Support information

Antioxidant and multi-sensitive PNIPAAm/keratin double network gels for self-stripping wound dressing application

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1. Physicochemical characterizations

The morphology of the hydrogel was obtained by scanning electron microscopy (SEM JSM-7600F). Fourier transform infrared spectrometry(FTIR) spectra were tested through Bruker alpha II infrared spectrometer (Germany). For standard mode, DSC measurement, the swollen hydrogels (each about 10-12 mg) were encapsulated in a crucible and heated in a power compensation DSC(Perkin-Elme, USA) with a dry nitrogen gas flow (30 mL/min) and a refrigerated cooling system. It was performed at least three times from 25 ºC to 40 ºC at a heating rate of 3 ºC/min.

The swelling rate(SR) of hydrogel was determined using the gravimetric method. The lyophilized hydrogel(m_d) was soaked in DW at 25 ºC until swelling equilibrium. Subsequently, sucked the water on the surface using filter paper and weighted immediately(m_s). The swelling rate was capable of calculating by the following formation:

$$SR = \frac{ms - md}{md} \times 100\%$$

Equilibrium swelling rates of the hydrogel at various temperatures were tested to verify thermo-sensitivity. The fully absorbent hydrogels were incubated in the bath at various temperatures for 12 h and then weighted(m_e). The equilibrium swelling rate was capable of calculating by the following formation:

$$ESR = \frac{me - md}{md} \times 100\%$$

2.Cell viability

Cell viability was gauged by MTT method culturing L929 cells on the surface of the hydrogel. Briefly, a different ratio of PNIPAAm/keratin hydrogel was prepared in a 24-well plate, followed by sterilization and disinfection using 75% ethanol for 24h. And then using PBS and DMEM to substitute it for 2 days. After that, L929 cells were seeded onto hydrogels(2×10^4 cells/well) and placed in the cell incubator(37 ºC, 5% CO_2) for culturing. Meanwhile, cells without any management were set as negative controls. After 3 days of culture, the supernatant was discarded, followed by putting 100 μL culture medium mixed with MTT(9:1) in each well culturing for another 4 h in the incubator. Subsequently, cells precipitated at the bottom of each well were washed three times using PBS solution and then dissolved by 100 μL DMSO for 30 min in dark.

Finally, the absorbance of the solution was tested by a microplate reader at 570 nm. The cell viability was calculated by the following formation:

$$\text{Cell viability} = \frac{OD_{ex}}{OD_{co}} \times 100\%$$

3. Cell proliferation

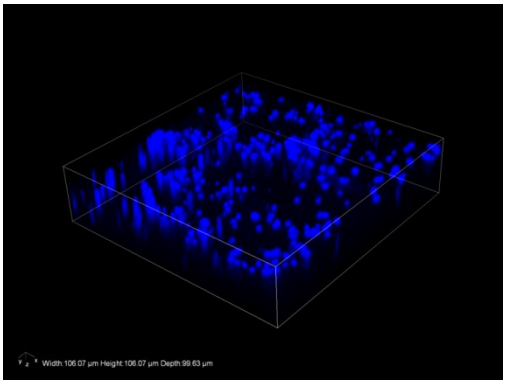

Fig. S1 3D CLSM images by DAPI staining.

3. Standard curve of CHX

Concentration-Absorbance Standard Curve and Standard curve equation for chlorhexidine acetate (A = -0.03953+45.48674C(mg/mL）were obtained according to different absorbance values of CHX at 260 nm. As shown in Fig S1, the linear correlation coefficient of the standard curve equation is R=0.99942, which indicated that the concentration of CHX has a good linear relationship with the absorbance.

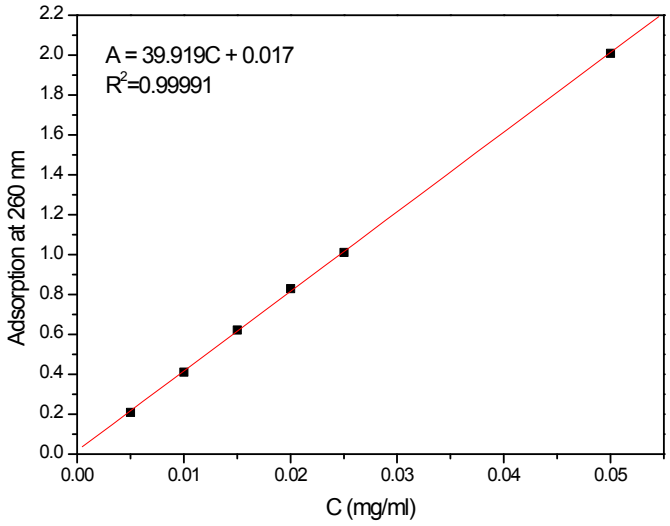

Fig. S2 Standard curve of CHX as a function of concentration at 260 nm.

4. Drug delivery kinetics

The kinetics of drug release was further estimated with equations as follows:

$$\ln(1-q_t/q_e)=-kt+c;$$

$$q_t/q_e=kt^{1/2}+c;$$

$$\ln(q_t/q_e)=n\ \ln t+c;$$

where q_e is the cumulative release of CHX in the hydrogel, q_t represents release amounts of CHX in a certain time, k_1-k_3, c_1-c_3 are slope and constant terms respectively. n is the diffusion index, which indicates the mechanism of drug release.

Table S1 Correlation coefficients of CHX release kinetics in different pH and GSH concentrations

	First-order model			Higuchi model			Ritger-peppas model		
	k	**R^2**	**c**	**k**	**R^2**	**c**	**n**	**R^2**	**c**
pH=4, 38℃, 20 mM H_2O_2	-0.6112	**0.9922**	0.1199	0.2807	0.7962	0.1927	0.1526	0.8330	-0.3381
pH=4, 38℃, 5 mM H_2O_2	-0.717	**0.9468**	0.6674	0.3089	0.9232	0.0674	0.4164	0.8396	-0.9194
pH=4, 38℃	-0.2888	**0.9439**	0.2443	0.3127	**0.9599**	-0.0703	0.8102	0.9375	-1.8854
pH=9, 38℃	-0.2776	**0.9838**	0.1106	0.3009	**0.9914**	-0.0108	0.5607	**0.9795**	-1.3355
pH=7, 38℃	-0.3095	0.8979	0.2947	0.3075	**0.9795**	-0.0532	0.6793	**0.9877**	-1.6148
pH=7, 31℃	-0.2025	0.8502	0.2700	0.2997	0.8504	-0.1606	1.0996	**0.9637**	-2.7122

5. Inhibition zone of bacteria

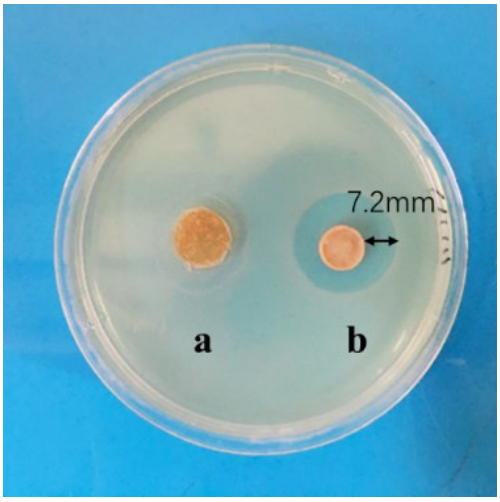

Fig.S3 Antibacterial inhibition zones of the PNIPAAm hydrogel (a) and drug-loaded PNIPAAm/keratin hydrogel (b) against E. coli

6. Histological analysis

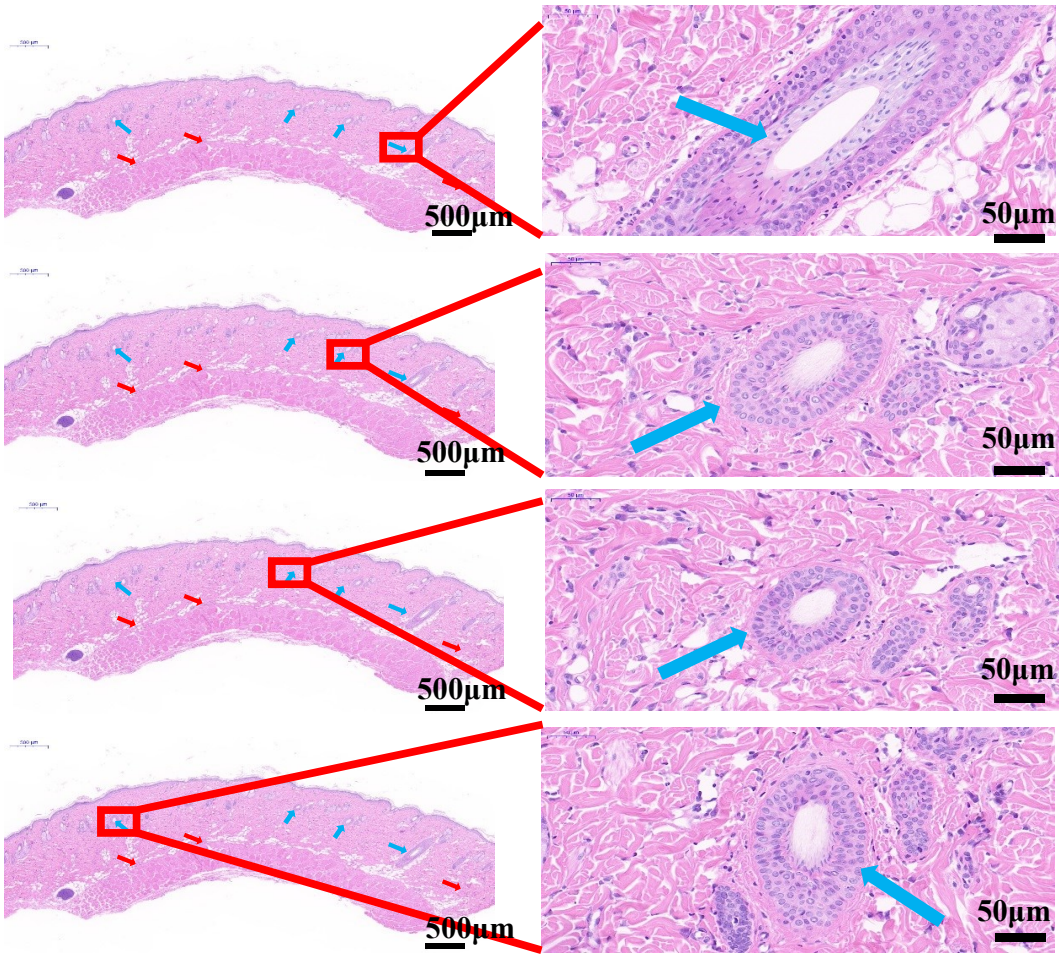

Fig.S4 The newborn hair follicles in wound area treated with CHX-loaded PNIPAAm/Keratin hydrogel(7/3) for 14 days

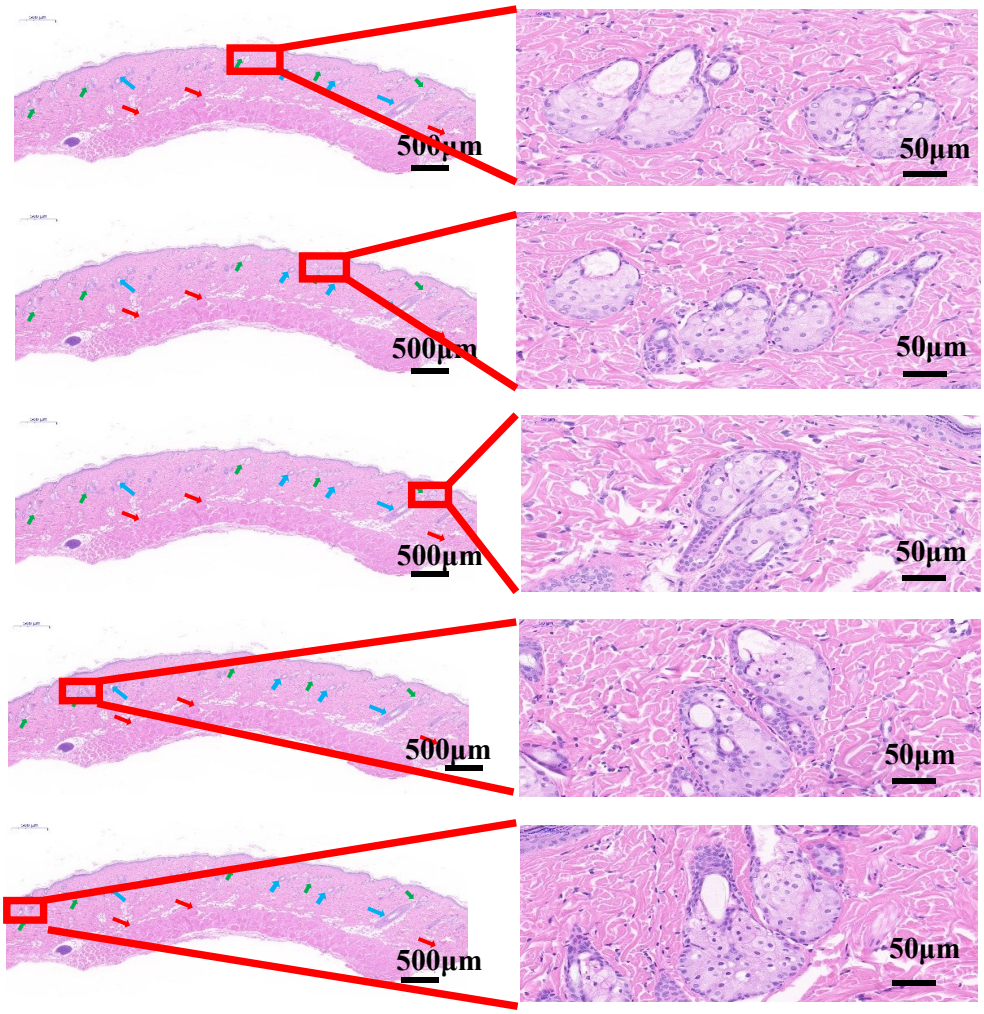

Fig.S5 The newborn sebaceous glands in wound area treated with CHX-loaded PNIPAAm/Keratin hydrogel(7/3) for 14 days