Supporting Information

Adipose-Derived Mesenchymal Stem Cells-Loaded Polysaccharide Hydrogel Promotes Wound Healing through Angiogenesis

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1 Oxidation degree of OD

To study the oxidation degree, OD (100 mg) was dissolved in 25 mL of hydroxylamine hydrochloride (0.25 M)-methyl orange (5 %) aqueous solution. After being reacted for 3 h at 25 ℃, the unreacted HCl was quantified by titration with NaOH (0.1 M). The oxidation degree of OD was computed according to the equation (S1)：

$$Oxidation\ degree\ (\%) = \frac{(V_N - V_B) \times M_N \times M_D}{1000 \times W_O} \times 100\% \quad \text{(S1)}$$

In this equation, V_N indicates the volume of NaOH used for titration, V_B indicates the volume of NaOH used in the control group, M_N indicates the molarity of NaOH, M_D indicates the molecular weight of dextran, and W_O indicates the weight of OD.

2. Porosity of the hydrogels

The porosity of the hydrogels was determined by the ethanol impregnation method. Specifically, the pre-weighed lyophilized hydrogels were immersed in 5 mL of ethanol for 30 min and weighed again. The porosity was calculated according to equation (S2):

$$\text{Porosity}\ (\%) = \frac{W_1 - W_0}{\rho V_0} \times 100\% \quad \text{(S2)}$$

In this equation, W_0 and W_1 denote the hydrogel weight before and after immersion in ethanol, respectively. V_0 is the volume of the hydrogel, and ρ is the density of ethanol (0.785 g/cm^3).

3·. Cytocompatibility Study

The cytocompatibility of different hydrogels was evaluated using the Cell Counting Kit-8 (CCK-8). Hydrogel extract solutions were prepared at a concentration of 100 mg/mL (in DMEM) according to the Chinese National Standard GB/T 16886.5. L929 cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 12 h at

37°C and 5% CO_2. Then, the old medium was replaced with 100 μL of different hydrogel extracts. Pure DMEM served as the control group. After 1, 3, and 5 days of incubation, L929 cells were washed with PBS three times, and cell viability was detected using the CCK-8.

***3. In Vitro* Hemolysis Assay**

Two milliliters of mouse whole blood were centrifuged (3000 rpm, 5 min), and the red blood cells (RBCs) were washed three times with PBS and resuspended in 50 mL of PBS for later use. Then, 0.3 mL of the RBC suspension was added to 1.2 mL of PBS containing $O_5C_{2.5}$ hydrogel at concentrations of 25, 50, and 100 mg/mL. PBS and deionized water were set as negative and positive controls, respectively. After incubation at 37°C for 2 h, the mixtures were centrifuged. The absorbance of the supernatant at 541 nm was collected using a UV-Vis-NIR spectrophotometer (Lambda 25, Perkin Elmer, USA). The absorbances of the negative control, positive control, and hydrogel-treated groups were recorded as D_n, D_p, and D_t, respectively. The hemolysis ratio was calculated according to equation (S3):

$$Hemolysis\ ratio\ (\%) = \frac{(Dt - Dn)}{(Dp - Dn)} \times 100\% \qquad \text{(S3)}$$

4. qPCR Analysis of Stemness Gene Expression

To evaluate the effect of 3D culture on ADSCs stemness, quantitative real-time PCR (qPCR) was performed. After 7 days of culture in $O_5C_{2.5}$ hydrogel, total RNA was extracted from encapsulated ADSCs using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using a PrimeScript RT reagent kit (Takara, Japan). qPCR was carried out using SYBR Green Premix Ex Taq (Takara, Japan) on a QuantStudio 6 Flex real-time PCR system (Applied Biosystems, USA). The primer sequences used were as follows: POU5F1 forward, 5'-

TGTCTCCGTCACCACTCTG-3'; reverse, 5'-CACCCTTTGTGTTCCCAAT-3'; SOX2 forward, 5'-TGATGGAGACGGAGCTGAA-3'; reverse, 5'-GGGCTGTTTTTCTGGTTGC-3'. GAPDH was used as an internal control. Relative gene expression levels were calculated using the 2^ΔΔCt method and normalized to ADSCs cultured on tissue culture plates (day 0).

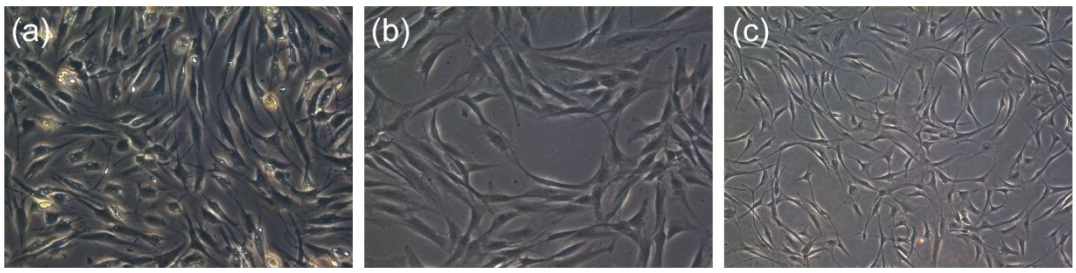

Figure S1. Morphological images of ADSCs at different passages. (a) Primary generation, (b) Passage 3 (P3), (c) Passage 5 (P5).

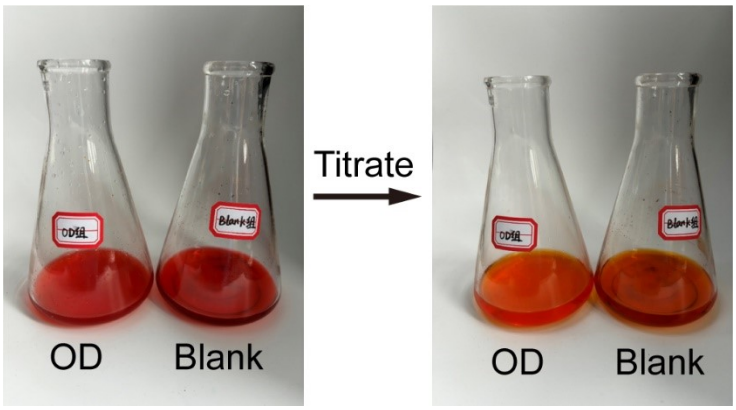

Figure S2. The oxidation degree of OD was determined by hydroxylamine hydrochloride titration.

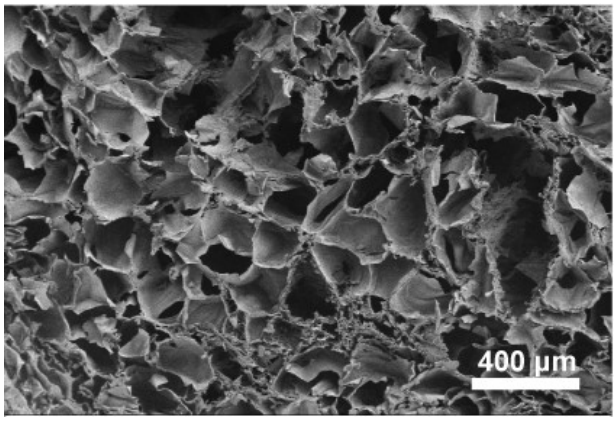

Figure S3. SEM image of $O_5C_{2.5}$ hydrogel.

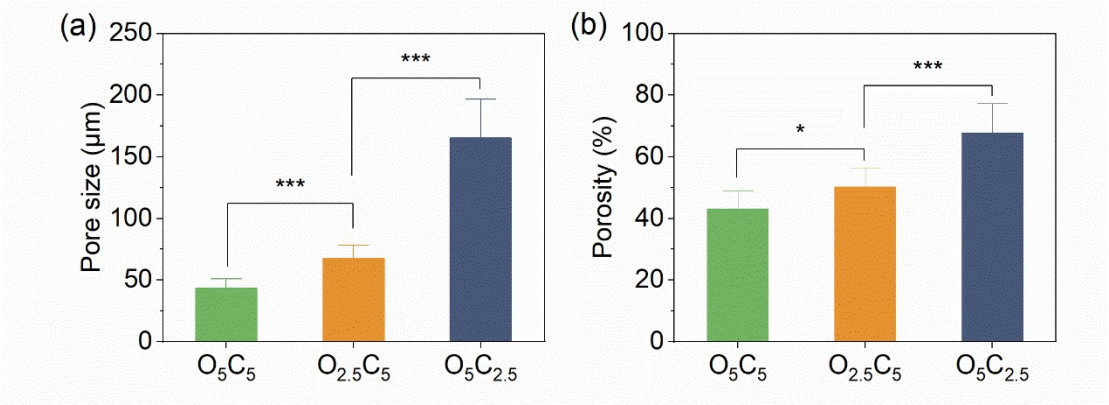

Figure S4. Pore size and porosity of the three OC hydrogels (n = 3). Data represent the mean ± s. d. (*p < 0.05, **p < 0.01, ***p < 0.001).

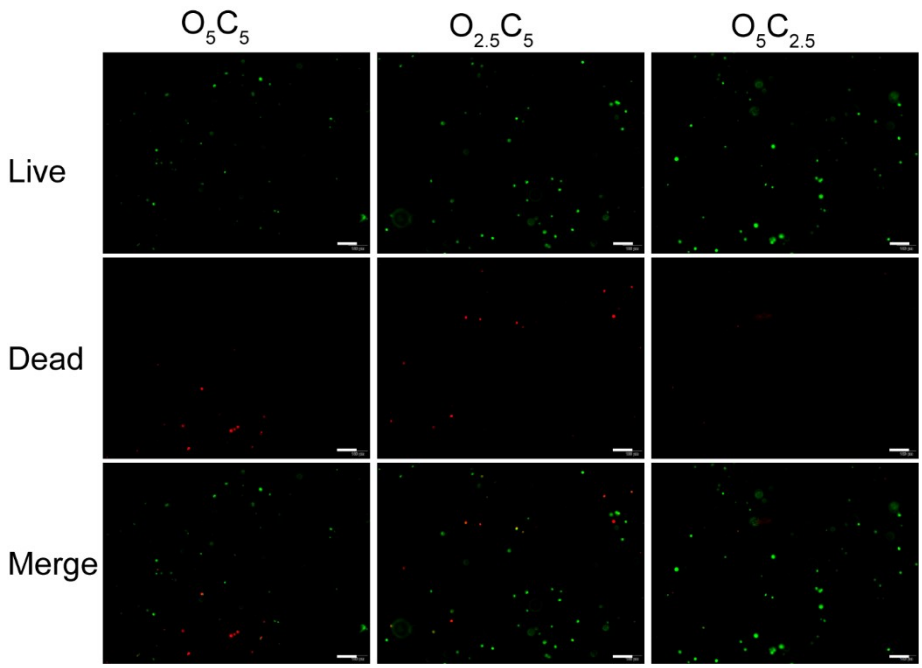

Figure S5. The images of live/dead staining after 3 day of culture (bar = 100 μm).

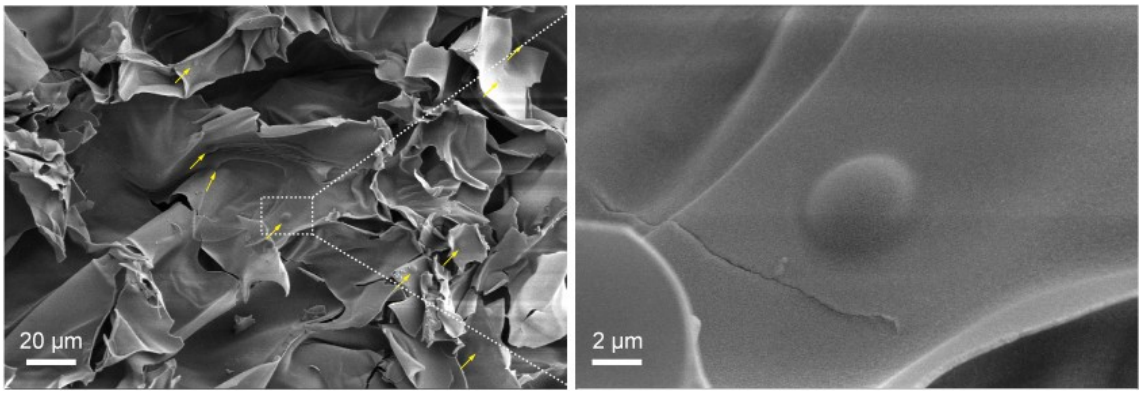

Figure S6. SEM images of OC-ADSCs hydrogel (cells were indicated by yellow arrows for observation).

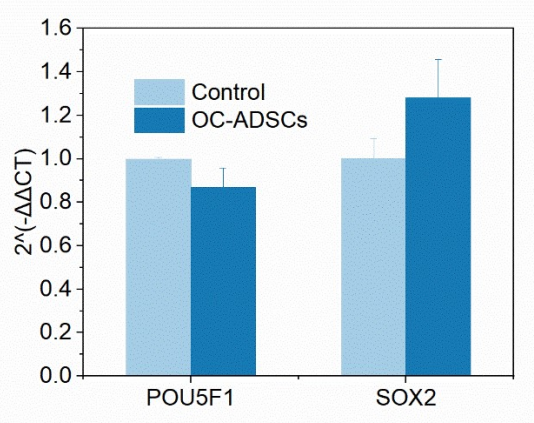

Figure S7. qPCR analysis of pluripotency marker expression (POU5F1 and SOX2) in ADSCs encapsulated in O5C2.5 hydrogel (n = 3).

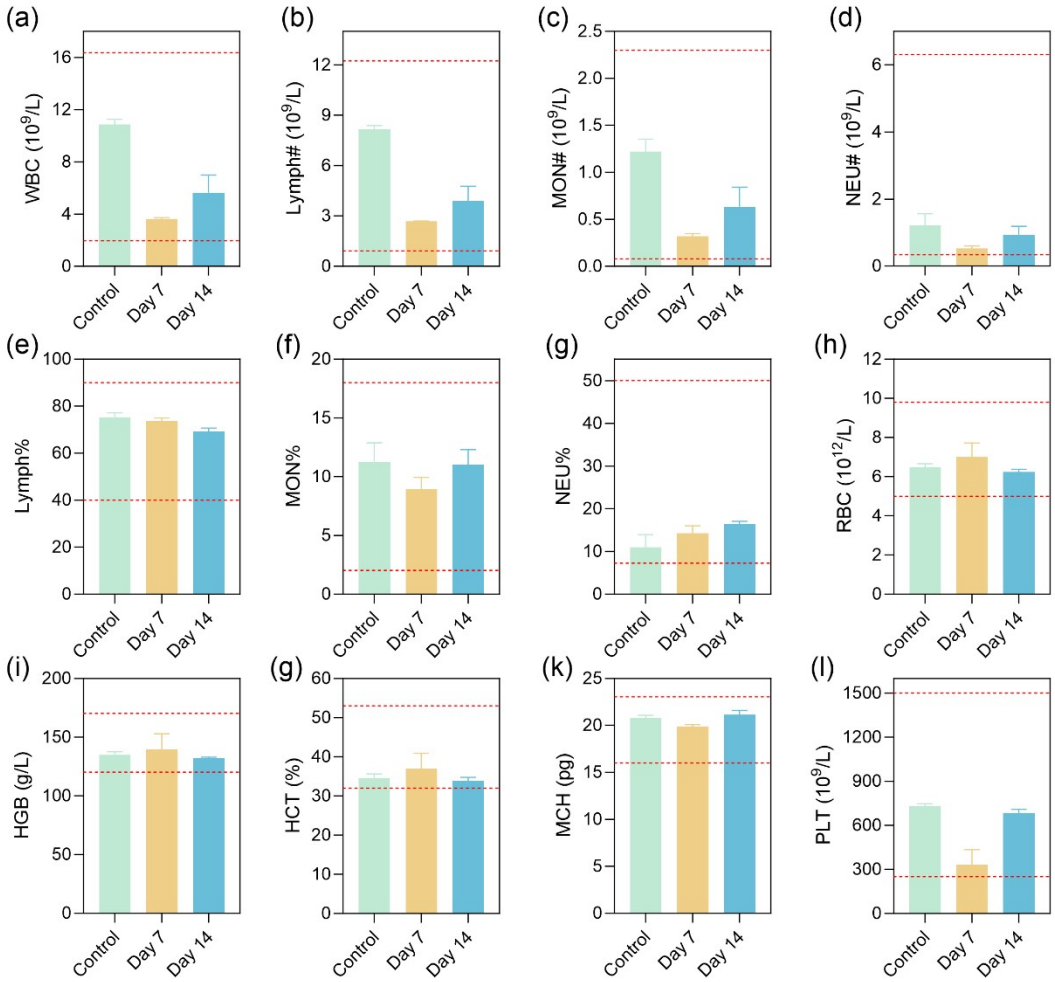

Figure S8. Routine blood test indicators at different time points after implantation of

the OC-ADSCs hydrogel in rats. (a) White Blood Cell count (WBC), (b) Lymphocyte count (Lymph#), (c) Monocyte count (Mon#), (d) Neutrophil count (NEU#), (e) Lymphocyte percentage (Lymph%), (f) Monocyte percentage (Mon%), (g) Neutrophil percentage (NEU%), (h) Red Blood Cell count (RBC), (i) Hemoglobin (HGB), (j) Hematocrit (HCT), (k) Mean Corpuscular Hemoglobin (MCH), (l) Platelet count (PLT). The red dotted line indicates the reference range.

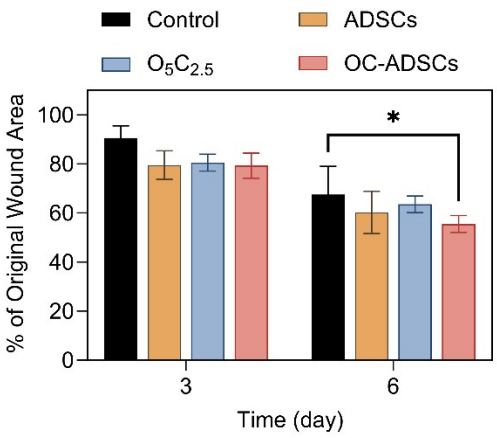

Figure S9. Statistical chart of wound area at days 3 and 6.