Electronic Supplementary Information (ESI)

Shape Memory Histocompatible and Biodegradable Sponges for Subcutaneous Defect Filling and Repair: Greatly Reducing Surgical Incision

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Experimental Details

Experimental details for the construction of sponges and sponge sheets from different hot-pressing treatments

ECH-crosslinked HEC/SPI sponges (EHSS) were prepared by crosslinking HEC/SPI blend solutions (the dry weight ratio of 3:7) with ECH and the following freeze-drying process. Briefly, 10 g SPI powder were dispersed into 60 g water and 30 g 5 wt% NaOH aqueous solution was then added to obtain a viscose 10% SPI aqueous solution. A 2% HEC aqueous solution was prepared by dissolving 2 g HEC in 98 g deionized water with magnetic stirring. HEC and SPI solutions were mixed mechanically with the dry weight ratio of 3:7, and then ECH (20% of the total weight of original SPI and HEC powders) was added dropwisely into the blend solutions with constant stirring for 0.5 h, which were further degassed by centrifugation to obtain a transparent HEC/SPI blend solution. Then, the degassed solutions were poured into square molds and kept at 5 ºC in a refrigerator. The resultant hydrogels were frozen at -20 ºC for 12 h and -80 ºC for 24 h followed by freeze-drying. The freeze-dried sponges were soaked in 5 % acetic acid solution to remove the residual NaOH and ECH, and then rinsed with the running water overnight to remove acetic acid, the wet sponges were frozen at -80 ºC and freeze-dried again.

EHSS were soaked in the deionized water and cut into the required sizes, and treated by different temperature and pressure controlled by thermo-compressor, and the treated EHSS were divided into four groups according to the experiment: (1) Pressing group. The pressing temperature was kept at 30 ºC as the normal temperature

for 10 min with the variation of pressure, the resultant samples were coded as EHSS-P-n (P means the change of pressure, n corresponds to the pressure values (MPa), n = 5, 15, 30 and 45). (2) Hot-pressing group. The pressure were maintained at 30 MPa for 10 min with the variation of the hot-pressing temperature (T=30, 90, 130, 170 and 210^{o} C), the hot-pressed samples was denoted as EHSS-PT-n (P means the constant pressure, T means the hot-pressing temperature, n corresponds to the temperature values, n= 30, 90, 130, 170 and 210). (3) Temperature group. The samples were treated by different temperatures without pressure, the resultant samples was denoted as EHSS-T-n (t means the change of temperature, n means the temperature values (ºC), n=30, 90, 130, 170 and 210). (4) Hot-pressing cycles group. The sponges were hot pressed with the pressure of 30 MPa and temperature of 130 ºC for 10 min, soaked in water for 24 h and then freezing-dried, this process was coded as one cycle. The sponges was treated for 1-3 cycles and named as EHSS-C-n (C means the cycle treatment, n means the number of cycles, n=1, 2 and 3), obviously, EHSS-PT-130 equals to EHSS-C-1.

Characterization

The EHSS thin sheets were cut into 2×3 cm blocks, and the FTIR spectra was recorded by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, TNZ1-5700, Nicolet Co, America) over the wavelength range from 4000 to 600 cm^{-1}. The dried EHSS thin disks (before and after hot-pressing treatments) were used for XRD measurements, which were measured with a wide angle X-ray diffraction (WAXD) diffractometer (XRD-6000, Shimadzu, Japan). The patterns with

Cu K_α radiation (λ=0.15406 nm) at 40 kV and 30 mA were recorded over the region of 2θ from 5 to 40°, scanning rate was 2°/min. Thermogravimetric (TG) curves of EHSS, SPI and HEC were measured using a Mettler-Toledo TGA/DSC 1 STARe system with a heating rate of 20 °C/min under a nitrogen atmosphere of 40–500 °C. The morphology of the sponges was observed on a scanning electron microscope (SEM, VEGA3, TESCAN, Czech Republic) with 20 kV as the accelerating voltage. After being soaked in deionized water, the sponges were frozen in liquid nitrogen for 10 min, fractured immediately, and then vacuum-dried. The surfaces and cross-sections of the sponges were coated with gold for SEM observation with the accelerating voltage of 20 kV.

The compressive strength of the EHSS sponges at dry and wet states were tested on a universal testing machine (CMT6503, Shenzhen SANS Test Machine, China) according to ISO527-1995 (E) at a speed of 1 mm/min. The EHSS disk sponges with the diameter of 10 mm were vacuumed dried for 4h and then kept in sealed container with $CaCl_2$ for 5d or soaked in water for 24h, which were used for test as the dry state and wet groups, respectively. The mean values of the compressive strength of the sponges were calculated from four specimens. Because the mechanical properties are affected by environmental temperature and humidity, all measurements were made under the same conditions.

Cytocompatibility evaluation by MTT assay and direct culture method

According to ISO 10993-5: 2007, a cell line of mouse lung fibroblasts (L929, provided by China Centre for Type Culture Collection, Wuhan University, Wuhan,

China), was re-suspended in culture medium and plated (100 μL/well) into 96-well plates at a density of 1×10^3 cells/well. The plates were incubated at 37 ºC in a humidified atmosphere of 5 % CO_2 for 24 h, 48 h and 72 h. The medium was then replaced with sterilized extracts from the EHSS and EHSS-PT-n (n=30, 90,130,170 and 210) sponges. The culture medium with similar cells was used as a control. After incubating for 1d, the cells were treated with 10 μL/well of MTT and incubated for another 4 h at 37 ◦C in a humidified 5 % CO_2 atmosphere. Absorbance values were read in triplicate against a reagent blank at a test wavelength of 570 nm using a multiwell microplate reader (Tecan GENios, Tecan Austria GmbH, Salzburg, Austria). Cell viability was calculated using eq S1:

$$\text{Cell viability (\%)} = (A_{test}/A_{control}) \times 100 \qquad \text{(S1)}$$

where A_{test} and $A_{control}$ are the absorption values of the test and control groups, respectively.

The EHSS-PT-n (n = 30, 90, 130 and 210) sponges were cut into disks with the diameter of 1 cm, packaged with tin foil, labeled and high pressure steam sterilized at 121 ºC for 20 min. L929 cells in logarithmic growth phase were seeded in 6-well culture plate with the density of 1×10^5 cells/well, and the cells alignment reached 50-70% on the next day. EGFP-C1 plasmid and Lipofectamine ™2000 were added into 0.25 mL serum-free media respectively, which were blended carefully and incubated at room temperature for 5 min. The EGFP-C1 plasmid and Lipofectamine™ 2000 diluents were blended and incubated at room temperature for 20 min. The blend solution was added into 6-well culture plate with 1.5 mL serum-free media and cells.

After cultured at 37 ºC for 4-6 h, the medium was replaced with normal medium. After culture for 24 h, the neomycin was added with the concentration of 800 μg/mL, the medium was replaced every 2-3 days and fresh neomycin was added. A larger cell colony appeared after 2-4 weeks, which was seeded onto a new petri dish after digestion to obtain GFP-transfected L929 cells for the follow-up experiments.

When the growth condition of GFP-transfected L929 cells was good with suitable alignment, the primary culture medium was discarded, which was rinsed with sterilized PBS solution twice and then dissociated with desired 0.25 wt% trypsin solution. The medium in the plates was incubated at 37 ºC at a humidified atmosphere of 5 % CO_2 for 2-3 min. When cells gradually changed from shuttle-shape or polygon to round, the improved RPMI 1640 culture medium containing 10 % fetal bovine serum was added to end the dissociation process. Then, the suitable amount of improved RPMI 1640 culture medium was added to prepare the single cell suspension, whose concentration was diluted to 1×10^5 cells/mL.

The high pressure steam sterilized sponges were put into 24-well cell culture plate respectively. One drop of 100 μL cell suspension was added onto the surface of the composite material, and the same 100 μL cell suspension was added to the blank well as the negative control. After incubated for 2 h at 37 ºC in CO_2 atmosphere, the culture plates were taken out with the addition of 900 μL RPMI1640 cell culture medium containing 10% FBS, which were then incubated for 72 h. To observe the morphology of L929 cells, the EHSSs with cultured cells were taken out and rinsed with PBS solution twice, which were fixed with 25 wt% glutaraldehyde at 4 °C for 4

h.

In vitro degradation evaluation of EHSS

The EHSS sponges were cut into blocks with the diameter of 10 mm and thickness of 3mm, dried in a vacuum oven for 6 h, and then weighed to determine the initial dry mass (M_{d0}). After high pressure sterilization, the samples were put in the sterilized PBS at 37 °C, which was shaken for 1 h every day at 100 rpm/min. PBS was replaced every three days. Three samples at each point in time of 3, 7, 14 and 28 d were taken out and freeze-dried weigh the corresponding dry mass (M_{dt}). In vitro degradation rate was calculated according to Eq. (S2), and the mean values of in vitro degradation rate were calculated from three specimens:

$$\text{In vitro degradation rate (\%)} = [(M_{dt} - M_{d0})/M_{d0}] \times 100 \qquad \text{(S2)}$$

where M_{dt} and M_{d0} are the mass at time point t and the mass before degradation, respectively.

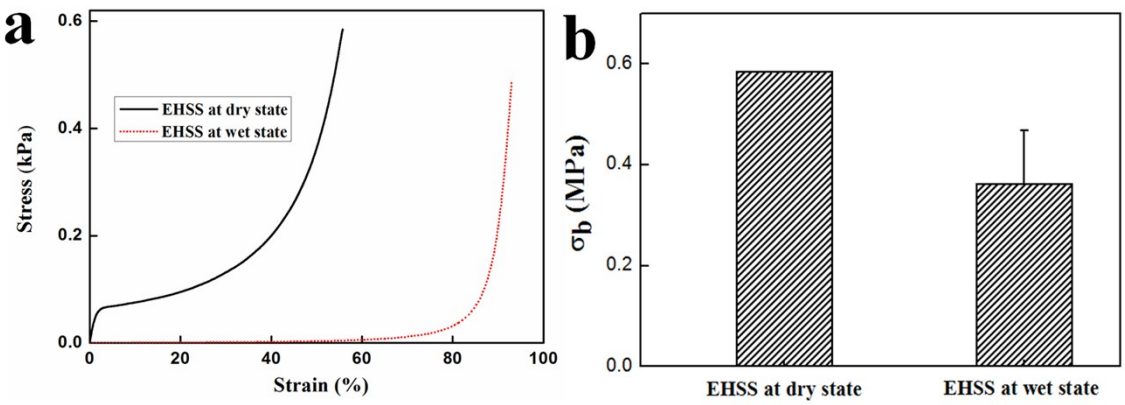

Fig. S1. The stress-strain curves of EHSS at dry and wet states (a) and the average compressive strength (b).

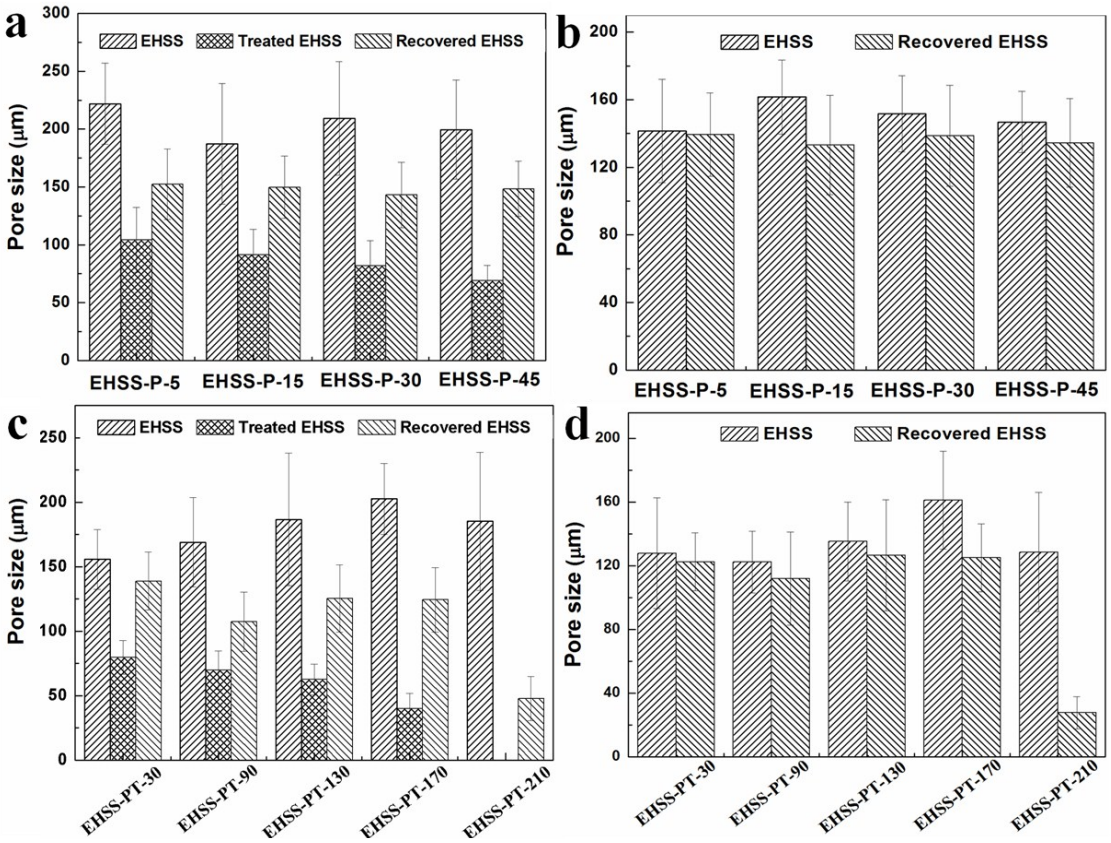

Fig. S2. The statistical size histograms of the average pore size for the surfaces of EHSS, Treated EHSS and the corresponding Recovered EHSS (a, c), and the corresponding cross-sections of EHSS and Recovered EHSS (b, d).

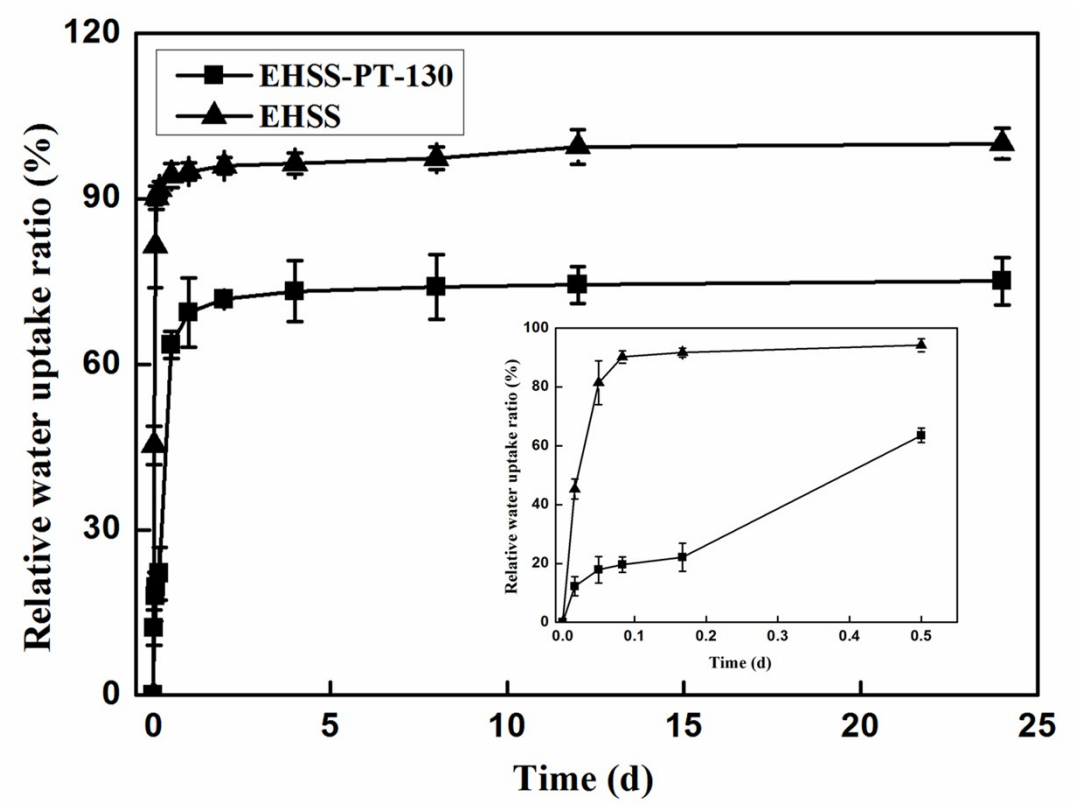

Fig. S3. The swelling kinetics of EHSS and EHSS-PT-130 in ultrapure water.

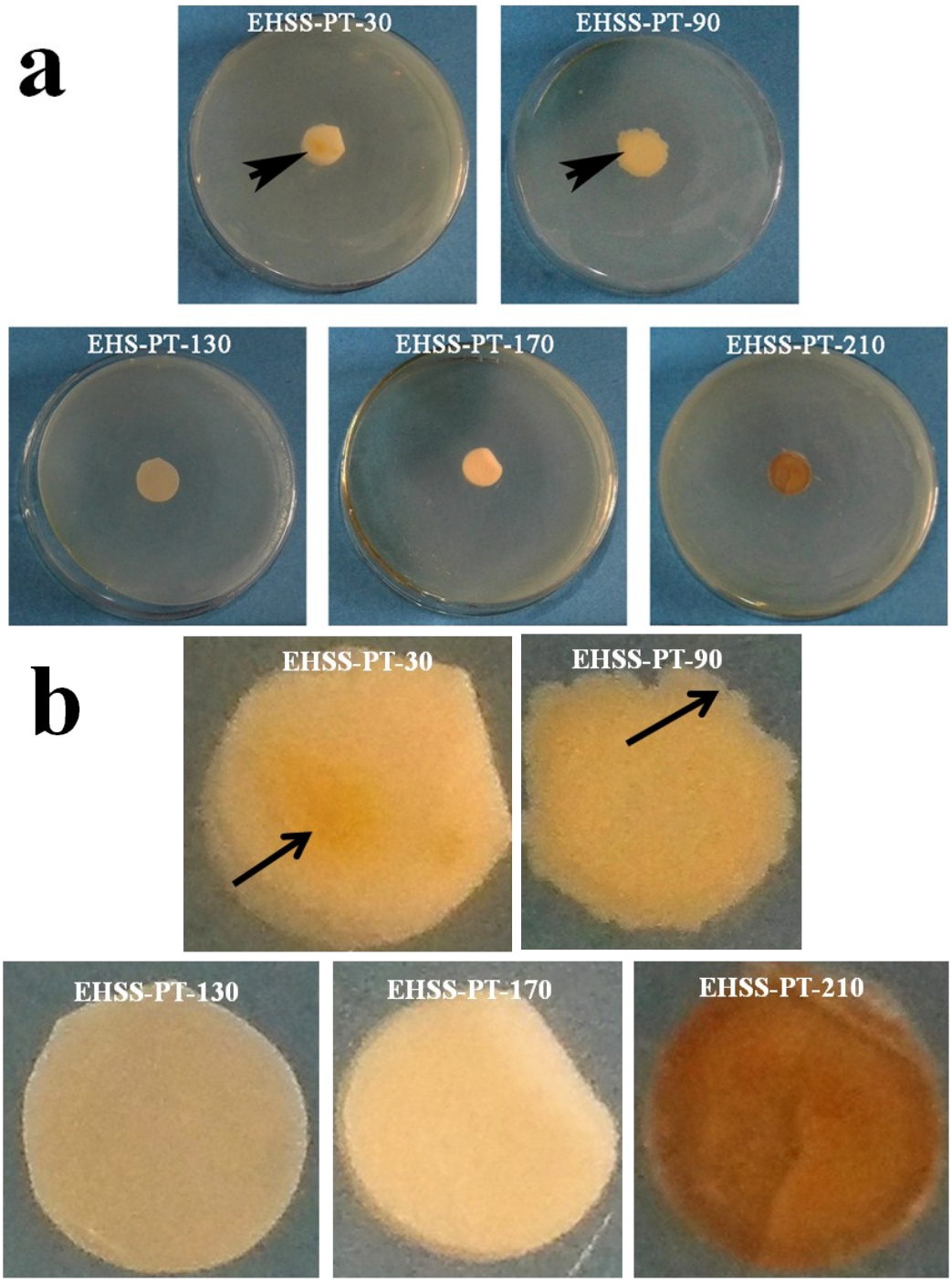

Fig. S4. The photographs of bacteria survival status for the EHSS-PT-n (n = 30, 90, 130, 170 and 210) sponges (a); The enlarged photograph of a (b). The black arrows indicate the bacterial colonies.

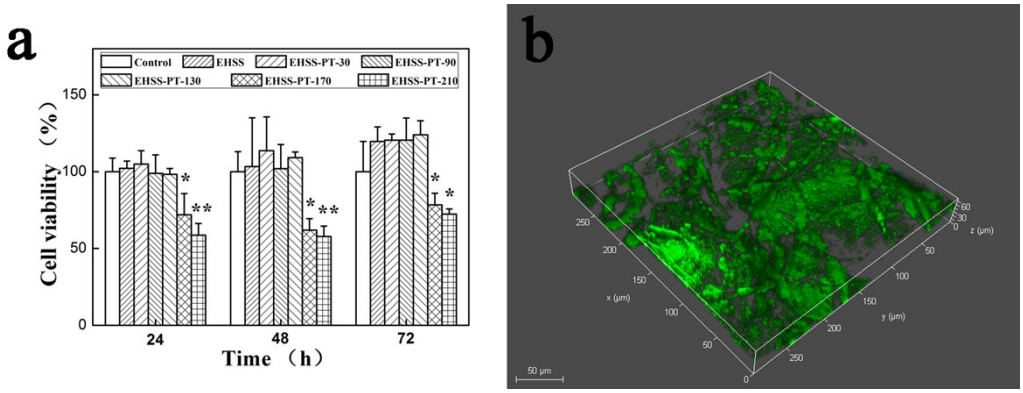

Fig. S5. Cell viability of L929 cultured in extracts from the EHSS and EHSS-PT-n (n =30, 90, 170 and 210) sponges for 24 h, 48 h and 72 h. *P＜0.05, **P＜0.01 (compared with the control at the same time) (a), and confocal microscope image of pure EHSS-PT-210 without cells (b).

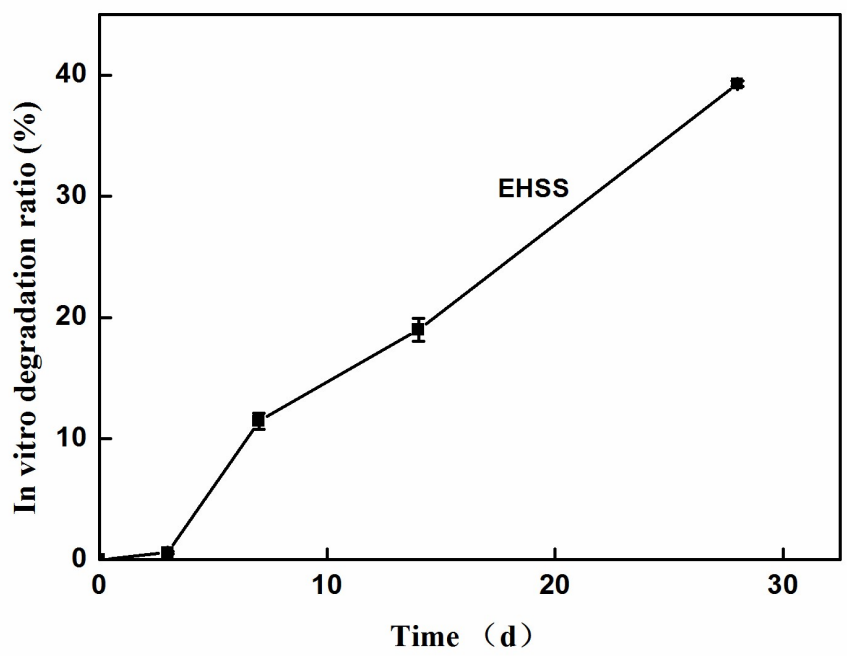

Fig. S6. Dependence of in vitro degradation ratio of EHSS soaked in PBS on soaking time.

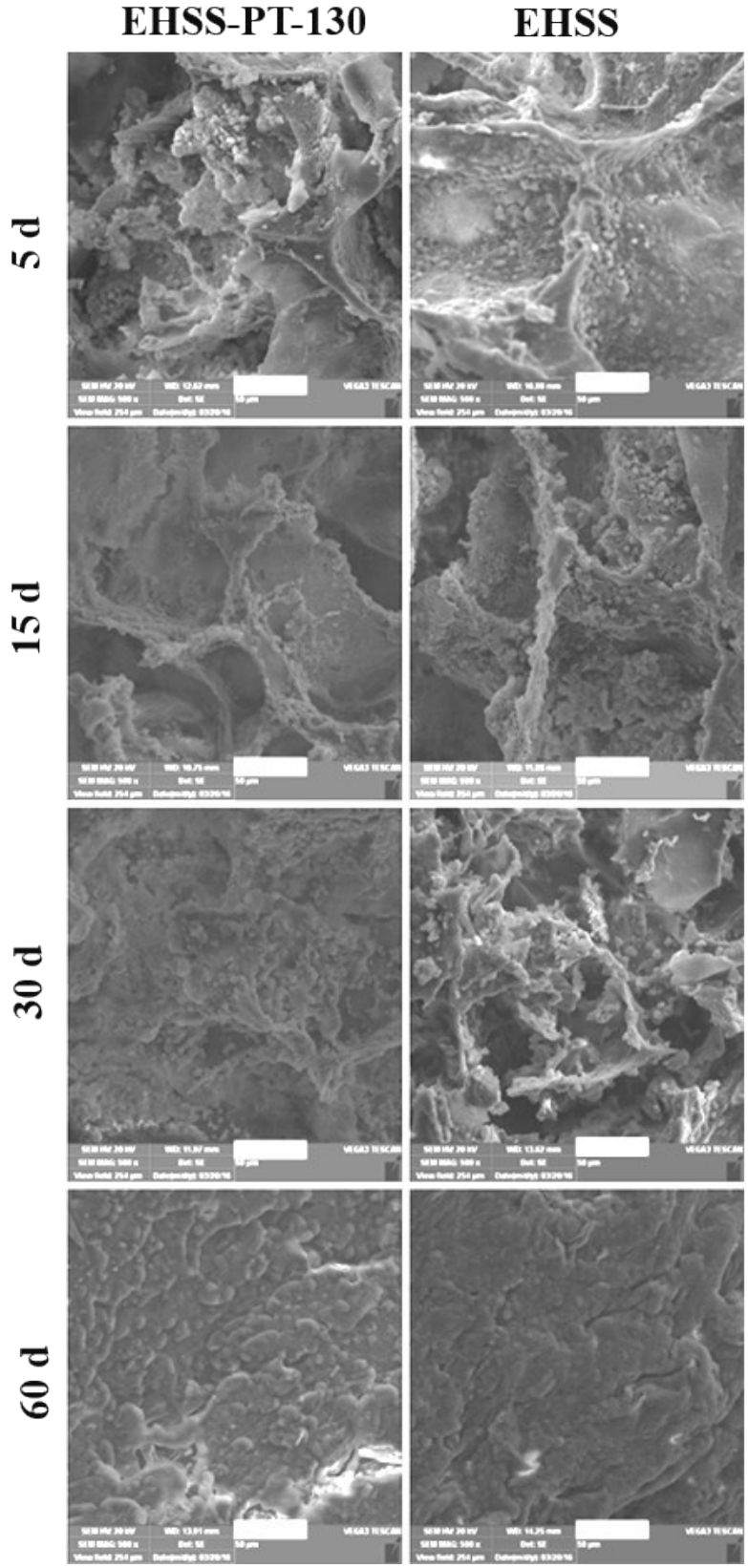

Fig. S7. SEM images of EHSS-PT-130 and EHSS sponges after implanted in rats for 5, 15, 30 and 60 days (b). Scale bar = 50 μm.