Supporting Information

Injectable *in Situ* Self-Cross-Linking Hydrogels Based on Hemoglobin, Carbon Quantum Dots, and Sodium Alginate for Real-Time Detection of Wound Bacterial Infection and Efficient Postoperative Prevention of Tumor Recurrence

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Number of pages: 11
Number of figures: 11

Contents

Experimental Section

***In Vitro* Antibacterial Effects and Bacterial Adhesion.** The antibacterial ability of SA@Hb@CQDs was studied with *Escherichia coli* (*E. coli*, Gram-negative, ATCC25922) and *Saphylococcus aureus* (*S. aureus*, Gram-positive, ATCC25923) as typical bacteria according to the previous study. *E. coli* or *S. aureus* were cultured in lysogeny broth (LB) medium (10 g of tryptone, 5 g of yeast extract, and 0.5 g of NaCl per L of ultrapure water). The original bacterial suspensions were incubated in a shaking incubator at 37 °C for 12-24 h. Afterwards, the bacterial suspensions were diluted with fresh LB medium and continued to grow until the concentration of the optical density 0.5 at 540 nm. *E. coli* or *S. aureus* (1×10^7 cfu mL^{-1}) bacterial suspensions were incubated with: (a) control, (b) H_2O_2, (c) SA@Hb@CQDs and (d) SA@Hb@CQDs + H_2O_2 (1 mM) at 37 ℃ for 24 h. Then, the colony count experiment was performed to evaluate the antibacterial ability by coating bacterial suspensions onto agar plates and incubating for another 24 h. Additionally, SA@Hb@CQDs and SA@CQDs hydrogel were made into film by mold and cut into size 1×1 cm^2. Then the films were contact with bacterial suspension for 3 h in 24-well plate. Afterwards, the films mixed with 2.5 vol% glutaraldehyde and performed serial dehydration with various concentrations of ethanol. The morphologies of *E. coli* or *S. aureus bacteria* were observed by SEM after sputter-coating bacteria with gold.

MTT assay. The mouse embryonic fibroblasts NIH3T3 were used to investigated the cell cytotoxicity of SA@Hb@CQDs. The SA@Hb@CQDs were placed into 96-well plate with well-grown NIH3T3 cells at a density of 10^4 cells mL^{-1}. After incubation for different days at 37 °C, the culture solution and sample were removed, and 20 μL 5 mg mL^{-1} of MTT solution was added for another 4 h incubation. The obtained formazan crystals were dissolved with 100 μL of DMSO and the solution was then measured at 570 nm on a Bio-Tek microplate reader.

Hemolysis Assay and Morphological Changes of RBCs. The blood with anticoagulation collected from rats was centrifugated at 1500 rpm for 8 min to separate

with whole blood (5 mL) and discard platelets and plasma. The obtained RBCs were washed repeatedly and redispersed in PBS at a volume ratio of 1:9. After that, SA@Hb@CQDs were incubated with equivalent RBCs at 37 °C for 3 h. The water and physiological saline (0.9%) were used as a positive and negative control, respectively. After all the mixtures were centrifuged at 3000 rpm for 15 min, a microplate reader (BioTek synergy 2) was used to detected the optical density (OD) at 545 nm of the samples. The hemolysis rate can be calculated by the following formula:

$$\textbf{hemolysis rate (\%)} = (OD_s - OD_n) / (OD_p - OD_n) \times 100\% \quad \textbf{(S1)}$$

Where OD_s OD_n and OD_p represent the adsorption intensity of samples, negative control and positive controls, respectively. Finally, a droplet of RBCs suspension incubated with different concentrations of samples after centrifuged were added onto clean slides to observe the morphological changes of RBCs under the optical microscope.

In Vitro **Hemostasis Tests.** To investigate hemostasis capability of SA@Hb@CQDs, the same volume of sponge and SA@Hb@CQDs (0.3 cm^3) hydrogel were placed into culture dish. Then, 60 μL of whole blood with anticoagulant was added onto the sample and the same blood was added directly into the culture dish as control group. After incubated at 37 ℃ for 5 min, 30 mL of deionized water was added into the container along the wall of culture dish to cause hemolysis of free red blood cells in water.
Analogously, the same volume of gelatin sponge and SA@Hb@CQDs were placed into beaker. After 60 μL of whole blood with anticoagulant was dropped onto the sample, all beakers were put into 37 ℃ water bath kettle for 5 min to start the clotting process. Then 30 mL of deionized water was added into the container along the wall of culture beaker and the relative absorbency of diluted blood sample was measured by ultraviolet measurement at 540 nm. The blood clotting index (BCI) of materials can be calculated with optical density (OD) of supernatant at 540 nm microplate reader (BioTek synergy 2) and the calculation formula is as follows:

$$\textbf{BCI} = OD_{sample} / OD_{control} \times 100\% \quad \textbf{(S2)}$$

Where OD_{sample} and $OD_{control}$ were absorbency of blood solution touched with samples and pure blood solution respectively. 60 μL of whole blood containing anticoagulant diluted with 30 mL water was assumed to be 100 as reference. The smaller the BCI value, the stronger the hemostasis ability.

In addition, the same volume of gelatin sponge and SA@Hb@CQDs was placed into a test tube and treated with 37 °C water-bath for 3 min. After adding 1 mL of anticoagulant whole blood and 60 μL of $CaCl_2$ (0.25 mol/L), the tube was tilted every 15 s to record the blood clotting time when no blood was flowing. The control group (blood only) was also performed.

Cell Culture Experiments. All cell lines were obtained from the Jiangsu KeyGEN BioTECH Corp., Ltd. and cultured under hypoxic environment (5% CO_2 at 37 °C). The cell culture medium was DMEM media containing 10% FBS and 1% streptomycin.

Animal Models. Female Balb/c mice (6-8 weeks old) were injected with 4T1 or hepatic carcinoma (HCC) cells to obtain animal models. All animal experiment procedures were conducted in line with the guidelines approved and supervised by the Ethics Committee of Nanjing Normal University.

***In Vivo* Wound Healing Model.** Female Balb/c mice (6-8 weeks old) were divided into four groups: (a) PBS (control); (b) H_2O_2; (c) SA@Hb@CQDs; (d) SA@Hb@CQDs + H_2O_2 (1 mM) (n=5). All mice were cut out a wound of d = 5 mm (~78 mm^2) by surgical procedure on the dorsal thoracic midline of mice after anesthesia. Afterwards, the wounds were infected by *S. aureus* in logarithmic phase with 1×10^7 CFU mL^{-1}. 100 μL of SA@Hb@CQDs solution were injected directly to the wound area. After 24 h, 1 mM H_2O_2 solution was followed. Other groups were treated with the same condition except H_2O_2. The wounds were monitored with UV light at day 1, 3, 5, 8 and the body weight was measured with 2 days intervals. Wound tissues and main organs were collected for analysis after the mice were euthanized at day 8. In addition, at day 1,4,8, the bacterial samples of each group were collected from the wound area using a sterile swab. The collected samples were added into 5 mL of liquid beef extract peptone medium and shaken for 24 h at 37 °C. 30 μL of bacterial suspension of the

culture solution were spread on the agar culture plate and incubated at 37 °C for 24 h. Skin tissues were fixed with 10% paraformaldehyde solution and followed with hematoxylin and eosin (H&E) staining for histological analysis.

Subcutaneous Tumor Model. The 4T1 tumor-bearing mice (1×10^6 cells in 100 μL PBS) were separated into 2 groups (n = 5 per group) at random when the tumor volumes had become to about 100 mm^3 and each group received subsequently the following treatments: (a) injection of PBS in situ; (b) SA@Hb@CQDs and transformed into semisolid gels. The changes of body weight and tumor volume were recorded every 2 days and the tumor volume was calculated by the following formula: $width^2\times length/2$. The whole treatment completed after 14 days and all tumors as well as major organs were dissected from sacrificed mice. The collection were embedded with paraformaldehyde and stained with H&E and DAPI agents for histological analysis in optical microscope pictures. Moreover, 100 μL of SA@Hb@CQDs containing ICG (5 mg/mL) (SA@Hb@CQDs-ICG) was also injected to test the degradation behavior of hydrogels. At day 1, 4, 7, 11, 13, 15, 17, 20, and 24, mice treated with SA@Hb@CQDs-ICG were performed dynamic fluorescence imaging. After 24 days, the mice were sacrificed to collect the main organs for DAPI staining and confocal laser scanning microscopy (CLSM) was subsequently conducted.

Surgical Tumor Recurrence Model. Female Balb/c mice bearing HCC cells (1×10^6 cells in 100 μL PBS) were selected as animal models to evaluated the anti-recurrence efficiency of SA@Hb@CQDs. After the tumor volume increased to about 100 mm^3, all tumor lesions were removed surgically. Mice were randomly divided into 2 groups (n=5): (a) injection of PBS in situ; (b) implant of SA@Hb@CQDs in situ (all operation wound was treated with biological glue for stitch). The weights of mice were measured every two days to monitor the recurrence mice. On the 18th day the treatment was finished and all mice were sacrificed to harvest major organs and tumors for H&E assay DAPI staining.

Statistical Analysis. All quantitative data were represented by mean ± s.d. The statistical significance between two groups and multiple groups was defined as $P<0.05$

by two-tailed student's t test or one-way ANOVA t-test. Two-way ANOVA analysis was performed when an additional factor or variant was involved in the experiments.

Supporting Figures

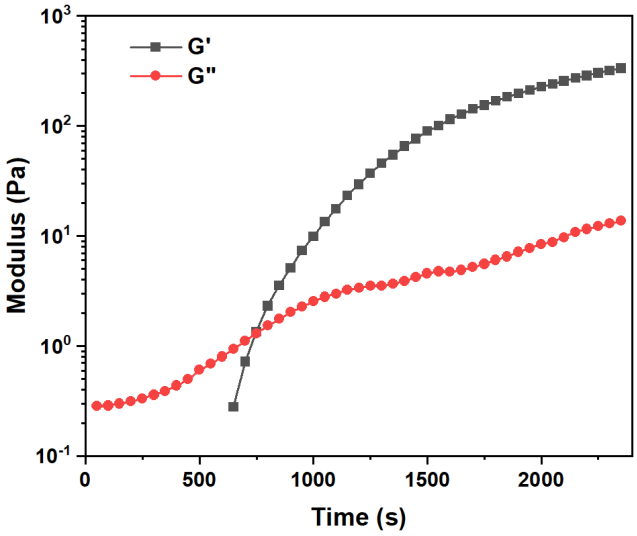

Figure S1. Rheological behavior of of SA@Hb@CQDs.

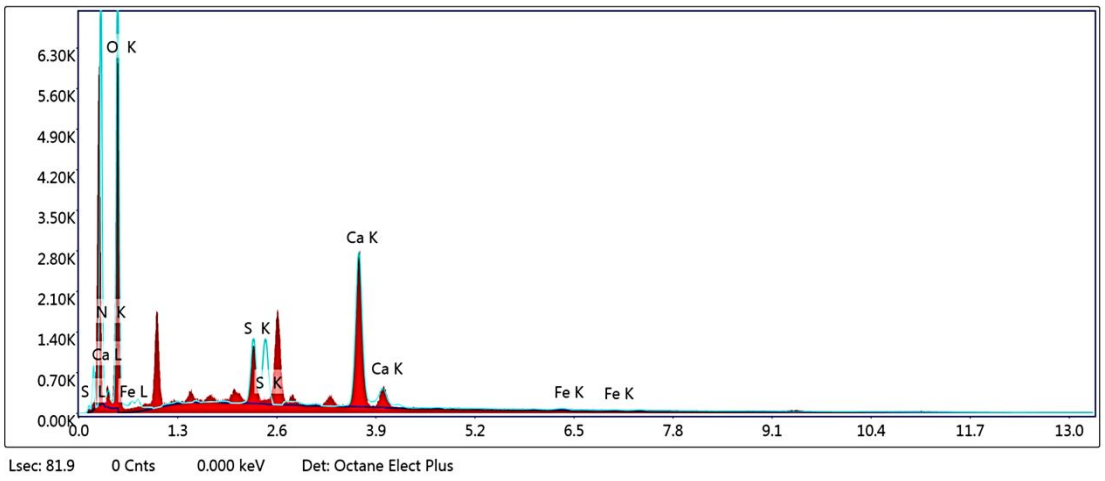

Figure S2. EDS spectra of SA@Hb@CQDs.

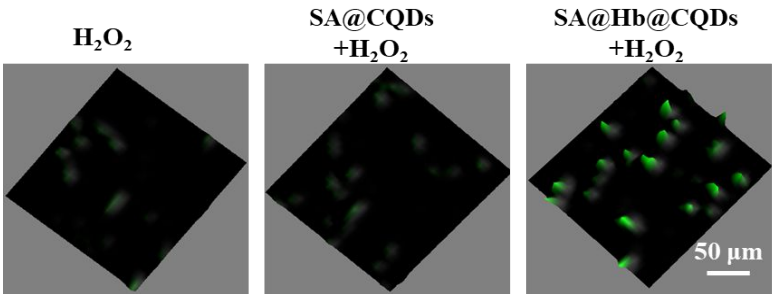

Figure S3. Intracellular levels of ROS detected by DCFH-DA under CLSM Scale bar: 50 μm.

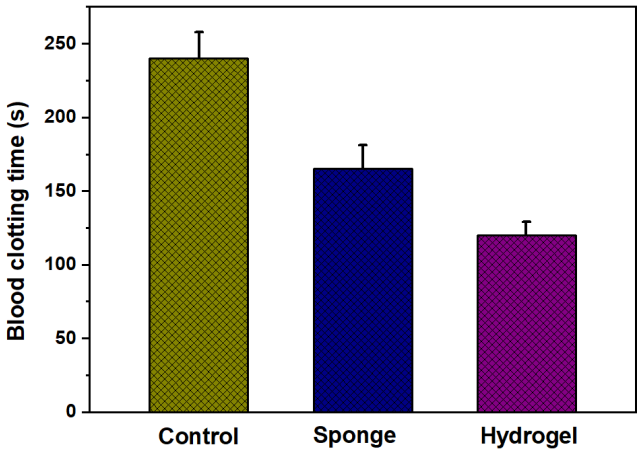

Figure S4. Corresponding blood clotting time of different materials.

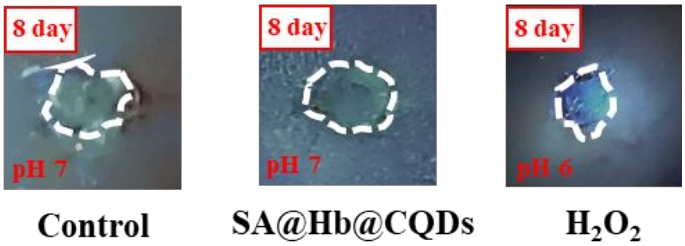

Figure S5. Photographs of *S. aureus* infected wounds of mice treated with PBS, SA@Hb@CQDs and H_2O_2 under UV light at day 8.

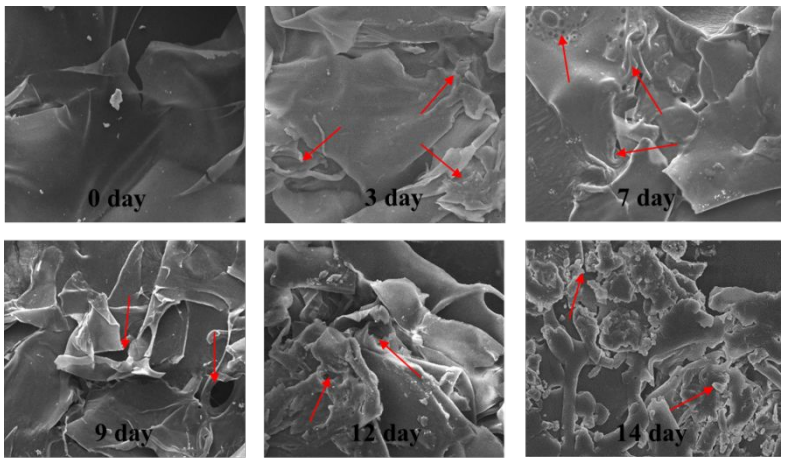

Figure S6. SEM images of SA@Hb@CQDs hydrogel incubated in acid PBS (pH=6) containing 1 mM of H_2O_2 at 37 ℃ for 0, 3, 7, 11, 18, and 24 days.

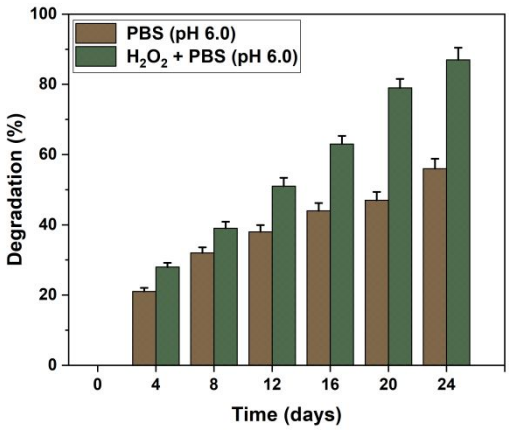

Figure S7. Degradation ratio of SA@Hb@CQDs hydrogel incubated in acid PBS (pH=6) followed with or without 1 mM of H_2O_2 at 37 ℃ for different days.

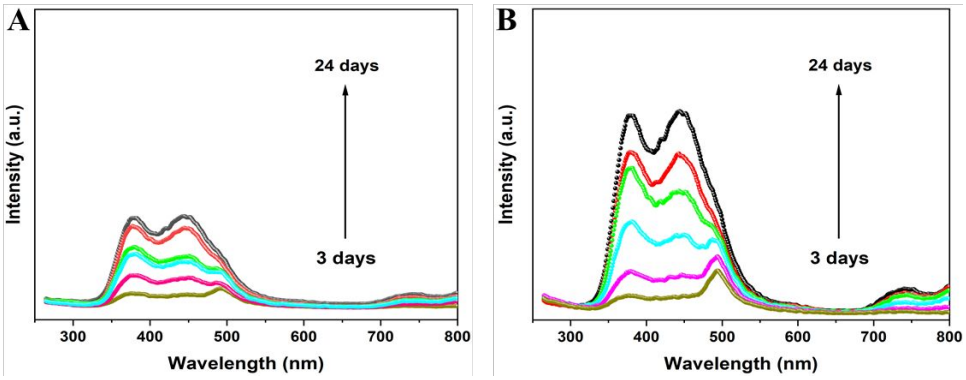

Figure S8. Fluorescence spectrum of SA@Hb@CQDs hydrogel incubated in acid PBS (pH=6) followed without (A) or with (B) 1 mM of H_2O_2 at 37 ℃ for different days (Ex=250 nm).

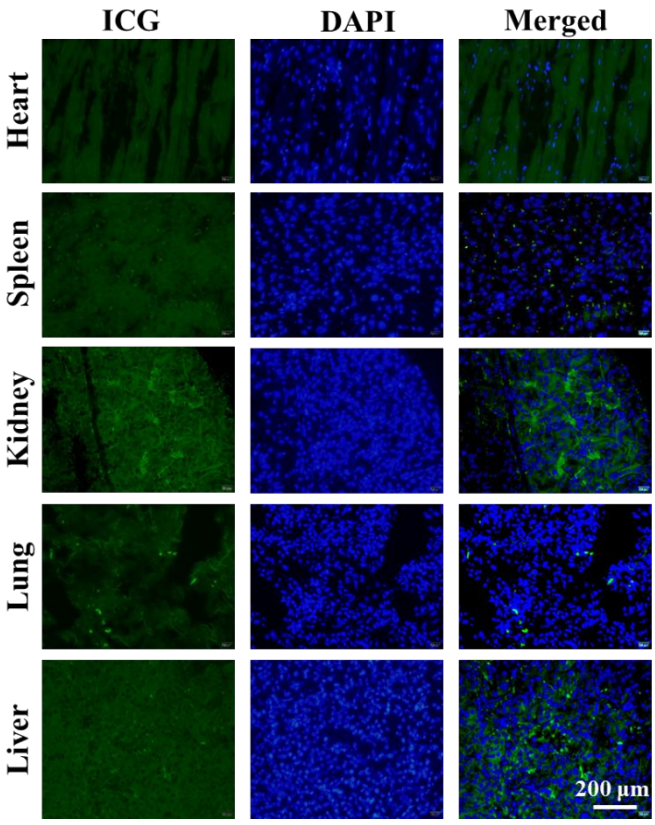

Figure S9. Confocal images of mice's main organs treated with ICG-SA@Hb@CQDs hydrogel and stained with DAPI.

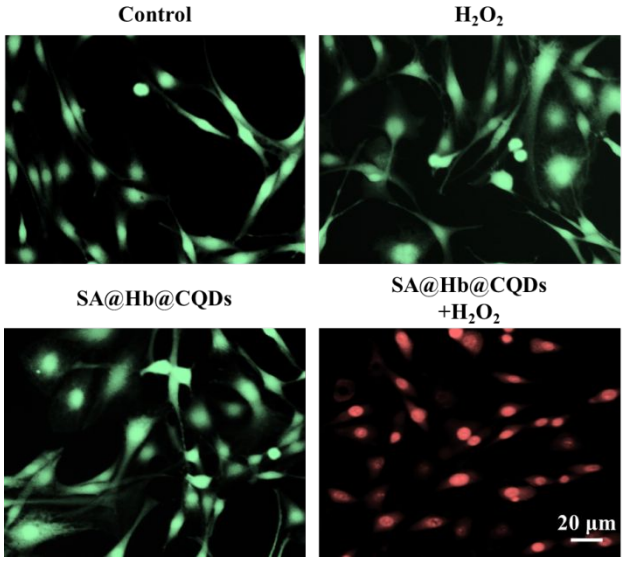

Figure S10. Live/dead staining images of 4T1 cells followed with various treatment.

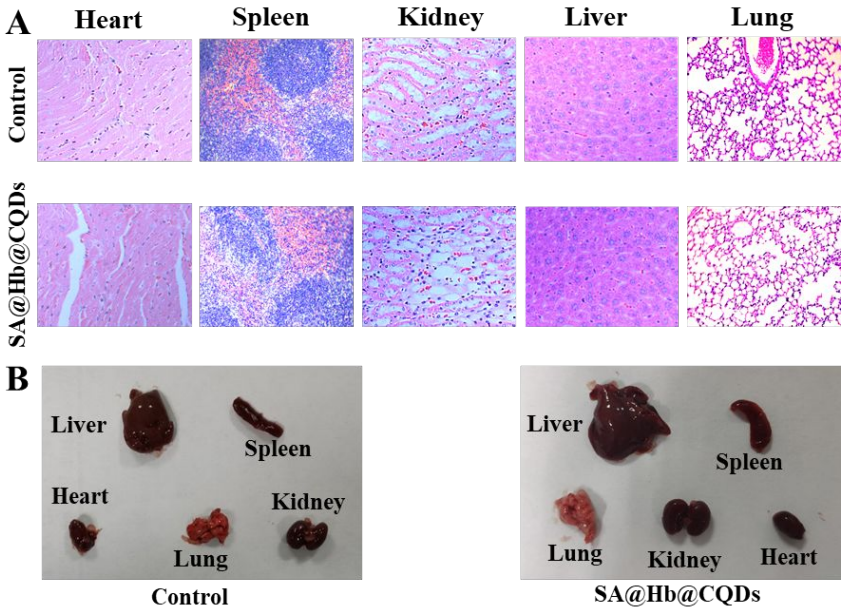

Figure S11. (A) H&E images and (B) photographs of vital organs from mice treated with PBS and SA@Hb@CQDs.