Electronic Supplementary Information

Photocrosslinked Hybrid Hydrogel Based on Chitosan/Hyaluronic Acid /ZnO toward Wound Sealing

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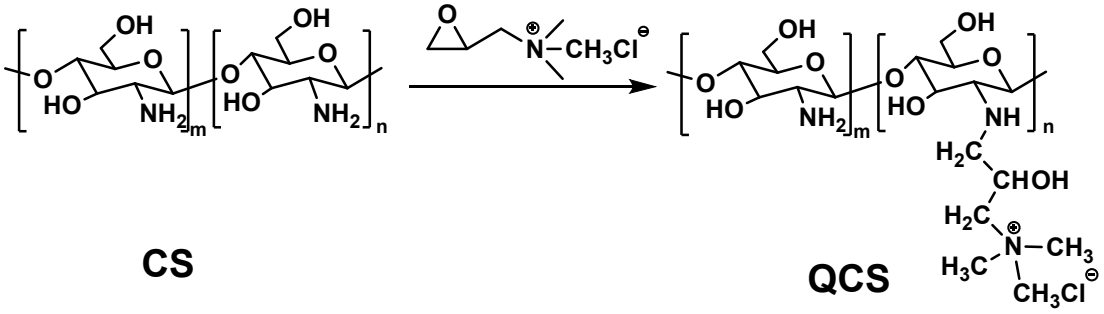
1. Synthesis and characterization

Synthesis of QCS: Chitosan (CS, 2.5 g) was dissolved in100 mL of isopropanol, and the pH was adjusted to 8~9 by 1 wt% NaOH followed by the addition of glycidyl trimethyl ammonium chloride (GTA). The mixture was stirred at 60°C for 24 h, then dialyzed for 3 days to remove unreacted GTA, and finally freeze-dried for another 3 days to yield quaternized chitosan (QCS).



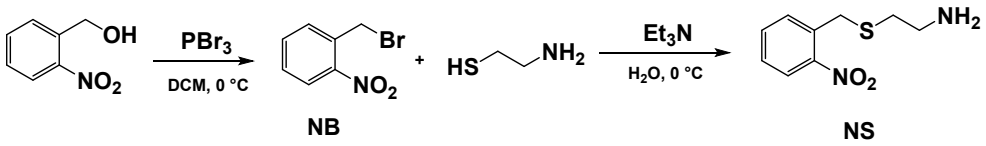
Scheme. S1 Synthetic route of QCS.

The degree of quaternization (*DQ*) of QSC was determined to be 67.2 % by the analytical method using 8% K_2CrO_4 as the indicator. To perform the titration, 1 mL of the indicator was added to the QSC solution, followed by slow titration with 0.05 mol/L silver nitrate solution. The endpoint of the titration was marked by the solution turning brick red.

$$DQ = \frac{V \cdot C \cdot 10^{-3}}{V \cdot C \cdot 10^{-3} + \left(W - V \cdot C \cdot MW_1 \cdot 10^{-3}\right)/MW_2} \times 100\% \quad \text{(Eq.S1)}$$

where, V (mL) represents the volume of the $AgNO_3$ aqueous solution required to reach the inflection point, C (mol/L) is the $AgNO_3$ aqueous solution concentration, W (g) is the weight of the QSC sample, MW_1 is the molecular mass of quaternary glucosamine (314 g/mol), MW_2 is the molecular mass of glucosamine and (162 g/mol), respectively.

Synthesis of NS: 2-Nitrobenzyl alcohol (10 mmol) was dissolved in dichloromethane (10 mL), and PBr_3 (1.2 mL) was added under an ice-water bath. The mixture was stirred for 60 min, then diluted with 10 mL dichloromethane and washed with deionized water. The aqueous phase was extracted with dichloromethane, and the combined organic phases were collected. The brown solid product o-nitrobenzyl bromide, was isolated using column chromatography. (1.48 g, yield of 68.5%). 2-Aminoethanethiol (5.5 mmol) was dissolved in deionized water (15 mL) and triethylamine (0.7 mL) was added to the solution. The mixture was cooled to 0°C, and then o-nitrobenzyl bromide (5 mmol, dissolved in 15 mL CH_3OH) was slowly added dropwise to the flask. The mixture was stirred at 0°C for 30 min, followed by stirring at 25°C for an additional 30 min. The organic phase was separated, dried, and purified by column chromatography to yield a yellow oily product, NS (0.65 g, 61.2% yield).



Scheme. S2 Synthetic route of photocage NS.

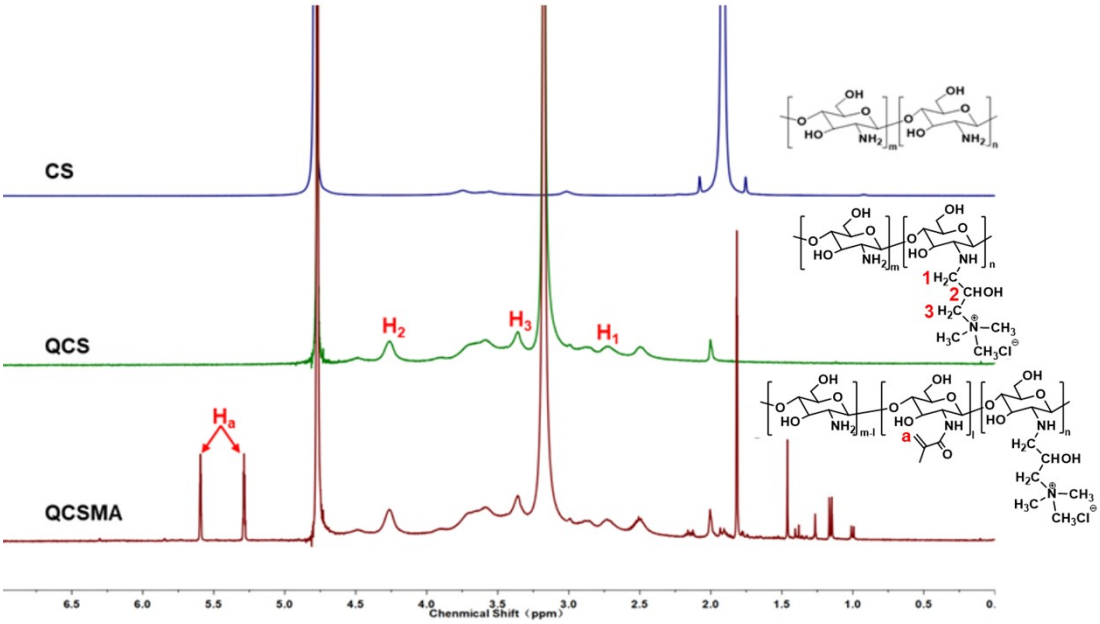


Fig. S1 ^{1}H NMR spectra of CS, QCS, and QCSMA.

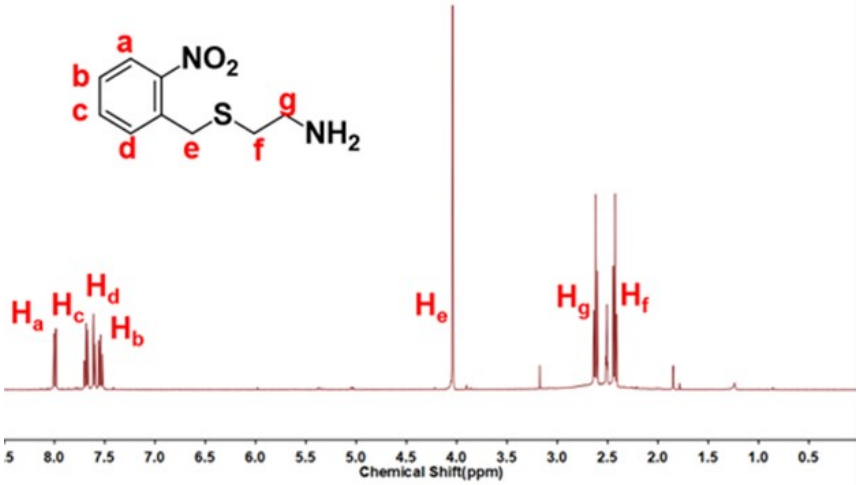


Fig. S2 ^{1}H NMR spectrum of NS.

$$Degree\ of\ NS\ (DN) = \frac{3I_a}{I_b} \times 100\% \qquad \text{(Eq.S2)}$$

where I_a represents the integral of the peaks corresponding to the protons of the NS group, and I_b represents the integral of the reference peak, the degree of substitution was determined to be 19.5%.

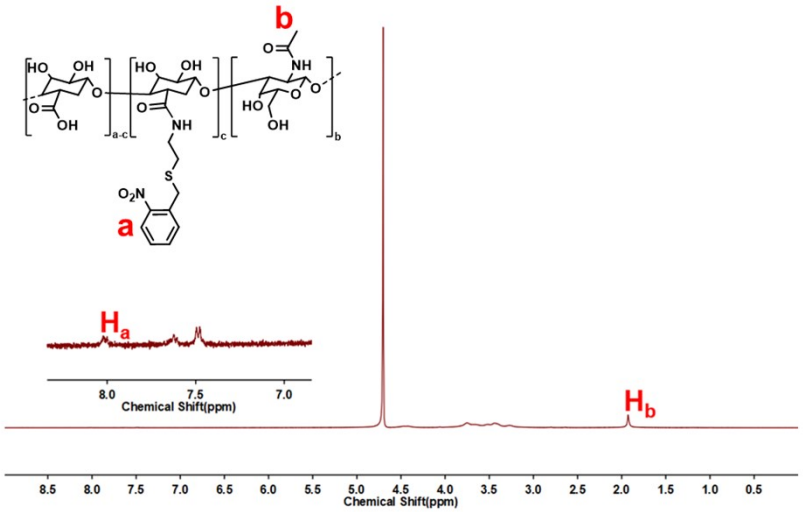


Fig. S3 ^{1}H NMR spectrum of HANS.

2. Results and Discussions

In order to investigate the reason for the decrease in the strength of the hydrogel after the addition of ZnO, we measured the UV-vis absorption spectra of ZnO and LAP, respectively, and it was assumed that the decrease in the strength of the hydrogel was due to the competition between the absorption of ZnO and the photoinitiator at 365 nm.

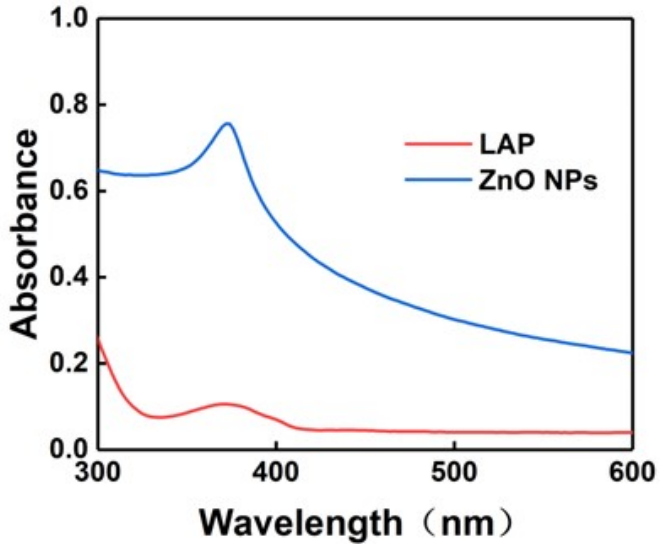


Fig. S4 UV-vis absorption spectra of photoinitiator LAP and ZnO NPs.

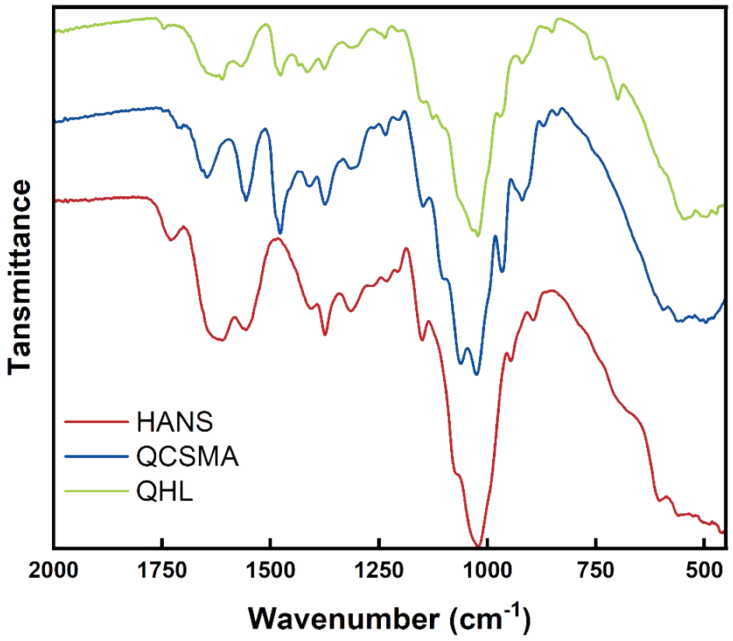


Fig. S5 FTIR spectra of HANS, QCSMA and QHL hydrogels.

The peaks at 1700 and 841 cm^{-1}, corresponding to the C=C bond, disappeared following photocrosslinking, confirming the successful formation of the hydrogel network. Furthermore, new weak peaks at 699 and 545 cm^{-1} emerged, which can be attributed to the formation of S–S bonds in the QHL/ZnO hydrogel.

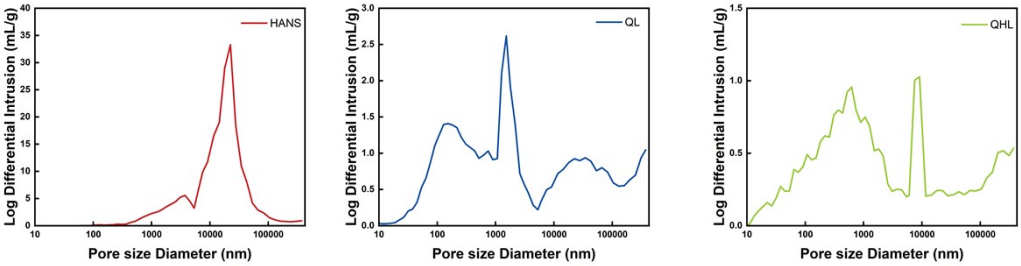


Fig. S6 Mercury intrusion porosimetry plots showing pore size distribution for different hydrogels.

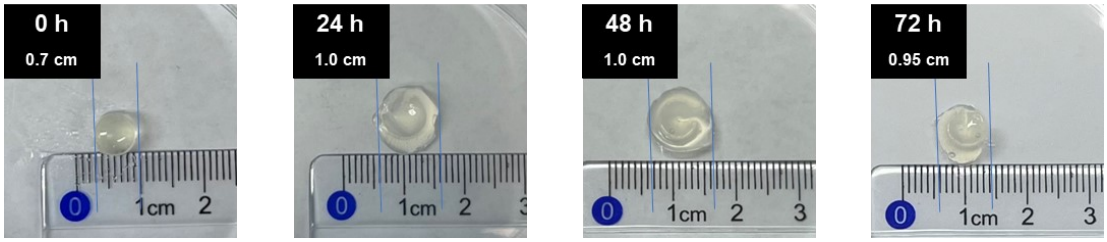


Fig. S7 Time-dependent morphological changes of QHL/ZnO hydrogel in 1 M HCl solution (pH = 0).

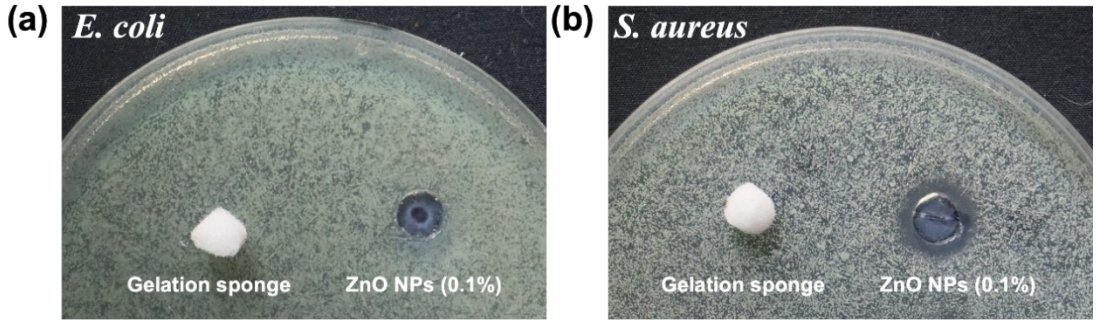


Fig. S8 The inhibition zone of hydrogels against (a) *E. coli* and (b) *S. aureus*.

Inoculation was performed by adding 100 μL of the hydrogel precursors to sample tubes, with 5 mL and 4.9 mL of the inoculum added to control and sample tubes, respectively, for incubation. After 18 h, the samples were diluted to 10^2, 10^3, and 10^4 times. And 100 μL aliquot of each dilution was spread onto agar plates and incubated for 24 h. Agar plates with dilutions yielding colony counts between 30 and 300 CFU were selected, photographed, and recorded. Bacterial concentration and antibacterial rate were calculated by Eq.3 and Eq.4 (ESI†), respectively.

Calculation of viable bacterial concentration:

$$Bacterial\ Concentration(CFU/mL) = \frac{Number\ of\ Colonies \times Dilution\ Factor}{Volume\ Plated(mL)} \quad \text{(Eq.S3)}$$

Calculation of antibacterial rate:

$$Antibacterial\ Rate = \frac{K_{Control} - K_{Sample}}{K_{Control}} \times 100\% \quad \text{(Eq.S4)}$$

Table S1 Antibacterial rates of hydrogels against *E. coli* and *S. aureus*.

Samples	*E. coli*		*S. aureus*	
	Bacterial concentration（CFU/mL）	Antibacterial rate（%）	Bacterial concentration（CFU/mL）	Antibacterial rate（%）
Control	2.94×10^5	/	2.57×10^5	/
GelMA/HANS	2.41×10^5	18%	1.84×10^5	28.4%
QCSMA/HANS	0.11×10^5	96%	0.11×10^5	96%
QCSMA/HANS /ZnO	0	99%	0	99%

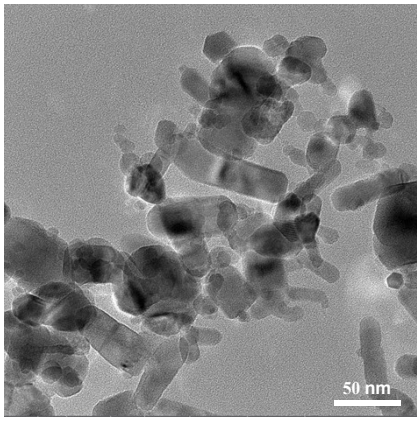


Fig. S9 TEM image of ZnO NPs.