Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Hydration of hydrogels regulates vascularization *in vivo*

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1 The freeze-dried temperatures were set at −80 and −196 °C to fabricate xerogels with different porous structures

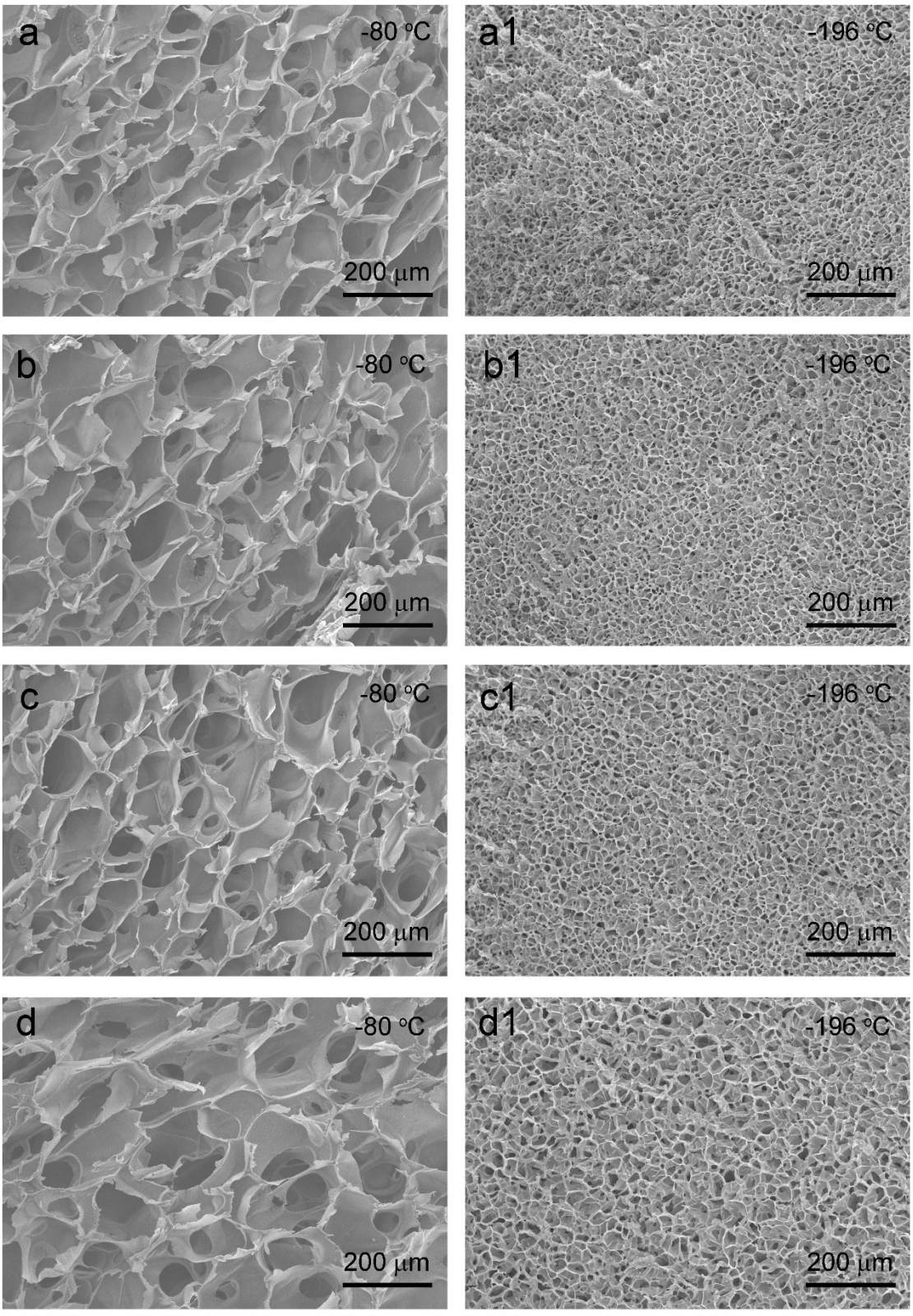

Fig. S1 Characterizations of PLGA based xerogels. The cross-linkers of xerogels

a−a1, b−b1, c−c1, and d−d1 were OEG_1, OEG_2, OEG_3, and OEG_9, respectively. The degree of cross-linking was ~50%, and the freezing temperatures of a−d and a1−d1 were −80 and −196 °C.

2 Preparation of FITC-labeled protein

The fluorescein isothiocyanate-labeled albumin from bovine serum (FITC-BSA) was synthesized by the method described previously.[1] 50.0 mg of BSA was dissolved in 250.0 mL of 0.1 M sodium carbonate buffer solution, and then 125.0 mL of 1.0 mg mL^{-1} FITC in anhydrous DMSO solution was added slowly to the protein solution with continuously stirring. After the FITC solution was totally added, the reaction was incubated for 8 h in the dark ice water bath. Then, certain amount of ammonium chloride (NH_4Cl) was added to the reaction solution and further stirred for another 2 h to quench the conjugation reaction. The FITC-BSA solution was added into dialysis bag (molecular weight cutoff (MWCO) = 3500 Da). After dialysis against deionized water for 3 days in the dark, 0.2 mol mL^{-1} FITC-BSA solution was prepared for further protein diffusion experiments.

(1) Relationship of fluorescence intensity with FITC-labeled protein concentration

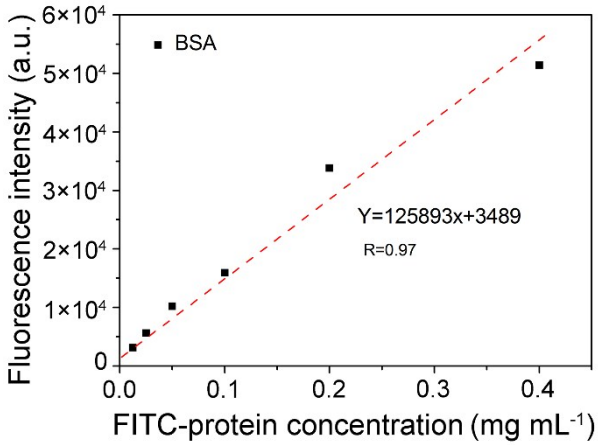

Fig. S2 Fluorescence intensity varied with FITC-labeled protein concentration.

(2) Protein absorption process in hydrogels

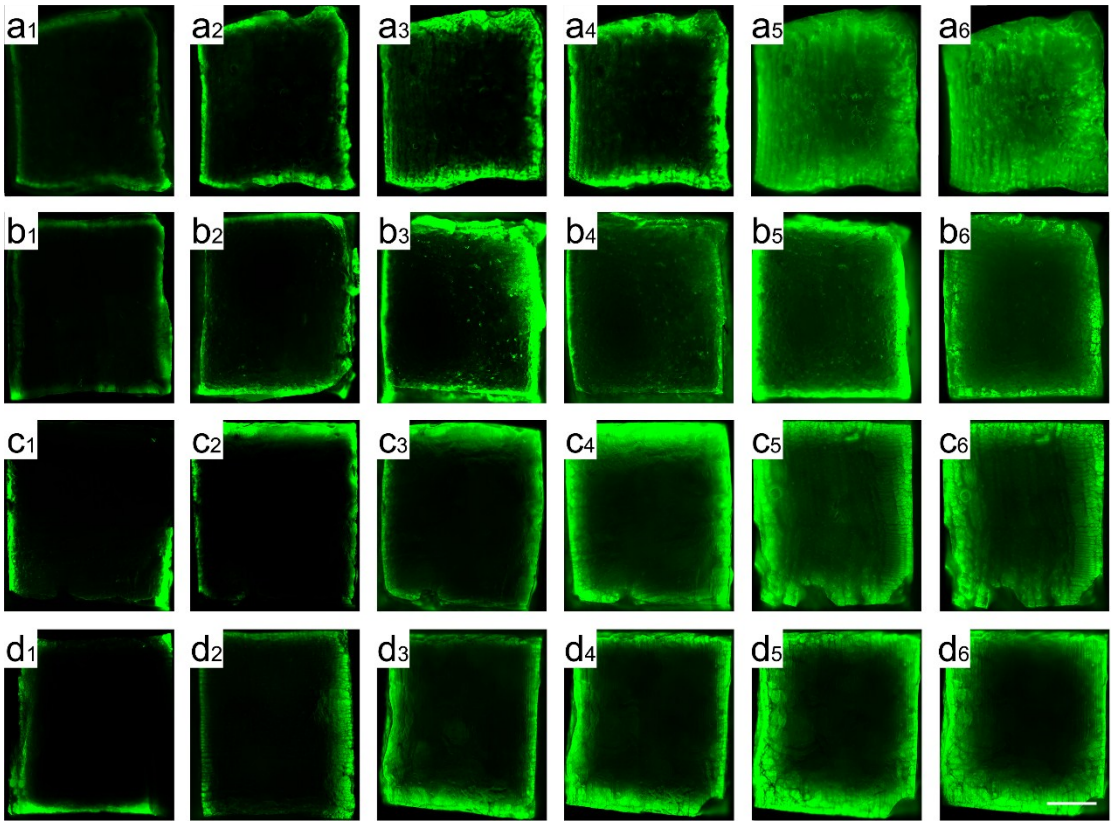

Fig. S3 Protein absorption process in hydrogels. Fluorescence micrographs of OEG-cross-linked hydrogels incubated in FITC-BSA solution at predetermined times. Micrographs of OEG_1 (a_1–a_6), OEG_2 (b_1–b_6), OEG_3 (c_1–c_6), and OEG_9 (d_1–d_6) hydrogels were recorded after 0, 10, 20, 30, 60, and 120 min. Bar scale: 1 mm.

(3) Protein release process in BSA-loaded hydrogels

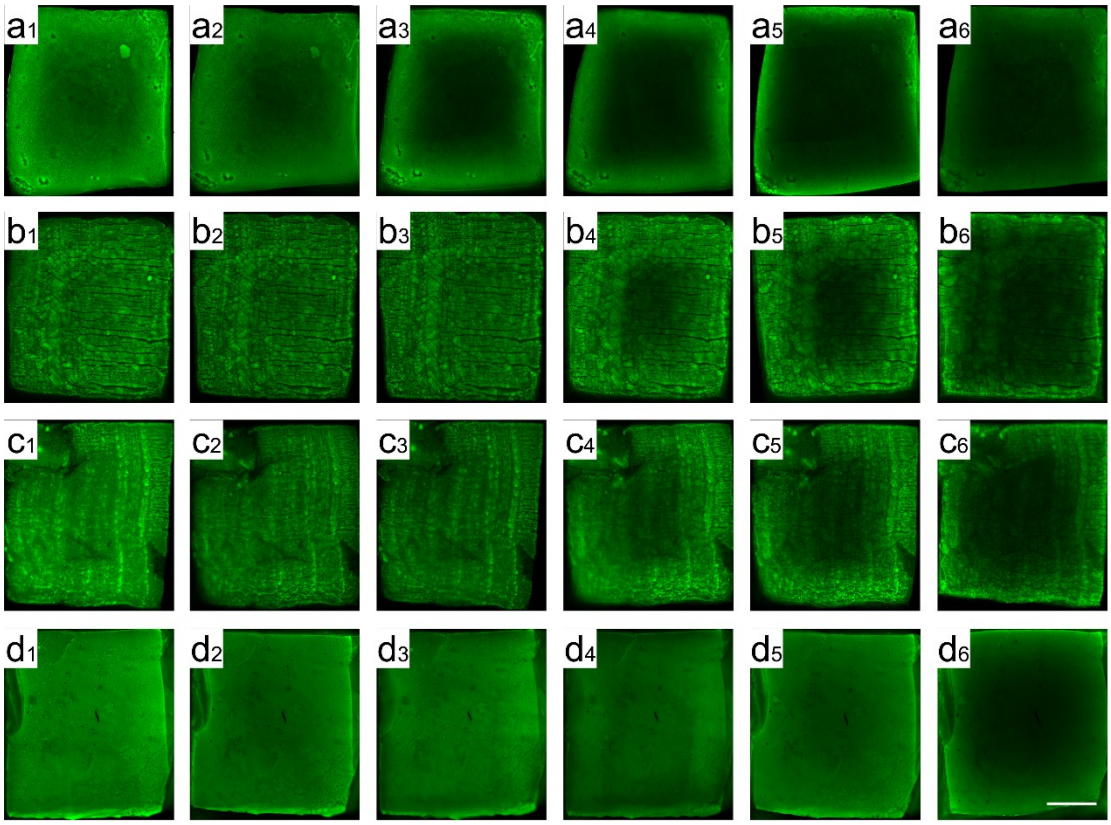

Fig. S4 Protein release process in BSA-loaded hydrogels. Fluorescence micrographs of FITC-BSA-loaded OEG hydrogels incubated in PBS solution at predetermined times. Micrographs of OEG_1 (a_1−a_6), OEG_2 (b_1−b_6), OEG_3 (c_1−c_6), and OEG_9 (d_1−d_6) hydrogels in protein release were recorded after 0, 10, 30, 60, and 120 min, and overnight. Bar scale: 1 mm.

3 Nitrogen gas adsorption/desorption in xerogels

The nitrogen gas (N_2) adsorption/desorption of the xerogels was analyzed isotherm at 77 K, by Quantachrome Instruments (Autosorb iQ Station 1, Quantachrome, Boynton Beach, USA).[2] The samples were pretreated at 60 °C overnight in a vacuum line. The N_2 adsorption volume was calculated from the amount adsorbed at a maximum relative pressure ($P/P_0 = 1$).

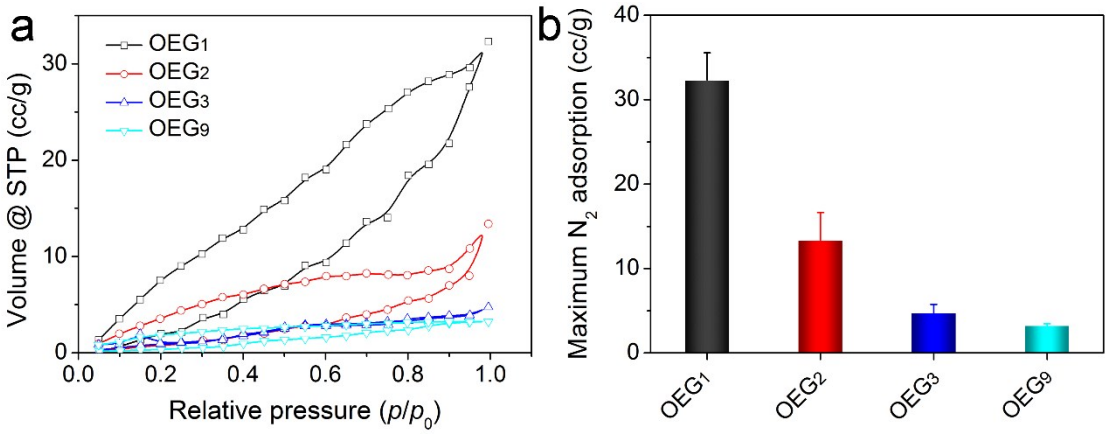

Fig. S5 a, The N_2 adsorption/desorption curves. b, The maximum N_2 absorption in OEG xerogels at a maximum relative pressure ($P/P_0 = 1$).

4 Adhesion behavior in PLGA-based hydrogels

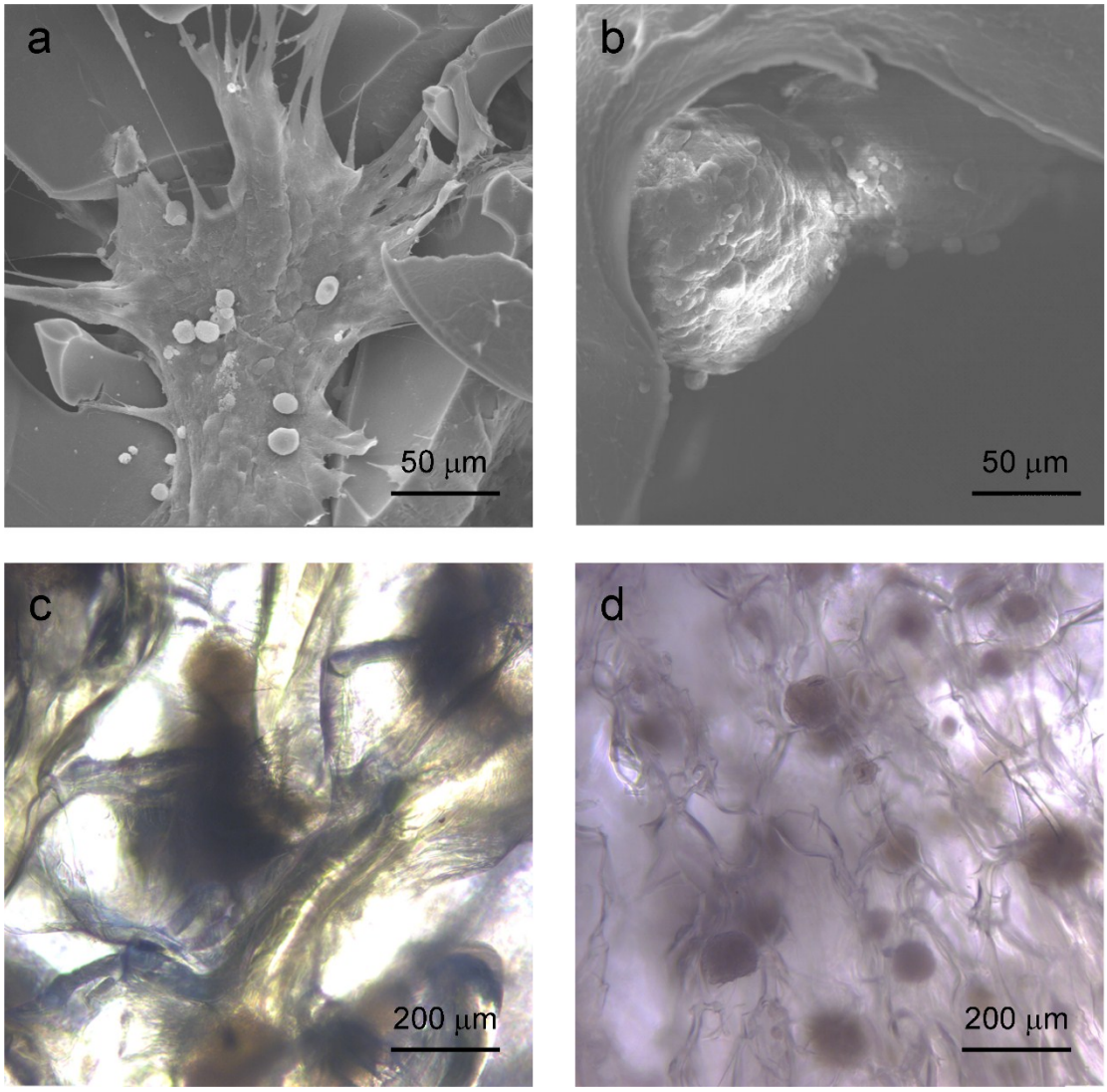

Fig. S6 Observation of ASC adhesion behavior in PLGA-based hydrogels. The cell/hydrogel constructs were observed by scanning electron microscope (SEM) at 3 days. The spindle-shaped ASCs were observed to attach along the wall of OEG_1 hydrogel (a), while the spheroid-shaped ASCs aggregated together in the pores of OEG_9 hydrogel (b). c−d, The digital microscopy photographs of cellular adhesion behavior in the OEG_1 and OEG_9 hydrogels after seeding for three days, respectively.

5 Cell viability in PLGA-based hydrogels

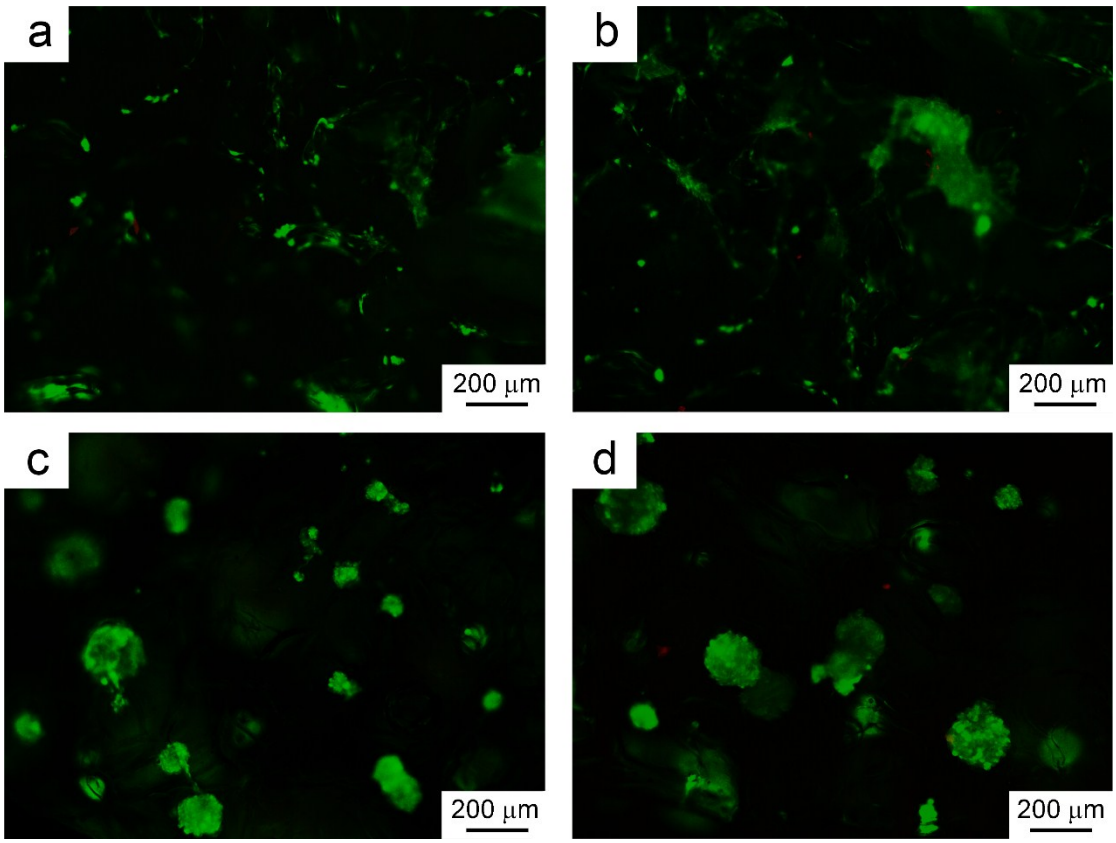

Fig. S7 Characterizations of ASC viability in OEG_1 and OEG_9 hydrogels. The live/dead staining showed that most of ASCs were viable after 3 and 7 days of *in vitro* culture in OEG_1 (a, b) and OEG_9 (c, d) hydrogels.

6 Angiogenesis upon hydrogels subcutaneously implantation in nude mice for 1 week

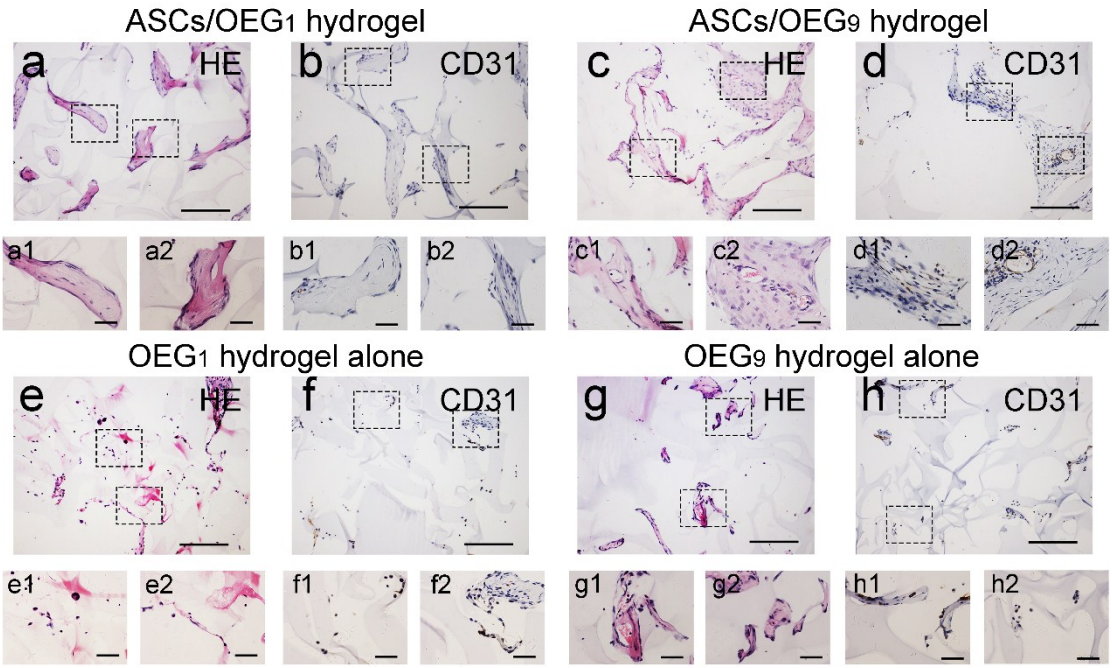

Fig. S8 ASCs/OEG hydrogel constructs (experiment groups) and OEG hydrogel alone (control groups) at week 1 after subcutaneously implantation into nude mice. H&E staining in the center of vascularized tissues with (a) ASCs/OEG_1 hydrogel constructs, (c) ASCs/OEG_9 hydrogel constructs, (e) OEG_1 hydrogel alone and (g) OEG_9 hydrogel alone, and a1−a2, c1−c2, e1−e2, g1−g2 were higher-magnification images selected from the vascularized areas outlined by the rectangles in a, c, e, g, respectively. CD31 immunohistochemical staining images of the experiment groups (b, d) and control groups (f, h), and b1−b2, d1−d2, f1−f2, h1−h2 were higher-magnification images selected from the vascularized areas outlined by the rectangles in b, d, f, h, respectively. (Bar scales: 200 μm for a−h, 50 μm for a1−a2, b1−b2, c1−c2, d1−d2, e1−e2, f1−f2, g1−g2, h1−h2)

Notes and references

(1) J. Wu, Z. C. Xiao, C. C. He, J. J. Zhu, G. Ma, G. Z. Wang, H. Y. Zhang, J. Xiao,

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(2) T. H. Kim, M. Eltohamy, M. Kim, R. A. Perez, J. H. Kim, Y. R. Yun, J. H. Jang, E. J. Lee, J. C. Knowles, H. W. Kim, *Acta Biomater*., 2014, **10**, 2612−2621.