Supporting Information

An agar-polyvinyl alcohol hydrogel loaded with tannic acid with efficient hemostatic and antibacterial capacity for wound dressing

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Materials and methods

Degradation ratio

The degradation test of all hydrogel specimens was carried out in vitro. Briefly, the hydrogel-samples were soaked in PBS until the swelling equilibrium was reached, and after recording the initial weight, they were incubated in a shaker at 37 ℃ for a specified time (1 d, 3 d, 6 d, 9 d, 14 d), weighed and recorded again, and the relative degradation ratio of the hydrogels were calculated as follows:

$$Degradation\ ratio\ (\%) = \frac{m_0 - m_1}{m_0} \times 100\%$$

Where m_0 is the initial weight of hydrogel, m_1 represents the weight of the corresponding hydrogel being degraded. Data from each specimen was calculated using triplicate measurement.

Biomechanical performance

The tensile strength of the hydrogel specimens was tested according to the method of Huang. Briefly, each sample from each group was trimmed as a test strap of 4 mm × 20 mm in size. Five tissue strips in each group were extended on an Instron material testing machine (Instron Co. USA) from 0 g load until the tissue strip ruptured at a constant speed of 10 mm min-1. After measurement, the ultimate tensile stress and the ultimate tensile strain were recorded before failure.

The compression strength was determined by a similar method. Briefly, the hydrogels with a height of 10 mm and a diameter of 18 mm were prepared for the compression test. The displacement rate was set as 5 mm min-1. The stress, strain, and compression modulus were recorded and calculated until the sample broke.

Cytotoxicity assay

L929 mouse fibroblast cells were seeded in DMEM with 10% FBS and antibiotics (1% penicillin-streptomycin). The cells were incubated at 37 ℃ in a humidified incubator with 5% CO_2. For sub-cultivation, 0.25% trypsin was used to detach cells from culture plate.

All specimens were sterilized by γ-rays for 15 min with an irradiation measurement of 25 KGy. Then, the sterilized samples were placed in 24-well plates and soaked overnight in DMEM medium without serum.

CCK-8 array was adopted to measure the cytotoxicity of AGAR@PVA-TA hydrogels. CCK-8 can be reduced to yellow formazan product by dehydrogenase in

cells. The quantity of formazan produced was directly proportional to the number of living cells. 200 μl P3 generation of L929 cells (1×10^5 cells/ml) were slowly dropped onto the surface of the hydrogels. After incubation for 3 h, 800 μl serum-containing medium was added to each well. There plates were incubated in the same environment as above, and the medium was changed every other day. CCK-8 was used to evaluate the cell proliferation after 1, 3 and 5 days. Results were the average of three independent measurements.

Hemolysis test

4 ml of rabbit whole blood (containing 3.8 wt% sodium citrate) was dispensed in 1.5 ml centrifuge tubes and centrifuge at 1500 rpm for 15 min at 4 ℃. The erythrocyte solution was obtained by diluting the lower red blood cells (RBCs) after centrifugation with isotonic PBS solution at a ratio of 1:7. About 1.6 ml PBS and 0.4 ml RBCs solution were added to the hydrogels in a 24-well plate, and all specimens were incubated at 37 ℃ for 3 h. Meanwhile, we dispersed the RBCs in isotonic PBS buffer as a negative control and dispersed the RBCs in distilled water as a positive control. After incubating for 3 h, all samples were centrifuged at 3000 rpm for 4 min, the 100 μl of corresponding samples supernatant was transferred to a 96-well plate, and the absorbance of their released hemoglobin was measured using a spectrophotometer at 570 nm. All experiments were performed in triplicate. The hemolysis rate of different hydrogels was calculated as follows:

$$Hemolysis\ rate\ (\%) = \frac{Suspensions_{abs} - Negative\ control_{abs}}{Positive\ control_{abs} - Negative\ control_{abs}} \times 100\%$$

The hydrogel specimens were removed from the above hemolysis rate determination experiment. Samples were gently washed in PBS solution to remove the unadhesive RBCs, and immersed in 2.5% glutaraldehyde solution and fixed at 4 ℃ for 24 h. The hydrogels were then gradient-dehydrated by ethanol (30%, 50%, 70%, 90%, 95%, 98% and 100%) and underwent critical point drying. Finally, the adhesion of red blood cells on the specimen surface was observed by SEM.

Antibacterial studies

The antibacterial ability of AGAR@PVA-TA hydrogels was determined by plate count method and agar disc diffusion test, and Staphylococcus aureus and Escherichia coli were used as model bacteria.

For plate count method, the bacteria were first incubated in Nutrient Broth (NB)

at 37 ℃ until the exponential growth phase. The bacterial broth was then diluted with saline and a concentration of 1×10^7 colony forming units (CFU)/ml was used for subsequent experiments. Each hydrogel specimen was made into a disk of 1 cm diameter and 4 mm thickness, sterilized and placed in a 12-well plate. 30 μl of the above bacterial suspension and 270 μl of saline were added to the surface of each hydrogel and co-cultivated for 3 h at 37 ℃, the blank well was considered as a control group. Subsequently, the above co-cultured bacterial suspension was diluted about 1000 times and inoculated with 100 μl of this dilution on NB agar plates and incubated at 37 ℃ for 24h. the reduction rate of bacteria was calculated by counting the numbers of CFUs on the agar plate. The bacterial reduction rate of hydrogels was calculated as follows:

$$Reduction\ rate\ of\ bacteria\ (\%) = \frac{q_{control} - q_{sample}}{q_{control}} \times 100\%$$

Where q_{sample} and $q_{control}$ were the number of CFUs on the agar plate for the hydrogel sample group and the control group. Data from each sample was calculated using triplicate measurement.

In the agar disc diffusion test, the above bacterial solution was diluted 10 times and inoculated on NB agar plates, and the solution was spread evenly on the agar plates with an applicator, then the hydrogel was placed on the agar plates and the inhibition area was measured after incubation at 37 ℃ for 24 h. All experiments were performed in triplicate.

Results and discussions

Degradation ratio

It could be seen from Figure S1 that the degradation ratio of all hydrogel-samples was low at 14 days, and only the degradation ratio of blank group (AGAR@PVA hydrogel) exceeded 10% (11.04%). This indicated that all hydrogel-samples had good stability, and the stability increased with the increase of TA content. This was because after being subjected to freeze-thaw cycle, the agar and PVA in hydrogel had formed a large number of hydrogen bonds, which endowed the hydrogels with good stability, and TA could further increase the density of hydrogen bonds, making the stability of hydrogels further improved.

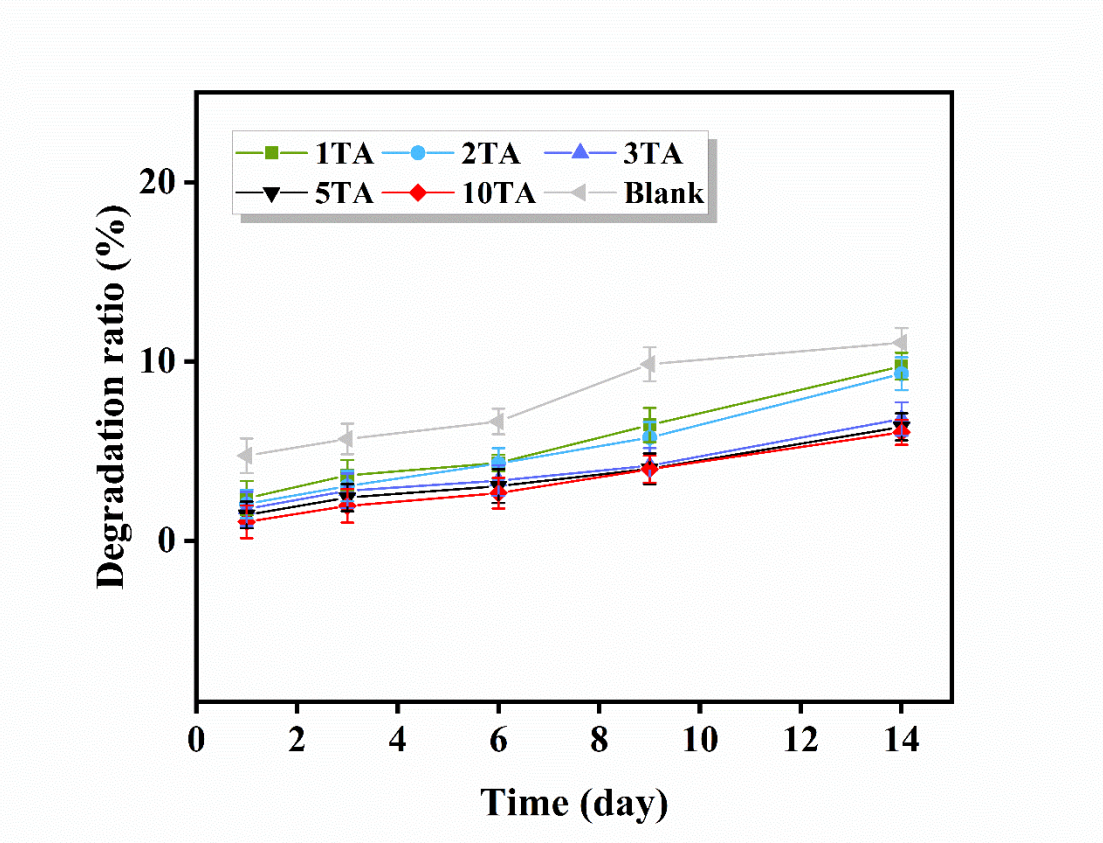

Figure S1. The degradation ratio of hydrogels with different TA concentrations.

Agar disc diffusion test

The antibacterial effect of the hydrogel specimen was also confirmed using the agar disc diffusion test. The areas of inhibitory zone of specimens were shown in Figure S2.

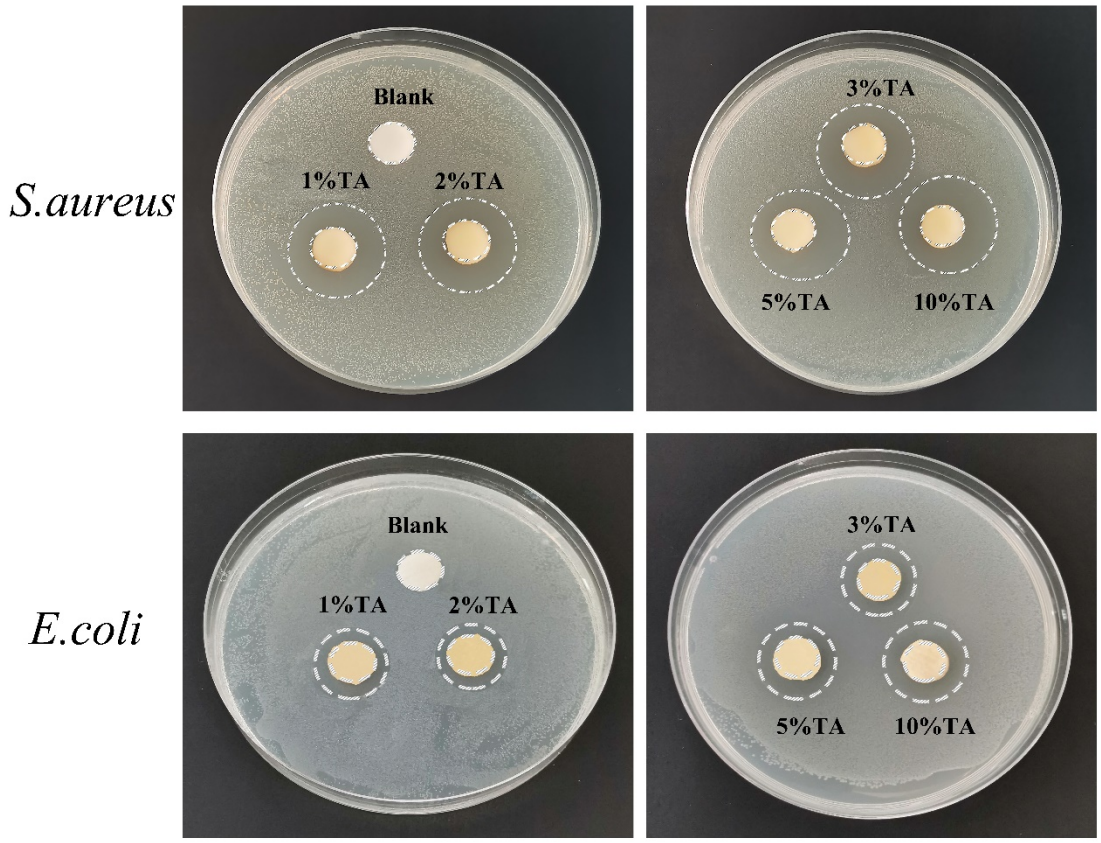

Figure. S2. Areas of inhibitory zones of different hydrogels against E. coli and S. aureus.

As can be seen from the figure, AGAR@PVA hydrogel has no antibacterial activity, but with the increased of TA concentration in hydrogel, the antibacterial ability of AGAR@PVA-TA hydrogels increased and showed better antibacterial effect against *S. aureus* and *E. coli*.