

## High Strength and Antibacterial Polyelectrolyte Complex CS/HS Hydrogel Films for Wound Healing

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## Tensile tests

The elastic modulus  $E$  was calculated from the slope of the initial linear region of the stress-strain curve (within 5%-10%), while the work of extension was estimated from the area below the tensile stress-strain curve until fracture.

The hydrogel samples for tear test were cut into rectangular shape ( $w = 2\text{-}2.5$  mm,  $d = 7.5$  mm,  $L = 30$  mm, with an initial notch of 20 mm long) and then subject to the test method established in ref <sup>[1]</sup>. During the test, the lower arm was fixed, and the upper arm was pulled upward at 10 mm/min constant velocity. Failure energy ( $G$ ) is calculated as  $G = 2F_{ave}/w$ , where  $F_{ave}$  is the average force throughout the test and  $w$  is the width of the hydrogel sample. Elongation and change of elastic energy stored in the pulled arms were ignored.

To analyse the dependence of hydrogels on strain rate, strip samples of the same shape and size were subjected to the uniaxial tensile test with different strain rates (0.014, 0.035, 0.083, 0.14, 0.35 and 0.83 S<sup>-1</sup>). Cyclic loading-unloading tests were conducted to examine the self-recovery property of the hydrogel films, especially for those that were fully swelled in physiological saline, at 30% strain and under different recovery times (0-240 min). The dissipated energy was obtained from the area between the loading-unloading profiles. The hysteresis ratio, defined as the ratio of the area of the second stretch cycle to the area of the first, was used to characterize the self-healing efficiency of the hydrogel films. Unless otherwise stated, the stress in this study refers to engineering stress,  $\sigma$ , defined as the tensile force divided by the cross-

section area of the undeformed sample.

### **Antimicrobial test**

0.1 mL aliquots of serially diluted suspension of *E. coli* were placed onto the solid agar. A hydrogel film sample of 10 mm in diameter was then placed on the surface of the agar dish and incubated at 37 °C for overnight. As shown in Figure 7a, the transparent concentric circle around the hydrogel is defined as the inhibition zone. The experiment was carried out in duplicate.

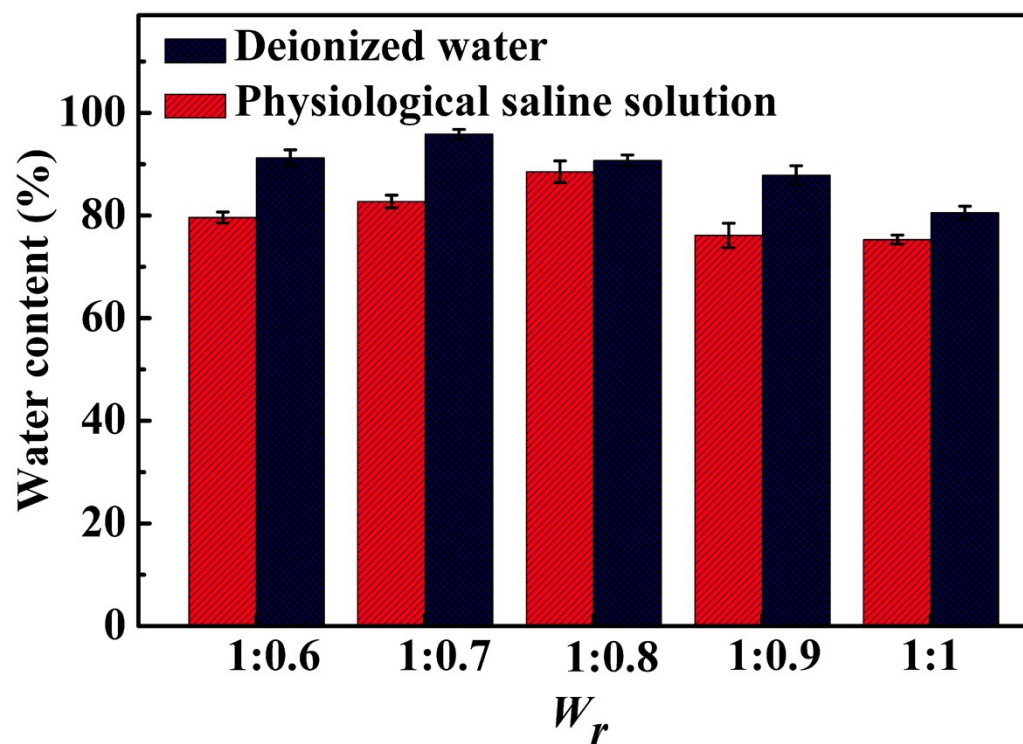
### **In Vivo Wound Healing**

Eight SD rats (Sprague-Dawley rats, male, 200 g) were randomly divided into four groups. 20 mm diameter full-thickness excisions were performed on both sides of the back of each rat after the rats were anesthetized with 4% chloral hydrate, their back was sterilized, and the wound sites were fixed with metal rings. The wounds on the left side of the rats' back were left untreated and recorded as negative control, while those on the right side were treated with UV sterilized CS, CS/HS (1:0.5), CS/HS (1:1.1) and CS/HS (1:1.2) hydrogel films (the deionized water used for preparing such hydrogel films were sterilized at 121 °C for 30 mins using an Asahi Medical Autoclave), with the CS treated wounds being recorded as positive control. Transparent dressing and bandages were used to protect the treatment on the wound surfaces. The wound healing process was captured with a digital camera at day 0, 7, and 21. Wound area was calculated using image analysis software (Image J). The

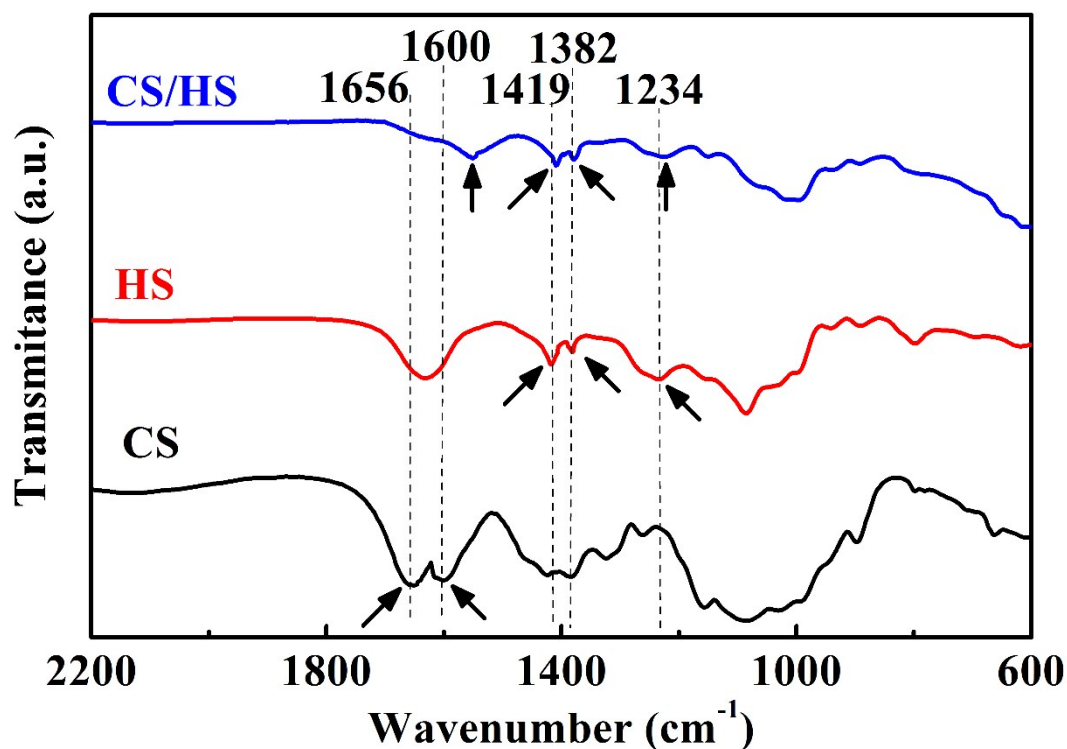
wound closure rate was defined as the percentage of the closed area to the initial wound area. It was calculated as wound closure rate (%) =  $(A_0 - A_t) / A_0 \times 100\%$ , where  $A_0$  represents the initial wound area on day 0 and  $A_t$  represents the remaining area of wound at each time points. Rats were sacrificed by cervical dislocation at day 7 and 21, and the wound tissue was collected for histological analysis. The skin samples were fixed with 4% paraformaldehyde, dehydrated with gradient alcohol, embedded in paraffin. and sectioned into 8 mm-thick sections for further analysis. Then the Haematoxylin-eosin (H&E) techniques were carried out for morphological analysis.

### **Statistical analysis**

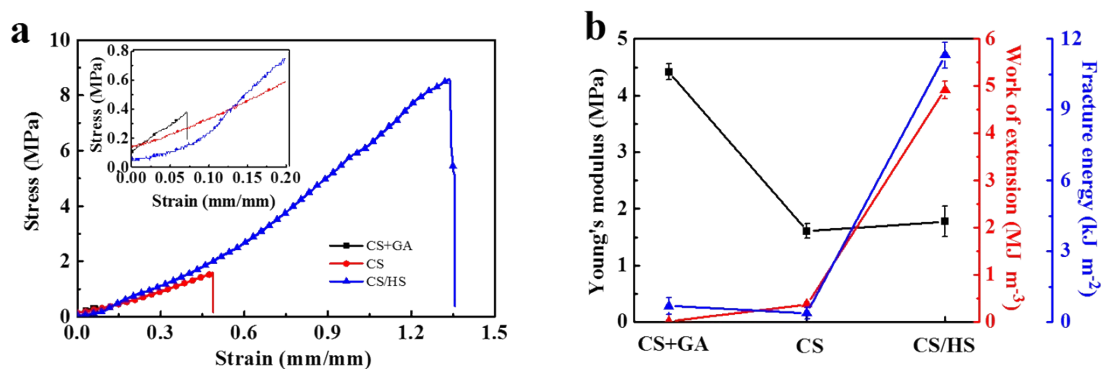
Statistically significant differences among sample groups were determined by using one-way analysis of variance (ANOVA), performed with a Student's t-test program, and the differences were considered significant when  $P < 0.05$  (\*) or  $P < 0.01$  (\*\*).



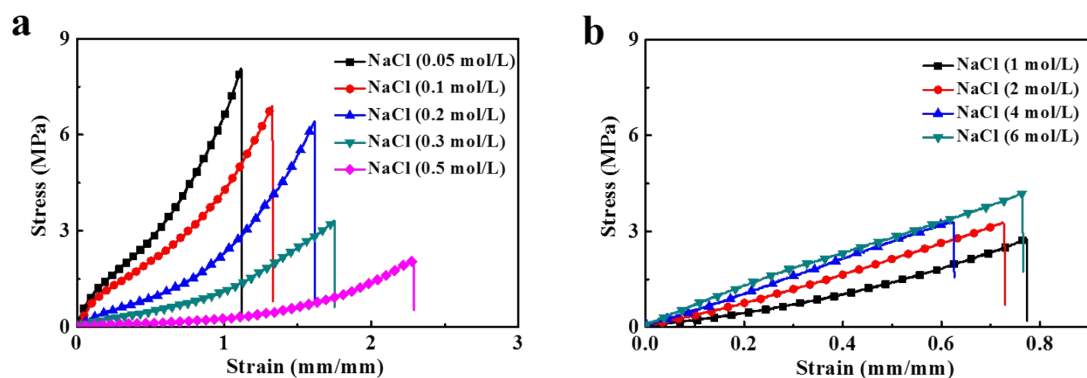
**Figure S1.** Water content of CS/HS hydrogel films of different  $w_r$ 's soaked in deionized water and physiological saline, respectively.



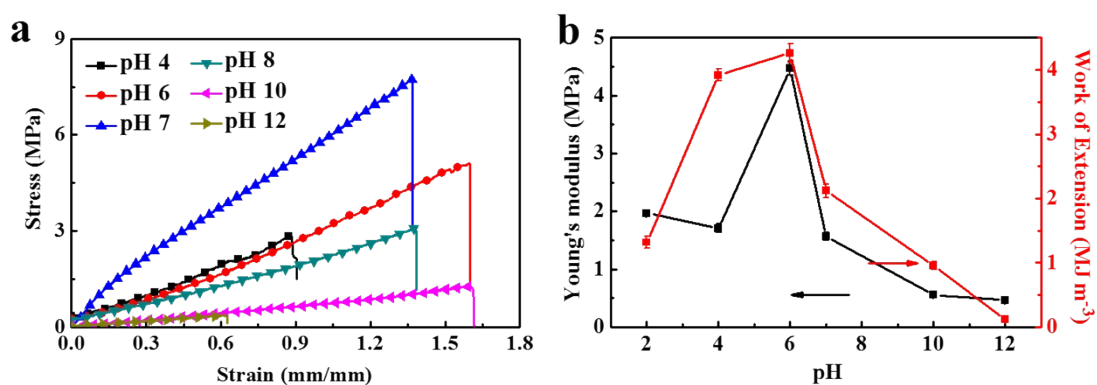
**Figure S2.** FTIR spectra of CS film, HS powder, and CS/HS hydrogel film ( $w_r = 1:0.7$ ).



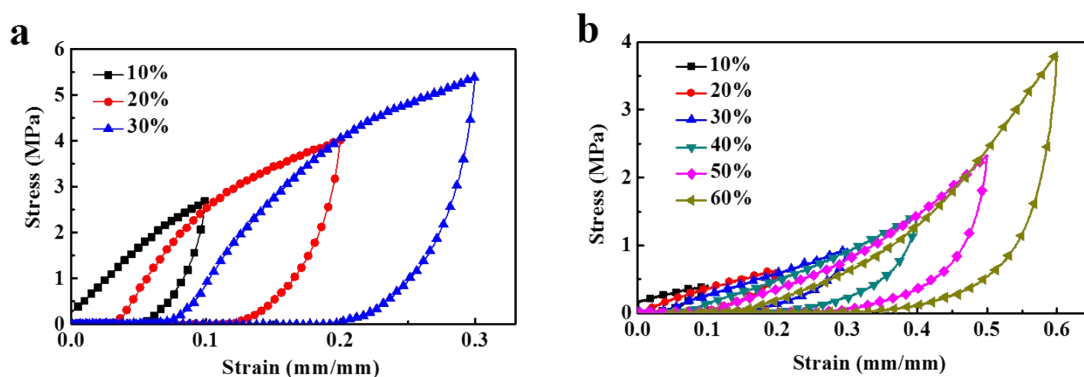
**Figure S3.** (a) Stress-strain curves of chemically crosslinked chitosan hydrogel films (CS+GA), chitosan hydrogel films (CS), and CS/HS hydrogel films of  $w_r = 1:0.7$  soaked in deionized water and (b) elastic modulus, work of extension, and fracture energy for CS+GA, CS, and CS/HS hydrogel films.



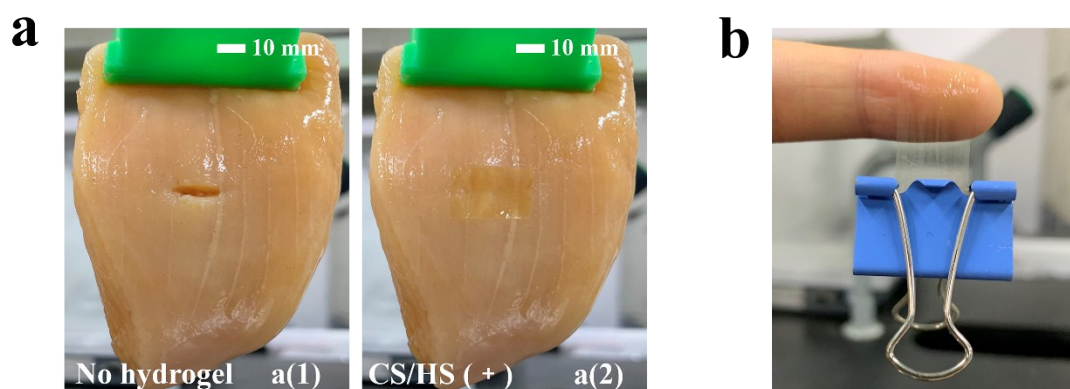
**Figure S4.** Typical stress-strain curves of CS/HS hydrogel film of  $w_r = 1:0.7$  soaked in low (a) and high (b) concentration NaCl solutions.



**Figure S5.** (a) Stress-strain curves of CS/HS hydrogel film of  $w_r = 1:0.7$  soaked in physiological saline at different pH's, (b) Young's modulus and work of extension of CS/HS hydrogel film soaked in physiological saline of different pH's.



**Figure S6.** Cyclic loading-unloading curves at different strains for CS/HS hydrogel film of  $w_r = 1:0.7$  soaked in deionized water (a) and in physiological saline (b).



**Figure S7.** Adhesion of the CS/HS hydrogel to biological tissues. (a) Adhesion behavior of the CS/HS (+) hydrogel to the surface of a piece of chicken breast tissue. a (1) An open notch of  $\approx 10$  mm length was made on the tissue, and a (2) the hydrogel adhered to the tissue and closed the notch. (b) The CS/HS hydrogel could adhere to the surface of human skin and withstood 20 g weight.

## Reference

- [1] F. Luo, T. L. Sun, T. Nakajima, T. Kurokawa, Y. Zhao, K. Sato, A. B. Ihsan, X. F. Li, H. L. Guo, J. P. Gong, *Adv. Mater.* **2015**, 27, 2722.