Supporting Information

Injectable and degradable PEG hydrogel with antibacterial performance for promoting wound healing

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S1. Ellman's test

A series of L-cysteine standard solutions with different concentrations (0, 0.01, 0.02, 0.03, 0.04 mM) was prepared, and then added DTNB standard solution and incubated for 5 min at 25 °C, their absorbance at 410 nm were finally measured (the spectrum was shown in Figure S1A) via UV-1800 ultraviolet-visible spectrophotometer (Shimadzu, Japan). Based on the obtained data, the standard curve was drawn of different absorbance corresponding to different thiol content as shown in Figure S1B. The thiol content of the crosslinkers in the raw materials was set as a control, and the residual thiol content at different

time points during the gel forming process was measured. The residual thiol content (RTC) was calculated by the following formula: $RTC(\%) = \frac{C_t}{C_i} \times 100$, where C_t and C_i refer to the thiol content at the corresponding time point and the thiol content of the crosslinkers in the raw materials respectively. As shown in Figure S1C, after mixing the crosslinkers and the crosslinking monomer, the residual thiol content decreased rapidly. After 10 min, the rate of residual thiol content decrease gradually slowed down, and after 60 min, the residual thiol content approached to zero. The results showed that the cross-linking process began immediately after the crosslinkers and the crosslinking monomer were mixed, and the initial crosslinking rate was extremely fast. After 10 min, most of residual thiol content in the crosslinkers had participated in the reaction. The residual thiol content approached to zero at 60 min, which proved that the hydrogel has achieved maximum cross-linking.

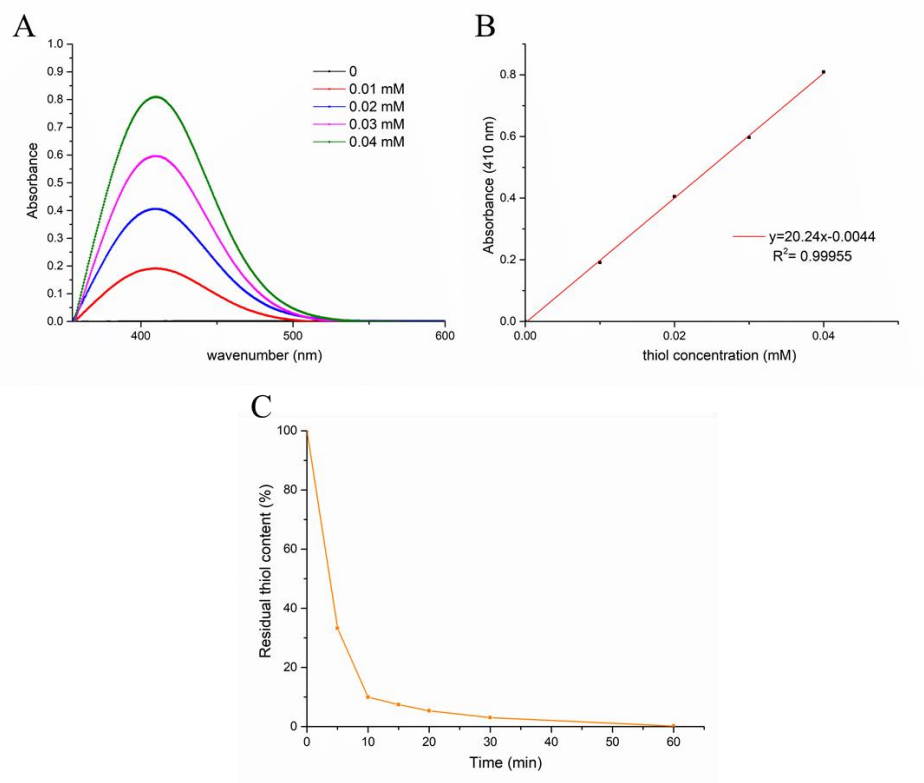

Figure S1. (A) UV spectra corresponding to different thiol content. (B) The standard curve of different absorbance in 410 nm corresponding to different thiol content. (C) The residual thiol content at different time points during the gel forming process.

S2. Biocompatibility of PEG hydrogel *in vitro*

S2.1 Raw materials toxicity assay

According to the international biological evaluation of medical devices standard ISO 10993-1 (3rd edition), raw materials' toxicity was tested by the cytotoxicity test method of the extracts. The three raw materials were separately dissolved in complete medium (high-glucose DMEM with 10% FBS) to form a 10 g/L solution, then placed in an incubator with humidified atmosphere containing 5% CO_2 at 37 °C for 48 hours to obtain the extracts. The extracts were sterilized by a 0.22 μm PES syringe filter (Biosharp, China). Refer to the ISO 10993-5 (3rd edition) [1], HaCAT cells were taken to prepare cell suspensions and then seeded in 96-well plate at a density of 1×10^4 cells per well. After overnight incubation with atmosphere containing 5% CO_2 at 37 °C, the medium was refreshed.

Experimental group: add 200 μL of extracts with a concentration of 10 g/L.

Positive control group: add 200 μL complete medium with 10 g/L phenol containing.

Negative control group: add 200 μL complete medium with 10 g/L polypropylene (PP) containing.

Blank group: add 200 μL complete medium (high-glucose DMEM containing 10% FBS).

After culturing 48 h, CCK-8 experiment and LIVE/DEAD cell staining were performed to analyze cell viability and proliferation.

S2.2 Material toxicity assay

S2.2.1 Direct contact test

Same as above, HaCAT cells were taken to make cell-suspensions and then seeded into 12-well plates with density of 5×10^4 cells per well. After incubation overnight, different treatments were performed according to groups.

Experimental group: covered with 200 μL (gelled volume) hydrogel material that has been fully

washed and swollen with PBS. Control group: No treatment

After treatments, the cells were cultured for 48 hours and detected with microscopes at 4 h, 24 h, 36 h and 48 h respectively (Figure S2). After 48 h incubation, the cells were stained with crystal violet staining (Solarbio, Beijing, China) and visualized with microscope (Figure S3). Then the cytotoxic effects were evaluated according Table S1.

Our previous research work has proved that in order to meet different needs, the degradation performance of PEG hydrogels can be adjusted by changing the ratio of the degradable crosslinker and the non-degradable crosslinker. In the biocompatibility experiment in this study, we supplemented the experimental group with a degradable crosslinker PEG-diester-dithiol content of 0 and 100%. For the sake of convenience, we used the content of the hydrolyzable degradable crosslinker as the naming method of hydrogels. PEG-0%, PEG-50%, PEG-100% means that the content of degradable crosslinker in the hydrogel were 0%, 50% and 100% respectively.

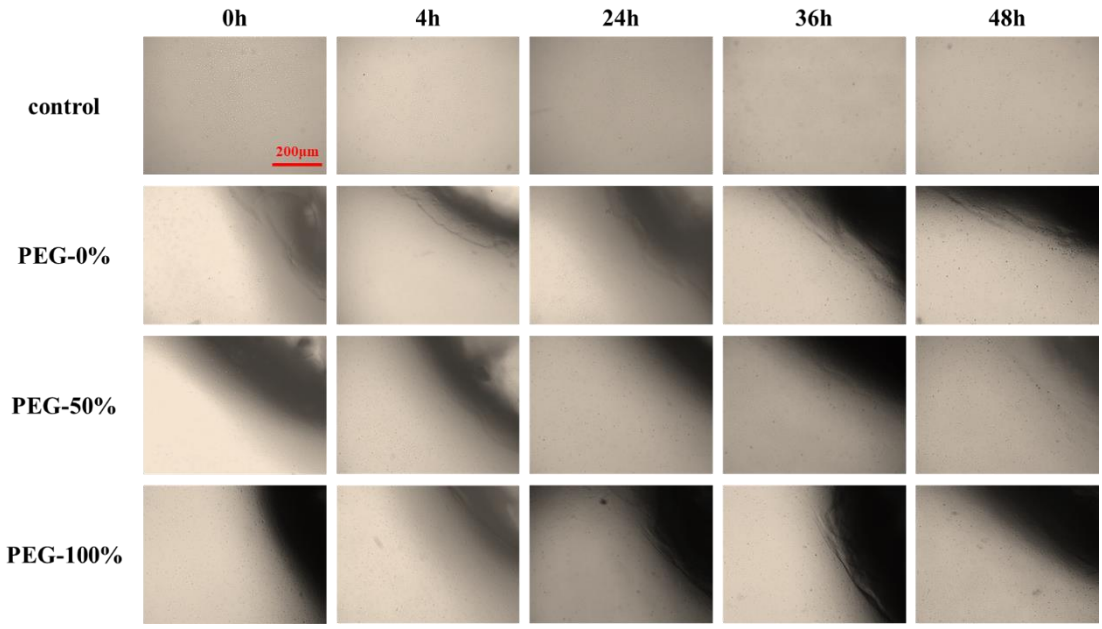

Figure S2. Direct contact test, the cells were detected with microscopes at 4h, 24 h, 36 h and 48 h respectively. scale bar: 200 μm.

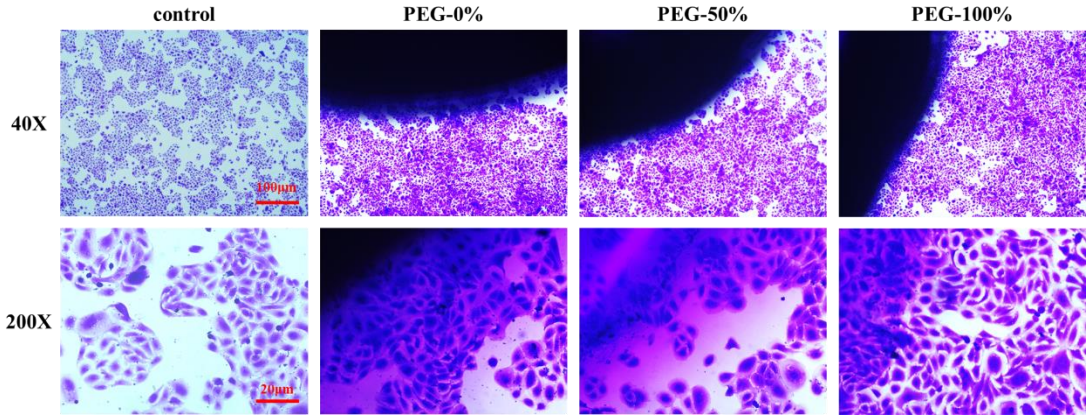

Figure S3. Direct contact test, the cells were stained with crystal violet staining and visualized with microscope.

Table S1. Reactivity grades for agar and filter diffusion test and direct contact test

Grade	*Reactivity*	*Description of reactivity zone*
0	*None*	*No detectable zone around or under specimen*
1	*Slight*	*Some malformed or degenerated cells under specimen*
2	*Mild*	*Zone limited to area under specimen*
3	*Moderate*	*Zone extending specimen size up to 1.0 cm*
4	*Severe*	*Zone extending father than 1.0 cm beyond specimen*

S2.2.2 Toxicity test of extracts

The operations of material extracts biocompatibility were same to the toxicity of raw material extracts. Herein, when the 10 g/L extract had been prepared, the lyophilized hydrogel material was weighed and then dissolved in the complete medium (high-glucose DMEM with 10% FBS).

Positive control: phenol was dissolved in complete medium to prepare a 10 g/L positive-control solution.

Negative control: polypropylene particles were dissolves in complete medium to prepare a 10 g/L negative-control solution.

Similarly, as shown in Figure S4, we supplemented the data of PEG-0% and PEG-100% and compared them with PEG-50%. All the experimental results showed that PEG-0% and PEG-100% have similar properties to PEG-50%, and the biological properties of the PEG hydrogel will not change with the content of the degradable crosslinker. Therefore, in actual application, the content of degradable crosslinker can be adjusted according to the approximate time of wound healing, so as to better achieve the purpose of promoting wound healing.

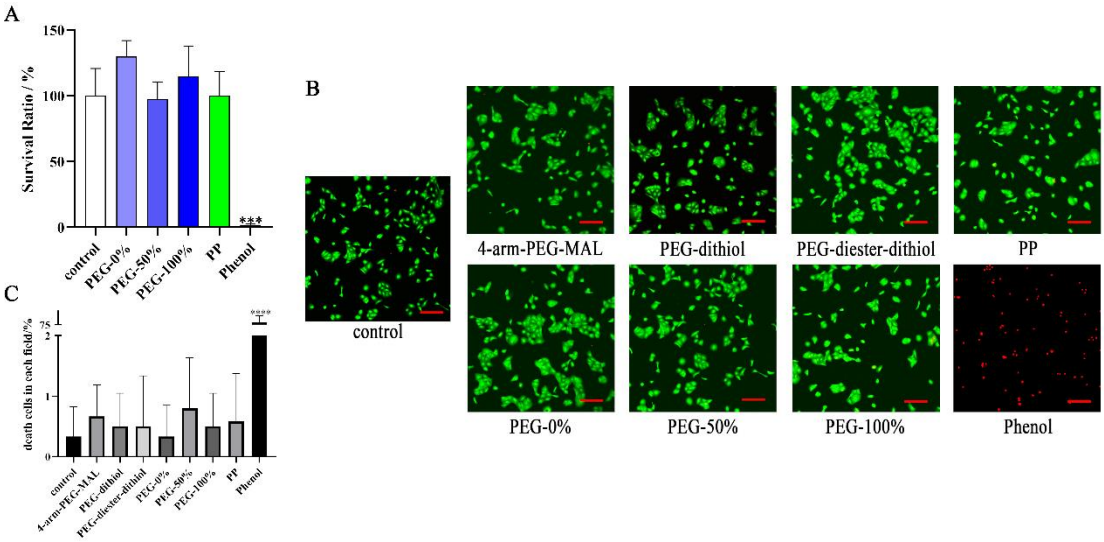

Figure S4. (A) The survival ratio of HaCAT cells treated with liquid extracts of PEG hydrogels (B) Representative micrographs of LIVE/DEAD staining of HaCAT cells treated with hydrogels or their raw materials, scale bar: 200 μm. (C) Statistical analysis of death cells in each field of LIVE/DEAD staining.

S2.3 Degradation products toxicity assay

The fully swollen hydrogels were put into complete medium for degradation. The degradation process was taken place in a shake at 37 °C with a speed of 200 R/min. The degradation products were obtained on the 7th day, 14th day, and 21st day respectively. Similar methods were used for cultivation. CCK-8 and LIVE/DEAD cell staining were used to observe cell viability and proliferation. In order to eliminate the influence of DMEM, the groups of medium whose storage period same as the degradation time were set synchronously.

Reference

[1] ISO 10993-5 (Third edition 2009-06-01): Biological evaluation of medical devices–Part 5: Tests for in vitro cytotoxicity.