Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Supporting information

Wool keratin/zeolitic imidazolate framework-8 composite shape memory sponge with synergistic hemostatic performance for rapid hemorrhage control

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Materials and methods

Density

To calculate the sponge density (ρ), the sponge was first cut into cylindrical shapes, and the mass (M) and dimensions (V) (length and diameter) of the cylindrical sponge were measured by a balance and a digital caliper, respectively. The formula for calculating density (ρ) was as follows (1):

$$\rho = \frac{M}{V} \quad (1)$$

Porosity

The initial weight of the sponge sample was weighed as M_0. Next, the sponge samples were soaked in a certain amount of ethanol for 30 min, then removed from the ethanol and weighed again as M_1. The formula for calculating porosity (P) was as follows (2):

$$P = \frac{M_1 - M_0}{\rho_e V_0} \times 100\% \quad (2)$$

V_0 was the sponge volume. ρ_e was the density of ethanol (0.785 g/cm^3).

Water/blood absorption ratio measurements

Before testing, sponge samples were cut into cylindrical shapes and placed in a freeze-dryer for lyophilization. The weight of the lyophilized sponge sample was recorded as M_{dry} (g). Then the lyophilized sponges were immersed in DI water/blood After 1min, the samples were then taken out and gently dabbed on filter papers to remove the excess water on the surface, and the wet weight of the samples M_{wet} (g) was immediately measured. All sample evaluations were performed in triplicate. The water/blood absorption ratio was calculated according to Equation (2).

$$Water/blood\ absorption\ ratio(\%) = \frac{(M_{wet} - M_{dry})}{M_{dry}} \quad (3)$$

In vivo hemostatic performance of the sponges.

Rat liver laceration model: Briefly, the abdominal epithelium tissue of anesthetized rats was cut to expose the liver. Then, a scalpel was used to make a linear incision trauma of about 1 cm in length on the liver. After bleeding, pre-weighed sponge samples were immediately applied to the trauma. Finally, the hemostasis time and hemostatic volume were recorded.

Rat liver penetrating injury model: Firstly, the abdominal fur of the anesthetized rat was shaved and then fixed on the operating table. Next, the liver of each rat was exposed through an abdominal incision, and the fluid around the liver was gently removed with filter paper. After a pre-weighed filter paper was placed under the liver, a bleeding hole was made in the liver using a perforator with an internal diameter of 6 mm. Then, the sponge samples were immediately placed in the bleeding hole. Finally, the hemostasis time and hemostatic volume were recorded.

Rat femoral artery puncture model: Firstly, the fur on the thigh of the anesthetized rats was shaved and its capsicum inguinal muscles were opened to expose the femoral artery. After that, a syringe needle punctured the rat's femoral artery to create a bleeding wound. Then, the test sample was immediately placed on the bleeding site and the wound was manually pressed with the pre-weighed sterile gauze. The sample was lifted gently to observe the bleeding until hemostasis was achieved. Finally, the hemostasis time and hemostatic volume were recorded.

Rabbit liver penetrating injury model: The rabbits were anesthetized with sumianxin injection (0.2 mL/kg, intramuscular injection) and fixed on an operating table. Next, the

abdominal cavity was opened along the midline of the abdomen to expose the liver, and the serous fluid surrounding the liver was carefully removed by gauze. The filter paper was placed beneath the liver, and then a 10 mm-diameter circular perforation wound was made on the liver. After bleeding, the sponge samples were filled into the wound cavity. Finally, the hemostasis time and hemostatic volume were recorded.

Rabbit ear artery hemorrhage model: Firstly, the fur on the rabbit ears was shaved to expose the central artery. Then, the rabbit ear artery was wiped with iodine, and a 1 cm bleeding wound was formed with a scalpel. After bleeding, the wound was covered with the pre-weighed sponge. Next, the sample was lifted gently to observe the bleeding until hemostasis was achieved. Finally, the hemostasis time and hemostatic volume were recorded.

Histomorphology analysis

The muscle tissues cut from the animal were immunohistochemistry stained with tumor necrosis factor-α (TNF-α), antibodies by a standard protocol. The slides were then examined using a fluorescence microscope. Quantitative analysis was finally performed. Analysis software (Image J) was used to measure the integrated density (ID) of each image and the corresponding tissue pixel area. The Areal Density was calculated as follows:

$$Areal\ Density = \frac{ID}{Area} \qquad (4)$$

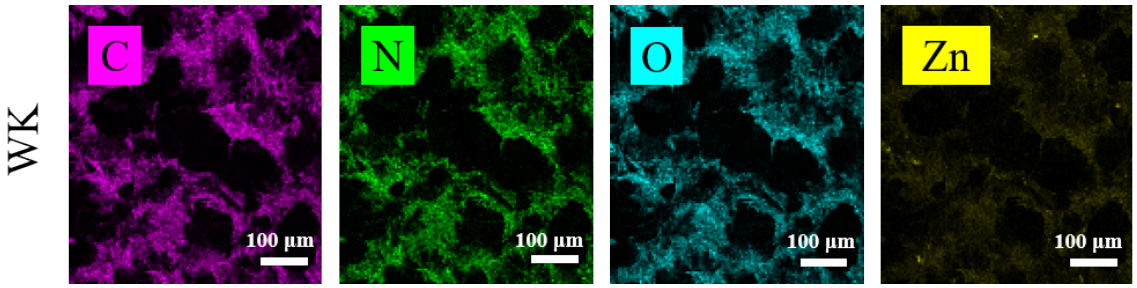

Fig. S1. Mapping analysis images for WK sponge.

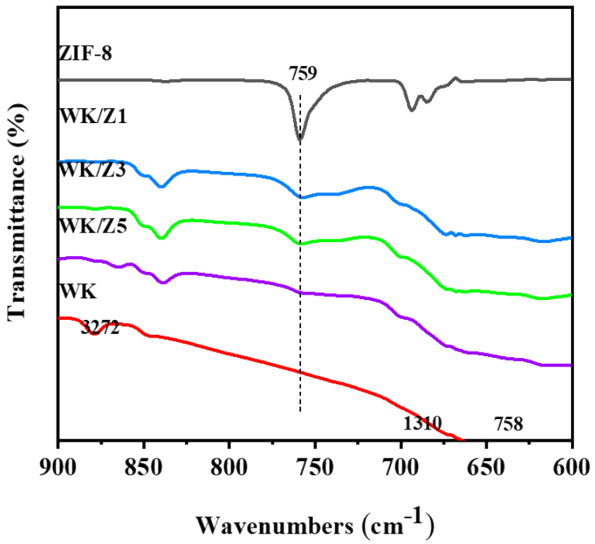

Fig. S2 FT-IR spectra of ZIF-8, WK, WK/Z1, WK/Z3, and WK/Z5 sponges.

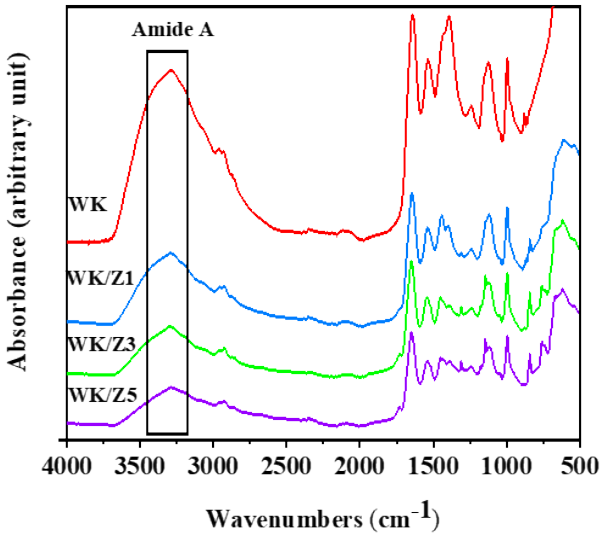

Fig. S3. The FT-IR spectra of ZIF-8, WK, WK/Z1, WK/Z3, and WK/Z5 sponges.

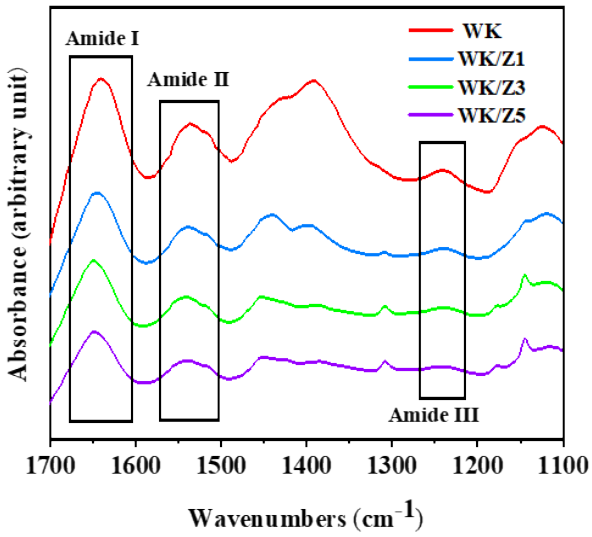

Fig. S4. The FT-IR spectra of WK, WK/Z1, WK/Z3, and WK/Z5 sponges.

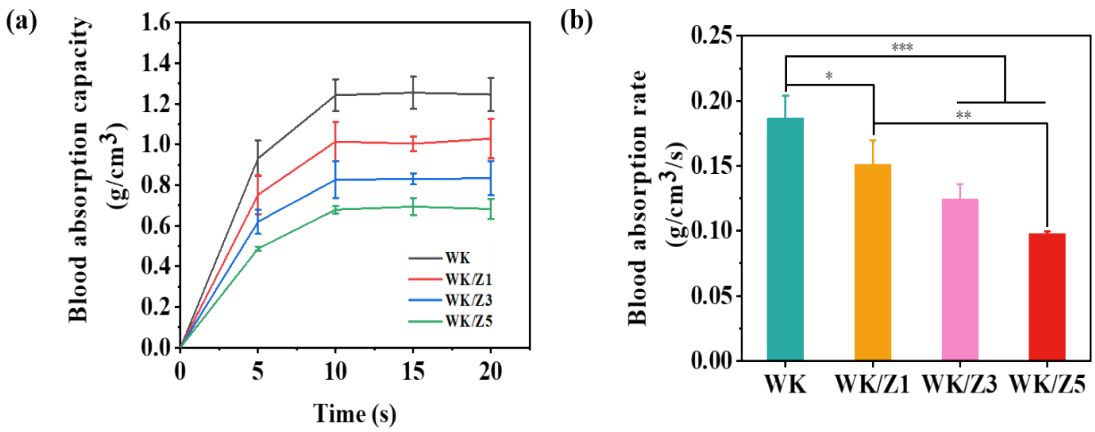

Fig. S5. Blood absorption capacity-time dynamic curves and absorption rate of different sponges.

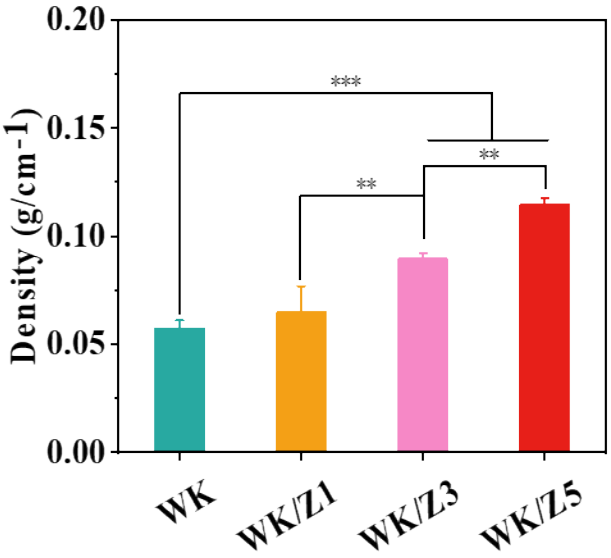

Fig. S6. Densities of WK, WK/Z1, WK/Z3, and WK/Z5 sponges.

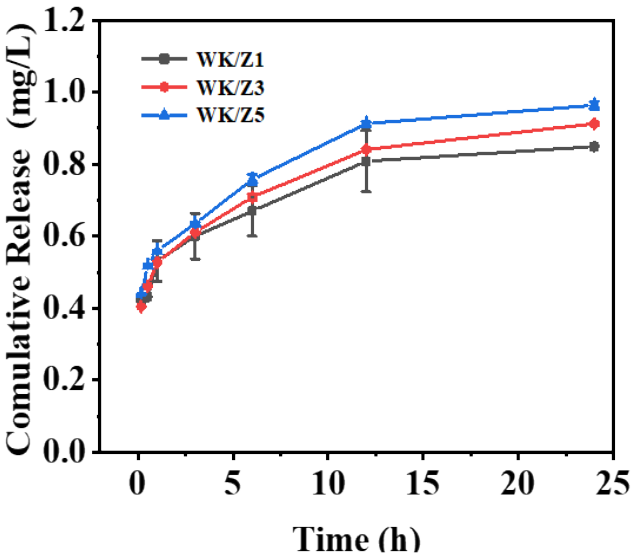

Fig. S7 Cumulative release of Zn^{2+} from WK/Z1 sponge, WK/Z3 sponge, and WK/Z5 sponge in the ultrapure water for 24h. (*p < 0.05, **p < 0.01, ***p < 0.001).

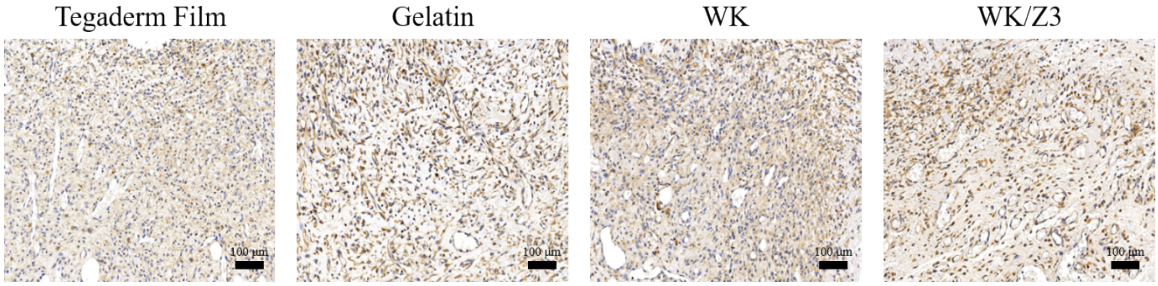

Fig. S8 Immunohistochemical staining of Tegaderm film, gelatin sponge, WK sponge, and WK/Z3 with TNF-α(a).

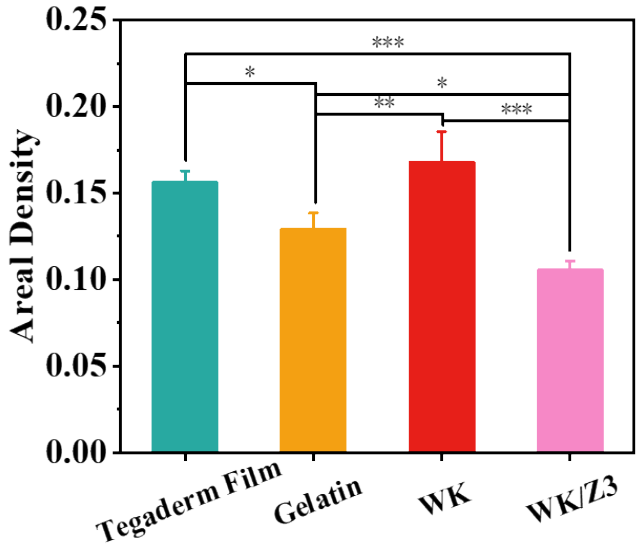

Fig. S9 Statistical analysis of TNF- α. (*p < 0.05, **p < 0.01, ***p < 0.001).