robustness and excellent elasticity.

To further evaluate durability, cyclic loading tests were conducted, as shown in Figure S4. After 100 loading–unloading cycles at low strain (0.1%), representing the intrinsic elastic regime, and at high strain (50%), corresponding to practical deformation conditions, the hydrogel maintained a stable storage modulus without observable attenuation, demonstrating excellent fatigue resistance. Meanwhile, the energy dissipation calculated from the hysteresis loops exhibited a relative fluctuation of less than 5%. This behavior is attributed to the presence of reversible dynamic crosslinks, including Schiff base and borate ester bonds, which enable efficient energy dissipation while preserving the structural integrity of the hydrogel network.

These findings indicate that the addition of TA and STB substantially improves the mechanical performance of QCMCS/OSA hydrogels. In conclusion, QCMCS/OSA/TA/STB hydrogels exhibit superior self-healing and mechanical properties.

3.7. Responsive Degradation Behavior of QCMCS/OSA/TA/STB Hydrogels

The degradation behavior of borate ester-crosslinked QCMCS/OSA/TA/STB hydrogels was examined *in vitro* under simulated diabetic wound conditions, featuring changes in pH, glucose, and reactive oxygen species, as presented in Figure 5a,b. A pH of 7.8 represents the mildly alkaline environment typical of unhealed diabetic wounds, while pH = 6.0 corresponds to the acidic conditions after re-epithelialization [39]. Under alkaline conditions (pH = 7.8), the hydrogel degraded moderately, retaining 55.23% of its mass after 24 h with minimal further loss through 96 h. In contrast, under acidic conditions (pH = 6.0), the degradation rate increased significantly due to the acid-labile nature of the borate ester bonds, resulting in only 42.11% mass retention at 24 h and 42.01% at 96 h, demonstrating pronounced pH responsiveness. The degradation was further accelerated by high glucose (16.6 mmol/L). At pH = 6.0, mass retention dropped to 42.01% within 8 h, while at pH = 7.8, degradation stabilized after 16 h, confirming glucose-dependent degradation behavior. In high ROS environments, only 27% of the hydrogel remained after 24 h, and under combined high glucose and high ROS conditions, retention further decreased to 24.05%, indicating synergistic acceleration of degradation. The degradation mechanism involves acid- and ROS-induced cleavage of borate ester bonds [40]. In acidic medium, protonation of the oxygen atom in the B–O bond reduces electron density and weakens the bond, ultimately hydrolyzing it into boric acid and alcohols. Under high ROS conditions, highly oxidizing radicals such as OH and O_2 —attack the B–O or C–O bonds via hydrogen abstraction or electron transfer, forming unstable intermediates that decompose and lead to bond breakage [41]. Together, pH and ROS triggers promote borate ester cleavage and subsequent hydrogel dissolution, highlighting the material's responsiveness to key diabetic wound microenvironmental cues.

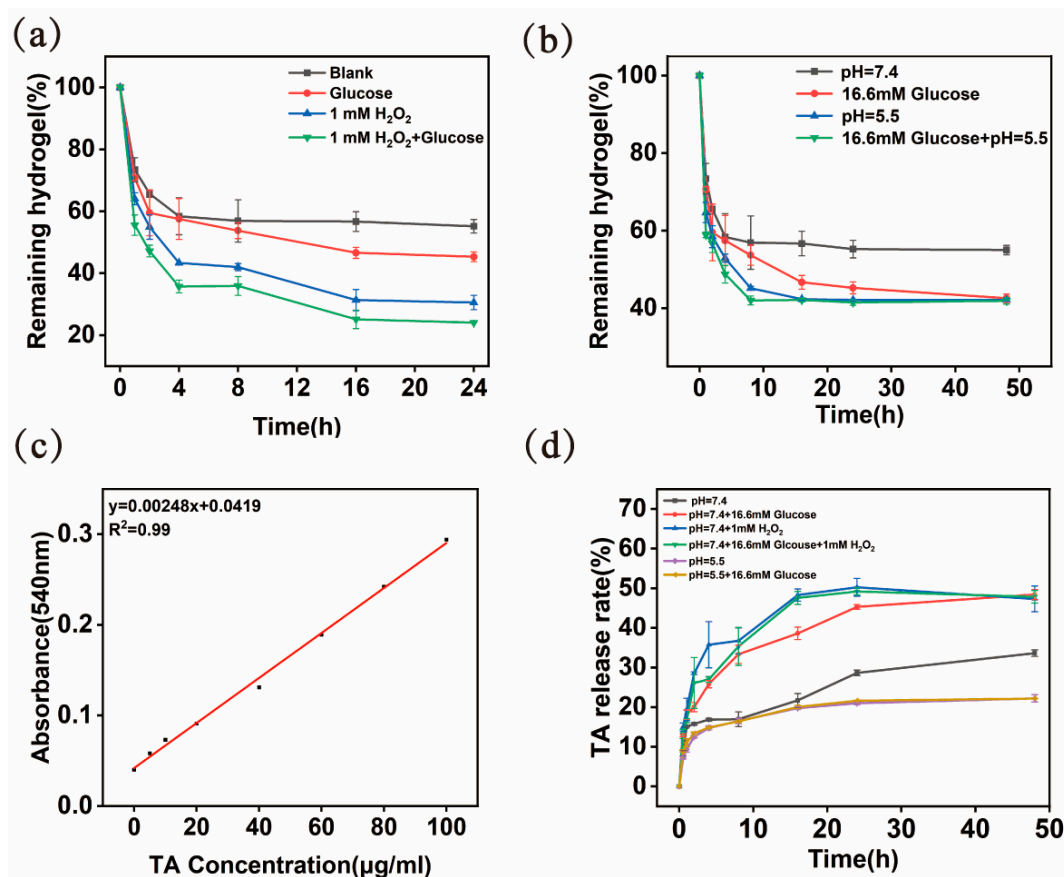


Figure 5. (a) In vitro degradation of QCMCS/OSA/TA/STB hydrogels under simulated diabetic wound conditions (16.6 mM glucose, 1 mM H₂O₂) at 37 °C; (b) Hydrogel degradation behavior at 37 °C in pH = 5.5 and 7.4 environments; (c) UV-visible standard curve for TA at 540 nm; (d) TA release kinetics from QCMCS/OSA/TA/STB hydrogels under different experimental conditions.

3.8. Responsive TA Release Behavior of QCMCS/OSA/TA/STB Hydrogels

TA exhibits potent antibacterial and antioxidant activities, which are primarily attributed to its abundant phenolic hydroxyl groups. These functional groups exert antibacterial effects by disrupting bacterial membrane integrity, inhibiting DNA replication, and chelating essential metal ions, thereby interfering with bacterial metabolism. In parallel, the antioxidant activity of TA arises from its ability to directly scavenge ROS and chelate transition metal ions, consequently suppressing metal-catalyzed ROS generation and alleviating oxidative stress-induced damage. However, the topical application of free TA is severely limited by its short biological half-life, rapid dilution by wound exudates, and the potential risk of systemic toxicity. In this context, sustained-release strategies have been demonstrated to substantially enhance the therapeutic efficacy of TA by modulating its local concentration and release kinetics [40].

To achieve effective TA delivery, the encapsulation efficiency (EE) of TA within the hydrogel was quantified by determining the residual TA content in the supernatant after hydrogel fabrication. The results revealed an EE of 62.77%, indicating the excellent loading capacity of the hydrogel network with minimal TA loss. This high encapsulation efficiency is attributed to the synergistic effect of the dual dynamic crosslinked framework. Reversible borate ester bonds formed between STB and the catechol groups of TA enable specific chemical anchoring, while Schiff base interactions between QCMCS and OSA generate a dense polymeric network that provides effective physical confinement. Together, these interactions significantly enhance TA retention and loading stability.

The stimulus-responsive release behavior of TA was subsequently investigated under different pH, glucose, and ROS conditions using the iron tartrate assay. As shown in Figure 5c,d, TA release was markedly faster at pH = 7.8 than at pH = 5.5. This pH-dependent behavior is closely related to the polyelectrolyte complex structure of the hydrogel network. Specifically, QCMCS, a cationic polymer, and OSA, an anionic polymer, interact through electrostatic attractions to form polyelectrolyte complexes. TA, a polyphenolic compound, carries a relatively lower degree of deprotonation under acidic conditions, which facilitates its electrostatic adsorption within the polyelectrolyte domains. This interaction effectively restricts TA diffusion and suppresses its release at lower pH values. As the pH increases, the phenolic hydroxyl groups of TA undergo progressive deprotonation to form phenoxide anions, leading to enhanced electrostatic repulsion within the complex and facilitating TA release from the hydrogel matrix [10].

To further elucidate the TA release mechanism, release profiles obtained over the pH range of 5–8 were fitted to four classical kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The goodness of fit was evaluated based on correlation coefficients (R^2), as summarized in Table S1.

Among these models, the Korsmeyer–Peppas model provided the highest R^2 values (0.96–0.98), suggesting that TA release from the hydrogel is predominantly governed by diffusion-controlled mechanisms. This behavior is likely associated with the moderate crosslinking density of the dual dynamic network, which permits sufficient molecular mobility of TA while maintaining structural integrity.

Further investigations demonstrated that the TA release rate was significantly accelerated in glucose-containing environments compared to the glucose-free PBS control, confirming the glucose-responsive nature of the QCMCS/OSA/TA/STB hydrogel. Under elevated glucose concentrations, the ortho-diol groups of glucose competitively interact with boronic acid moieties in the borate ester bonds, forming more thermodynamically stable glucose–boronate complexes. This competitive binding perturbs the dynamic equilibrium of the native borate ester bonds, thereby promoting bond dissociation and facilitating TA release [10,16].

Similarly, ROS responsiveness was evaluated by introducing H_2O_2 to simulate a high-ROS microenvironment. Under these conditions, TA release was significantly enhanced relative to the control group. Elevated ROS levels disrupt borate ester linkages through redox-mediated peroxidation, leading to network loosening and accelerated liberation of TA, thereby enabling its biological activity [42].

Collectively, the incorporation of TA into the hydrogel network through dynamic borate ester interactions, combined with the stimulus-responsive dissociation of these bonds under hyperglycemic and oxidative conditions, enables controlled and sustained TA release tailored to the diabetic wound microenvironment. This design provides a mechanistic basis for enhancing the local therapeutic efficacy of TA while minimizing premature loss, thereby offering a rational strategy for microenvironment-adaptive wound dressing development.

3.9. Antibacterial Properties of Hydrogels

In diabetic wounds, the hyperglycemic microenvironment accelerates bacterial multiplication, consequently impairing the healing process. In this context, hydrogel dressings, as a critical material for diabetic wound management, must exhibit exceptional antibacterial activity to effectively inhibit bacterial infections and establish a conducive microenvironment for wound healing [14]. In this study, the antibacterial properties of hydrogels with different compositions against *E. coli*, *S. aureus* and MRSA were evaluated, respectively, by the plate counting method (Figure 6a). As shown in Figure 6b–d, the survival rates of Con-

Control, QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB for *E. coli* were 99.99%, 44.91%, 38.10%, 4.61% and 13.23%. The survival rates of *S. aureus* were 99.99%, 32.76%, 27.87%, <1% and <1%. The survival rates for MRSA were 99.99%, 36.44%, 37.07%, <1% and 1.41%. To delineate the specific contribution of tannic acid (TA) to the antibacterial performance of the hydrogel, previous studies have reported that free TA alone exhibits only moderate antibacterial efficacy under comparable physiological conditions, with bacterial survival rates of approximately 49% for *E. coli*, 57% for *S. aureus*, and 57% for MRSA [4]. In contrast, the QCMCS/OSA/TA hydrogel developed in this work demonstrates markedly enhanced antibacterial activity, reducing the survival of *E. coli* to 4.61% and suppressing *S. aureus* and MRSA by more than 99%. This pronounced improvement indicates that the antibacterial performance of the hydrogel is primarily attributed to the synergistic interaction between QCMCS and TA, rather than the antibacterial action of TA alone.

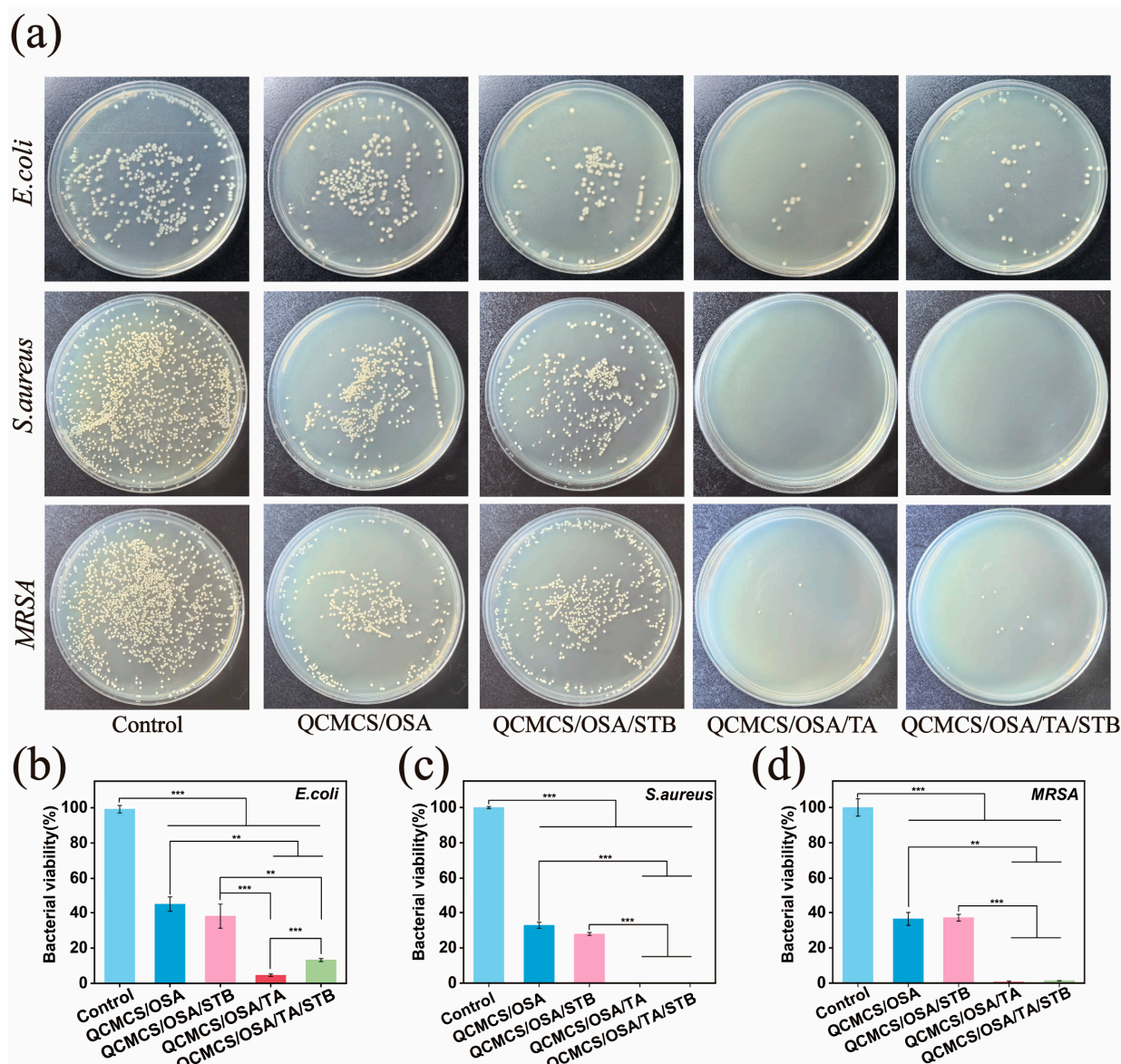


Figure 6. In vitro evaluation of the antibacterial performance of QCMCS/OSA/TA/STB hydrogels. (a) Representative agar plate images of viable bacteria after treatment with different samples; (b) *E. coli* colony counts (mean $\pm$ SD, $n = 3$, ** $p < 0.01$, *** $p < 0.001$); (c) *S. aureus* colony counts (mean $\pm$ SD, $n = 3$, *** $p < 0.001$); (d) MRSA colony counts (mean $\pm$ SD, $n = 3$, ** $p < 0.01$, *** $p < 0.001$).

As depicted in Figure S5, consistent with the plate counting assay, the optical density measurements confirm that QCMCS/OSA/TA and QCMCS/OSA/TA/STB hydrogels exhibit strong antibacterial activity. The positive charge of quaternized CMCS enables interaction with negatively charged bacterial membranes, leading to membrane disruption. This induces bacterial membrane rupture, resulting in increased permeability and leakage of intracellular components (e.g., amino acids and nucleotides), thereby inhibiting bacterial growth and reproduction and ultimately causing bacterial death [13]. During the proliferation of *E. coli*, *S. aureus*, and MRSA, ROS are generated. These ROS can cleave the borate ester bonds in the hydrogel, facilitating the release of tannic acid (TA) to exert its antibacterial effects [12]. Tannic acid (TA) can form hydrogen bonds and undergo hydrophobic interactions with bacterial surface proteins via its phenolic hydroxyl groups, inducing coagulation and precipitation of protein molecules. This process disrupts the structure and function of bacterial cell membranes, further inhibiting bacterial proliferation [43]. QCMCS and TA exhibit complementary antibacterial mechanisms. QCMCS primarily exerts antibacterial effects by disrupting bacterial cell membranes, inhibiting the activity of metabolic enzymes, and interfering with genetic materials. TA, on the other hand, acts against bacteria through pathways including precipitating bacterial proteins, inhibiting enzyme activity, chelating metal ions, and preventing bacterial adhesion. These two components exert distinct antibacterial mechanisms; their combination enables multi-targeted action against bacteria, leading to more comprehensive inhibition of bacterial growth and reproduction. Additionally, we observed that the QCMCS/OSA/TA/STB hydrogel exhibited stronger antibacterial activity against *S. aureus* and MRSA compared to *E. coli*, which aligns with the reported functional characteristics of TA in the literature [39,43,44]. TA penetration into *E. coli* is impeded by the bacterium's dual-layered cell envelope, limiting its effectiveness in disrupting metabolism and eliciting bacterial cell death [45,46].

3.10. Cytocompatibility of Hydrogels

The in vitro cytocompatibility of the corresponding hydrogels 72 h was analyzed through the CCK-8 assay and calcein AM/propidium iodide live/dead staining. As shown in Figure 7a, the CCK-8 assay revealed that the proliferation of human umbilical vein endothelial cells (HUVECs) cultured with these hydrogels for 24, 48, and remained highly viable, with cell survival rates exceeding 93% across all groups, showing no significant difference compared to the control group. In particular, the QCMCS/OSA/TA/STB group reached a cell survival rate of $98.49 \pm 1.4\%$ after 72 h, demonstrating excellent biocompatibility. Cell viability was further corroborated by live/dead staining using Calcein-AM and propidium iodide (PI). Fluorescence images in Figure 7b show that after 24, 48, and 72 h of culture with hydrogel extracts, the majority of HUVECs exhibited green fluorescence (indicating live cells), with only sporadic red fluorescence (dead cells) observed. These results align closely with the CCK-8 data, confirming the high cytocompatibility of the hydrogels. The excellent biocompatibility of the QCMCS/OSA/TA/STB hydrogel can be attributed to its mild dynamic borate ester cross-linking system and the low cytotoxicity of its polyelectrolyte complex components, thereby supporting its safe use in biomedical applications such as wound repair.

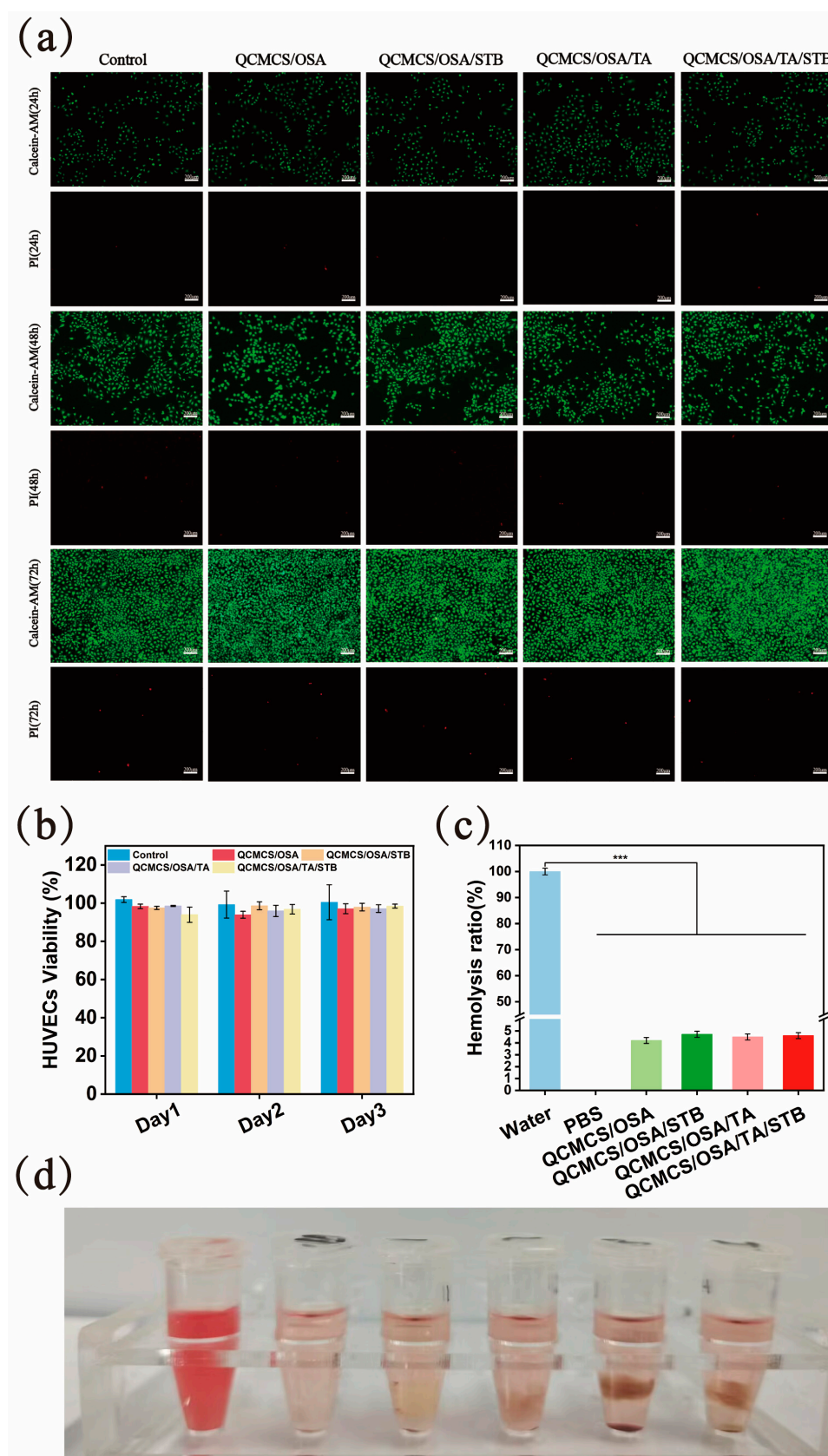


Figure 7. Hemostatic behavior and in vitro biocompatibility of QCMCS/OSA/TA/STB hydrogels. (a) Calcein-AM/PI staining of HUVECs at multiple time points (24–72 h); (b) HUVEC viability evaluation (mean $\pm$ SD, $n = 3$); (c) Hemolysis ratio determination (mean $\pm$ SD, $n = 3$, *** $p < 0.001$); (d) Representative image from the hemolysis experiment.

3.11. Blood Compatibility of Hydrogels

As widely used wound dressings in biomedical fields, hydrogels need to possess favorable cytocompatibility, particularly with regard to blood compatibility. Here, hemolysis assays were conducted to examine the blood compatibility of QCMCS/OSA, QCMCS/OSA/STB, QCMCS/OSA/TA, and QCMCS/OSA/TA/STB hydrogels. As shown in Figure 7c, significant hemolysis was observed in the positive control group (deionized water), while red blood cells in the negative control group (PBS) remained intact with negligible lysis. Importantly, all hydrogel groups displayed behavior similar to the PBS group: the supernatants remained clear and transparent, showing no visible signs of hemolysis. Quantitative results (Figure 7d) confirmed that the hemolysis ratios of all hydrogels were below 5%, corresponding to a slightly hemolytic classification according to ASTM F756-13A. These results revealed that all tested hydrogels demonstrated satisfactory hemocompatibility.

4. Conclusions

In conclusion, the QCMCS/OSA/TA/STB hydrogel, prepared through a straightforward physical blending process, demonstrates dual responsiveness to elevated glucose and ROS levels, allowing for the on-demand release of TA within the diabetic wound microenvironment to accelerate healing. TA enhances the structural stability, functionality, and bioactivity of the hydrogel via dual cross-linking through borate ester bonds and hydrogen bonding. The characteristic porous architecture of the hydrogel contributes to its outstanding physicochemical properties, including injectability, moldability, autonomous self-healing, swelling capacity, and controlled biodegradability. Moreover, the hydrogel exhibits exceptional biocompatibility, minimal hemolysis, and potent antioxidant and antibacterial performance. STB, a widely available chemical, plays an essential role in this system. Its borate ions ($\text{B}(\text{OH})_4^-$) condense with catechol-derived phenolic hydroxyl groups under alkaline conditions ($\text{pH} = 7.4$) to form dynamic borate ester bonds, which effectively shield and stabilize the catechol structures. In the specific microenvironment of diabetic wounds, characterized by high glucose and ROS, these borate ester bonds undergo cleavage, regenerating the active phenolic hydroxyl groups and thereby restoring TA's functional and bioactive properties, such as promoting wound repair. This multifunctional hydrogel presents a novel strategy for designing advanced polysaccharide-based biomaterials and shows significant promise for biomedical applications, particularly in the management of diabetic wound healing.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ma19040749/s1>, Figure S1: Photographs of the QCMCS/OSA/TA/STB hydrogel; Figure S2: Adaptation of QCMCS/OSA/TA/STB hydrogel to finger bending at 0° , 60° , and 90° ; Figure S3: Frequency sweep measurement of QCMCS/OSA/TA/STB hydrogels (fixed at 1% strain); Figure S4: Storage modulus variation in QCMCS/OSA/TA/STB hydrogels during cyclic loading at 0.1% and 50% strains; Figure S5: The OD value at 600 nm of *E. coli* (a) *S. aureus* (b) and MRSA (c) after co-culture with different sample. mean $\pm$ SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Table S1: Kinetic parameters obtained through fitting to various mathematical kinetic models of TA release profile in different pH at 37°C .

Author Contributions: Conceptualization, F.Y., C.W., Z.Z.; methodology, F.Y., J.Z.; software, F.Y., J.Z.; validation, J.Z.; formal analysis, F.Y.; investigation, F.Y.; resources, F.Y.; data curation, F.Y., C.W.; writing—original draft preparation, F.Y.; writing—review and editing, Z.Z.; visualization, F.Y.; supervision, Z.Z.; project administration, Z.Z.; funding acquisition, Z.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Animal Care and Use Committee of Wuhan University of Technology (protocol code WHUT2025–063), and all procedures followed the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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References

1. Wang, H.; Xu, Z.; Zhao, M.; Liu, G.; Wu, J. Advances of hydrogel dressings in diabetic wounds. *Biomater. Sci.* **2021**, *9*, 1530–1546. [\[CrossRef\]](#)
2. Xiong, Y.; Chu, X.; Yu, T.; Knoedler, S.; Schroeter, A.; Lu, L.; Zha, K.; Lin, Z.; Jiang, D.; Rinkevich, Y.; et al. Reactive Oxygen Species-Scavenging Nanosystems in the Treatment of Diabetic Wounds. *Adv. Healthc. Mater.* **2023**, *12*, 2300779. [\[CrossRef\]](#)
3. Zhang, Z.; Wang, Y.; Wang, C.; Yu, F.; Yuan, Y.; Zhao, Z. Poly(Lipoic Acid) Hydrogel Dressings With Adhesion, Self-Healing, and Self-Adaptive Properties via Dynamic Bonding. *J. Appl. Polym. Sci.* **2025**, *142*, e57021. [\[CrossRef\]](#)
4. Yang, Z.; Wang, C.; Zhang, Z.; Yu, F.; Wang, Y.; Ding, J.; Zhao, Z.; Liu, Y. A pH responsive tannic acid/quaternized carboxymethyl chitosan/oxidized sodium alginate hydrogels for accelerated diabetic wound healing and real-time monitoring. *Int. J. Biol. Macromol.* **2024**, *264*, 130741. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Liu, Y.; Zhu, M.; Ou, J.; Li, K.; Ju, X.; Tian, Y.; Niu, Z. Multi-responsive sodium hyaluronate/tannic acid hydrogels with ROS scavenging ability promote the healing of diabetic wounds. *Int. J. Biol. Macromol.* **2024**, *278*, 134896. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Li, Y.; Chen, P.; Wu, Z.; Shi, C.; Xu, Y.; Chen, X.; Chen, M.; Li, Y.; Yan, C.; Shi, Y.; et al. A nonradiographic strategy to real-time monitor the position of three-dimensional-printed medical orthopedic implants by embedding superparamagnetic Fe₃O₄ particles. *Interdiscip. Mater.* **2024**, *3*, 133–149. [\[CrossRef\]](#)
7. Pang, Z.; Li, Q.; Liu, K.; Wu, X.; Xu, H.; Chen, Z.; Dai, H. Efficacy of melanin-loaded lipoic acid-modified chitosan hydrogel in diabetic wound healing. *Carbohydr. Polym.* **2024**, *340*, 122215. [\[CrossRef\]](#)
8. Tian, M.; Fan, C.; Ge, R.; Liu, G.; Su, L.; Dong, H. Visible light-activated Ti-MOF loaded metformin-hydrogel composite dressing for accelerated chronic diabetic wound healing. *Chem. Eng. J.* **2025**, *506*, 160179. [\[CrossRef\]](#)
9. Gholamali, I.; Vu, T.T.; Jo, S.H.; Park, S.H.; Lim, K.T. Exploring the progress of hyaluronic acid hydrogels: Synthesis, characteristics, and wide-ranging applications. *Materials* **2024**, *17*, 2439. [\[CrossRef\]](#)
10. Wang, Y.; Yang, C.; Zhang, W.; Wang, X.; Zhao, Z.; Wang, Z.; Zhang, L. Multifunctional self-healing and pH-responsive hydrogel dressing based on cationic guar gum and hyaluronic acid for on-demand drug release. *Int. J. Biol. Macromol.* **2025**, *301*, 140326. [\[CrossRef\]](#)
11. Xu, J.; Chang, L.; Xiong, Y.; Peng, Q. Chitosan-Based Hydrogels as Antibacterial/Antioxidant/Anti-Inflammation Multifunctional Dressings for Chronic Wound Healing. *Adv. Healthc. Mater.* **2024**, *13*, 2401490. [\[CrossRef\]](#)
12. Zhao, J.; Lv, X.; Chen, Y.; Lu, X.; Xie, C. Self-adhesive and conductive hydrogel based on MIL-53 (Fe)-anchored graphene oxide for bioelectronics and wound healing. *Smart Mater. Med.* **2025**, *6*, 406–416. [\[CrossRef\]](#)
13. Chen, J.; Wang, Y.; Xu, W.; Wu, X.; Bao, Q.; You, L.; Xiong, C.; Wang, S. High performance breathable conductive hydrogel sensor based on sodium alginate and polyacrylamide with cross-linked dual network structures. *Int. J. Biol. Macromol.* **2025**, *307*, 142172. [\[CrossRef\]](#)
14. Wang, C.; Yu, F.; Yuan, Y.; Zhang, Z.; Yang, Z.; Wang, X.; Zhao, Z. Nanozyme-Crosslinked Poly(Thioctic Acid)-Based Hydrogel Adhesives with Coral-Like Structure for Promoting Wet Adhesion, Antibacterial Activity, and Wound Healing. *Adv. Healthc. Mater.* **2026**, *15*, e01866. [\[CrossRef\]](#)
15. Geng, H.; Zheng, X.; Zhang, Y.; Cui, X.; Li, Z.; Zhang, X.; Cui, J.; Meng, F.; Sun, L.; Ni, S. Microenvironment-Responsive Hydrogels with Detachable Skin Adhesion and Mild-Temperature Photothermal Property for Chronic Wound Healing. *Adv. Funct. Mater.* **2023**, *33*, 2305154. [\[CrossRef\]](#)
16. Ren, Z.; Wan, Q.; Zhu, Y.; Li, L.; Wang, K.; Zhao, F.; Jiao, K.; Tang, M.; Tay, F.; Wan, M.; et al. Atypical artificial cells: Novel biomimetic materials for combating cancer. *Interdiscip. Mater.* **2024**, *3*, 658–714. [\[CrossRef\]](#)

17. Wang, X.; Dai, J.; Shao, C.; Goto, T.; Dai, H. Emerging advances in fluorescence imaging and phototherapy of arthritis. *Interdiscip. Mater.* **2023**, *2*, 803–832. [[CrossRef](#)]
18. Gomez, C.G.; Rinaudo, M.; Villar, M.A. Oxidation of sodium alginate and characterization of the oxidized derivatives. *Carbohydr. Polym.* **2007**, *67*, 296–304. [[CrossRef](#)]
19. Zhao, G.; Gu, T.; Chen, L.; Wang, L.; Petrov, Y.V.; Baulin, V.E.; Tsivadze, A.Y.; Jia, D.; Zhou, Y.; Li, B. Synergistic adhesion enhancement of double-crosslinking chitosan hydrogel via catechol-Fe³⁺ coordination and Schiff base. *Carbohydr. Polym.* **2025**, *356*, 123380. [[CrossRef](#)]
20. Yang, C.; Wang, Y.; Zhang, Z.; Wang, C.; Yu, F.; Zhao, Z.; Wang, Z.; Zhang, L. Photothermal antimicrobial guar gum hydrogel cross-linked by bioactive small molecule lysine for infected wound healing. *Int. J. Biol. Macromol.* **2025**, *301*, 140328. [[CrossRef](#)] [[PubMed](#)]
21. Yin, Y.; Li, X.; Li, Y.; Miao, H.; Shi, G. A Metal Ion-Responsive Spiropyran-Based Fluorescent Color-Changing Hydrogel. *Materials* **2025**, *18*, 2573. [[CrossRef](#)]
22. Zhang, X.; Liu, Y.; Wang, H.; Li, J.; Chen, Z.; Zhao, Y. A boron-based probe-driven theranostic hydrogel dressing for visual monitoring and matching chronic wound healing. *Adv. Funct. Mater.* **2022**, *32*, 2109987. [[CrossRef](#)]
23. Wang, Y.; Quan, X.; Duan, X.; Yang, D.; Zhao, H.; Meng, K. Nanofiber membrane-foam composite sheet with dual pH-responsive functions of drug release and color change. *Smart Mater. Med.* **2025**, *6*, 334–346. [[CrossRef](#)]
24. Terriac, L.; Helesbeux, J.-J.; Maugars, Y.; Guicheux, J.; Tibbitt, M.W.; Delplace, V. Boronate ester hydrogels for biomedical applications: Challenges and opportunities. *Chem. Mater.* **2024**, *36*, 6674–6695. [[CrossRef](#)]
25. Su, K.; Li, J.; Wu, X.; Deng, D.; Gu, H.; Sun, Y.; Wang, X.; Huang, W.; Wang, Y.; Shang, X.; et al. One-step synthesis of hydrogel adhesive with acid-responsive tannin release for diabetic oral mucosa defects healing. *Adv. Healthc. Mater.* **2024**, *13*, 2303252. [[CrossRef](#)]
26. Wu, K.; Fu, M.; Zhao, Y.; Gerhard, E.; Li, Y.; Yang, J.; Guo, J. Anti-oxidant, anti-inflammatory and antibacterial tannin-crosslinked citrate-based mussel-inspired bioadhesives facilitate scarless wound healing. *Bioact. Mater.* **2023**, *20*, 93–110. [[CrossRef](#)]
27. Chen, Y.; Wang, C.; Zhang, Z.; Yu, F.; Wang, Y.; Ding, J.; Zhao, Z.; Liu, Y. 3D-printed piezocatalytic hydrogels for effective antibacterial treatment of infected wounds. *Int. J. Biol. Macromol.* **2024**, *268*, 131637. [[CrossRef](#)]
28. Ahmed, A.; Ullah, N.; Ahmed, W.; Haider, A.; senanu James-ocloo, O.; Lv, Z.; Zhang, X.; Han, X.; Wei, Y.; Hu, Y.; et al. Multifunctional alginate based hydrogel beads dressing for chronic wound healing: A review. *Int. J. Biol. Macromol.* **2025**, *339*, 149853. [[CrossRef](#)] [[PubMed](#)]
29. Xu, N.; Gao, Y.; Li, Z.; Chen, Y.; Liu, M.; Jia, J.; Zeng, R.; Luo, G.; Li, J.; Yu, Y. Immunoregulatory hydrogel decorated with Tannic acid/Ferric ion accelerates diabetic wound healing via regulating Macrophage polarization. *Chem. Eng. J.* **2023**, *466*, 143173. [[CrossRef](#)]
30. Chi, J.; Li, A.; Zou, M.; Wang, S.; Liu, C.; Hu, R.; Jiang, Z.; Liu, W.; Sun, R.; Han, B. Novel dopamine-modified oxidized sodium alginate hydrogels promote angiogenesis and accelerate healing of chronic diabetic wounds. *Int. J. Biol. Macromol.* **2022**, *203*, 492–504. [[CrossRef](#)] [[PubMed](#)]
31. Yu, J.; He, Y.; Wang, Y.; Li, S.; Tian, S. Ethylenediamine-oxidized sodium alginate hydrogel cross-linked graphene oxide nanofiltration membrane with self-healing property for efficient dye separation. *J. Membr. Sci.* **2023**, *670*, 121366. [[CrossRef](#)]
32. Shi, W.; Kong, Y.; Su, Y.; Kuss, M.A.; Jiang, X.; Li, X.; Xie, J.; Duan, B. Tannic acid-inspired, self-healing, and dual stimuli responsive dynamic hydrogel with potent antibacterial and anti-oxidative properties. *J. Mater. Chem. B* **2021**, *9*, 7182–7195. [[CrossRef](#)] [[PubMed](#)]
33. Yu, X.; Gholipourmalekabadi, M.; Wang, X.; Yuan, C.; Lin, K. Three-dimensional bioprinting biphasic multicellular living scaffold facilitates osteochondral defect regeneration. *Interdiscip. Mater.* **2024**, *3*, 738–756. [[CrossRef](#)]
34. Xiao, W.; Wan, X.; Shi, L.; Ye, M.; Zhang, Y.; Wang, S. A viscous-biofluid self-pumping organohydrogel dressing to accelerate diabetic wound healing. *Adv. Mater.* **2024**, *36*, 2401539. [[CrossRef](#)]
35. Contardi, M.; Ayyoub, A.M.; Summa, M.; Kossovaki, D.; Fadda, M.; Liessi, N.; Armirotti, A.; Fragouli, D.; Bertorelli, R.; Athanassiou, A. Self-Adhesive and Antioxidant Poly(vinylpyrrolidone)/Alginate-Based Bilayer Films Loaded with Malva sylvestris Extracts as Potential Skin Dressings. *ACS Appl. Bio Mater.* **2022**, *5*, 2880–2893. [[CrossRef](#)]
36. Schadte, P.; Rademacher, F.; Andresen, G.; Hellfritsch, M.; Qiu, H.; Maschkowitz, G.; Gläser, R.; Heinemann, N.; Drücke, D.; Fickenscher, H.; et al. 3D-printed wound dressing platform for protein administration based on alginate and zinc oxide tetrapods. *Nano Converg.* **2023**, *10*, 53. [[CrossRef](#)]
37. Xiong, Y.; Li, M.; Qing, G. Biomolecule-responsive polymers and their bio-applications. *Interdiscip. Mater.* **2024**, *3*, 865–896. [[CrossRef](#)]
38. Liu, F.; Li, G.; An, Z.; Wang, S.; Xu, S.; Liu, H. Dynamic Boronate Ester Based Hydrogel with Enhanced Mechanical Properties and Multi-Stimuli-Triggered Release for Tissue Repair and Antioxidant Therapy. *Gels* **2025**, *11*, 370. [[CrossRef](#)]
39. Hao, J.; Qin, C.; Wu, C. Three-dimensional multicellular biomaterial platforms for biomedical application. *Interdiscip. Mater.* **2023**, *2*, 714–734. [[CrossRef](#)]

40. Zou, P.; Lee, W.-H.; Gao, Z.; Qin, D.; Wang, Y.; Liu, J.; Sun, T.; Gao, Y. Wound dressing from polyvinyl alcohol/chitosan electrospun fiber membrane loaded with OH-CATH30 nanoparticles. *Carbohydr. Polym.* **2020**, *232*, 115786. [[CrossRef](#)]
41. Yang, W.; Zhong, W.; Yan, S.; Wang, S.; Xuan, C.; Zheng, K.; Qiu, J.; Shi, X. Mechanical Stimulation of Anti-Inflammatory and Antioxidant Hydrogels for Rapid Re-Epithelialization. *Adv. Mater.* **2024**, *36*, 2312740. [[CrossRef](#)]
42. Qiao, L.; Liang, Y.; Chen, J.; Huang, Y.; Alsareii, S.A.; Alamri, A.M.; Harraz, F.A.; Guo, B. Antibacterial conductive self-healing hydrogel wound dressing with dual dynamic bonds promotes infected wound healing. *Bioact. Mater.* **2023**, *30*, 129–141. [[CrossRef](#)]
43. Fu, D.; Yang, R.; Wang, R.; Wang, Y.; Li, Y.; Bian, H. A triple-crosslinked, self-healing, polyvinyl alcohol/nanocellulose hydrogel for versatile sensing applications. *Carbohydr. Polym.* **2024**, *334*, 122060. [[CrossRef](#)]
44. Yang, Z.; Zhang, J.; Wang, C.; Yu, F.; Yu, W.; Zhao, Z. A glucose responsive multifunctional hydrogel with antibacterial properties and real-time monitoring for diabetic wound treatment. *Biomater. Sci.* **2025**, *13*, 275–286. [[CrossRef](#)] [[PubMed](#)]
45. Dong, Z.; Xu, J.; Lun, P.; Wu, Z.; Deng, W.; Sun, P. Dynamic cross-linking, self-healing, antibacterial hydrogel for regenerating irregular cranial bone defects. *ACS Appl. Mater. Interfaces* **2024**, *16*, 39035–39050. [[CrossRef](#)] [[PubMed](#)]
46. Shang, D.; Zhang, X.; Zeng, S.; Jiang, K.; Gao, H.; Dong, H.; Yang, Q.; Zhang, S. Protic ionic liquid [Bim][NTf₂] with strong hydrogen bond donating ability for highly efficient ammonia absorption. *Green Chem.* **2017**, *19*, 937–945. [[CrossRef](#)]

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