Supporting Information

A Bio-inspired 3D Multilayered Shape Memory Scaffold with a Hierarchically Changeable Micropatterned Surface for Efficient Vascularization

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Additional Methods

Synthesis of 6a poly(ethylene glycol)–poly(ε-caprolactone)-acryloyl chloride (6a PEG-PCL-AC). The synthesis of 6a PEG-PCL-AC was carried out according to our previous article [1]. Curtly, 6 arm poly (ethylene glycol) – poly (ε-caprolactone) (6a PEG-PCL) was synthesized by ring-opening polymerization of ε-CL (20g, 175.44 mmol) with 6a PEG (10g, 1.67 mmol) as initiator and $SnCl_2$ (2 wt %) as catalyst at 140 °C. The obtained polymer was precipitated in cooled ethanol and dried under vacuum at room temperature for 48h. Then, 6a PEG-PCL was reacted with acryloyl chloride in the presence of triethylamine as acid binding agent (the mole ratio of 6a PEG-PCL: acryloyl chloride: triethylamine: = 1:6:6). Again, 6a PEG-PCL-AC was purified in supercooled mixed liquid ($V_{ethyl\ alcohol}$:V_{H_2O}=5:1) and dried under vacuum at room temperature for 48h.

***In Vitro* culture and immunostaining.** The ECs and VSMCs were obtained from human umbilical vein and human umbilical artery, severally. The membranes with three different topological structures (square, rectangular and flat) were prepared and sterilized by ultraviolet light irradiating for 12 h in 75% alcohol. After three-time rinses with PBS, ECs and VSMCs were seeded onto the different films at a density of 1×10^4 cells/well in 24-well plate (Costar) and cultured in F12 media with 10% fetal bovine serum (FBS) at 37 ℃/5% CO_2, respectively. For the cell viability study, the proliferation of ECs or VSMCs on different topological structures was quantified using Alamar Blue (AB) assay. F-actin and nuclei were labeled by rhodamine–phalloidin (Sigma, USA) and 4',6-diamidino-2-phenylindole (DAPI) (Sigma), respectively. The

relative cell number on square and rectangle was calculated by fluorescent photographs (200×), and at least 4 pictures was used. Cells morphology was further evaluated by SEM after dehydrated by gradient ethanol (25%, 50%, 70%, 75%, 90%, 95%, and 100%). Also, VECs and VSMCs on flat were stained with calcein (Sigma, USA) and propidium iodide (PI) (Sigma, USA) respectively.

To further study the influence of topology structure on the ECs expressing laminin and VSMCs expressing α-SMA, ECs and VSMCs were both seeded on the surfaces of membranes, which simultaneously possesses square and rectangle, at a density of 3×10^4 cells/well for co-culture. After 1 day's culture, the membranes were rolled up into tube with a condition of square region in the luminal layer and rectangular region in the outer layer for further 6 day's co-culture. After 6 day's co-culture, tubular vascular scaffolds were sectioned transversely using freezing microtome. For immunostaining, cells on the frozen sections were permeabilized with 0.1% Triton X-100 and following blocked with 5% bovine serum albumin (BSA). The cells were incubated with 1:100 diluted rabbit anti-human laminin antibody and α-smooth muscle actin (α-SMA) antibody (Abcam, USA) for 2 h at 37 ℃. After thoroughly washed with PBS, cells were further incubated with secondary antibody (Rhodamine-conjugated goat anti-rabbit for α-SMA and FITC-conjugated goat antirabbit for laminin, ZSGB-BIO, China) at room temperature for 2 h. Nuclei was labeled by DAPI (Sigma).

Platelet adhesion assay. Membranes (1×1 cm^2) with different micropatterns (square, rectangular and flat) were immersed in PBS at 24-well tissue culture plate and equilibrated at 37 ℃ for 1 h. After PBS was removed, 1 mL of fresh

platelet-rich-plasma (PRP) was added in tissue culture plat and incubated at 37 ℃ for 2 h. Then the PRP was removed and the unattached platelets were dislodged by rinsing with PBS. The specimens were immersed in 2.5 wt % glutaraldehyde solution at 4 ℃ for 4 h, and finally dehydrated by gradient ethanol (25%, 50%, 70%, 75%, 90%, 95%, and 100%). The platelets, adhering on the membrane, were observed by SEM (S-3500, Hitachi Instruments, Tokyo, Japan).

Additional Results

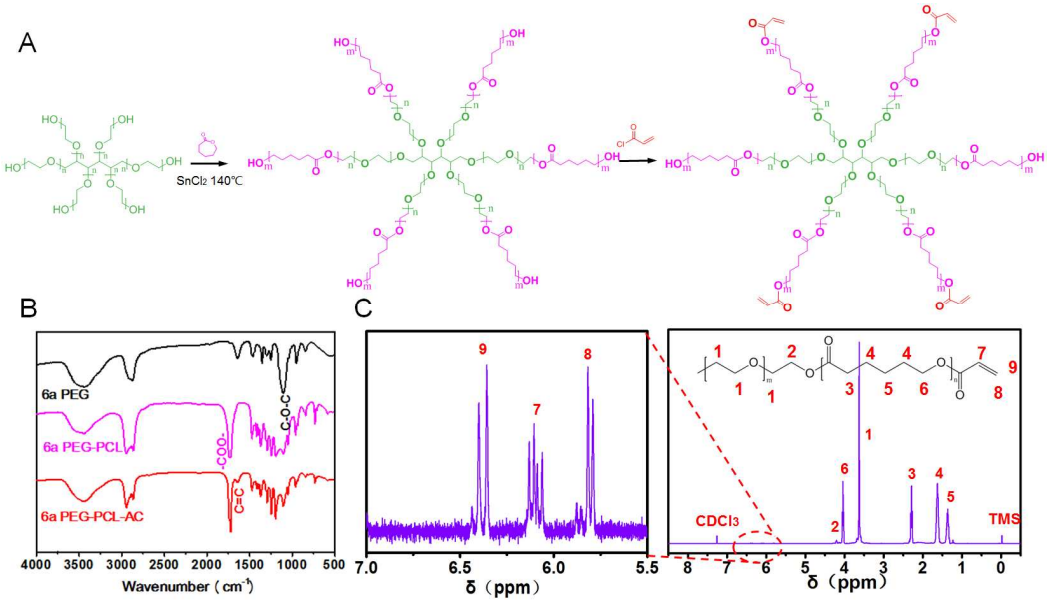

Figure S1. Scheme of the synthesis and characterization of 6a PEG-PCL-AC. (A) Synthetic procedure of 6a PEG-PCL-AC. (B) FT-IR spectra of 6a PEG, 6a PEG-PCL and 6a PEG-PCL-AC. (C) ^{1}H NMR spectra of 6a PEG-PCL-AC.

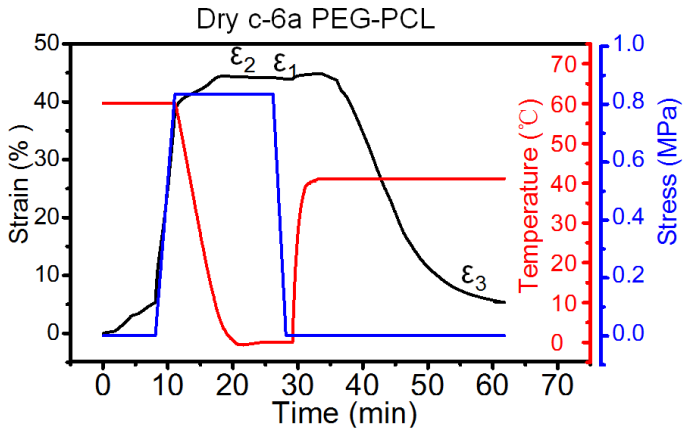

Figure S2. Quantitative thermal mechanical demonstration of c-6a PEG-PCL sheet on dry state.

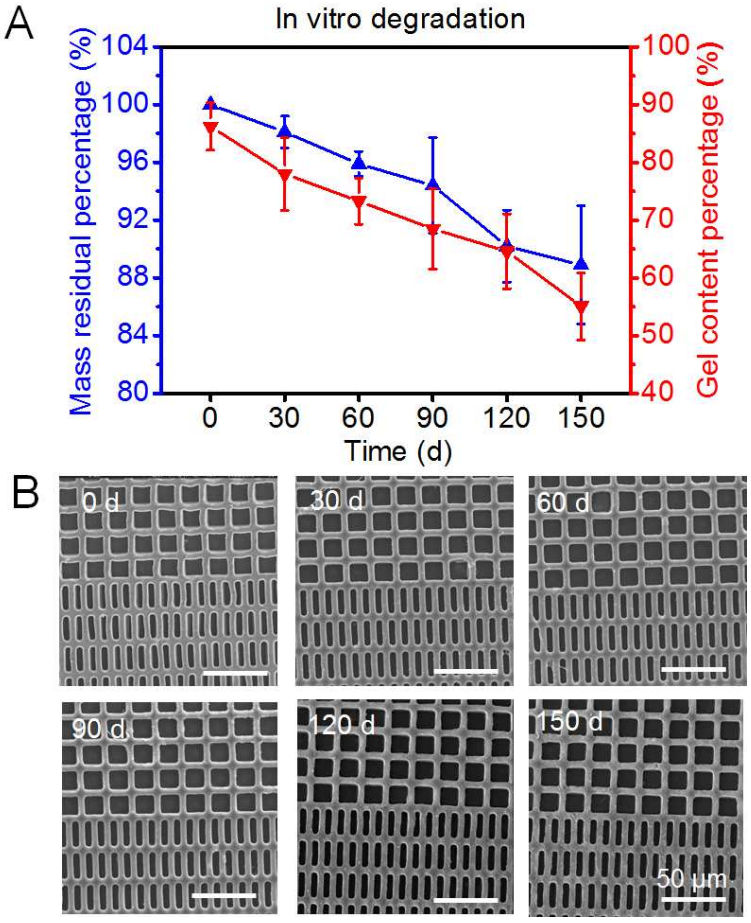

Figure S3. *In vitro* degradation of vascular grafts at each time point. (A) Change in mass and gel content implying molecular chain breakdown of c-6a PEG-PCL. (B) The surface morphology were monitored by SEM.

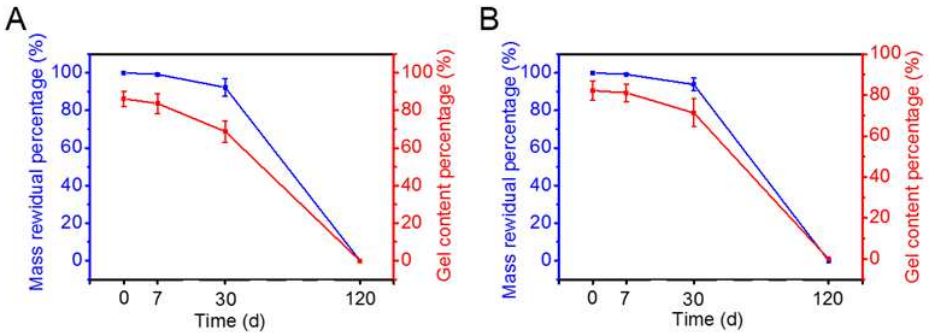

Figure S4. The in vivo degradation of vascular grafts with pattern (A) and no-pattern (B).

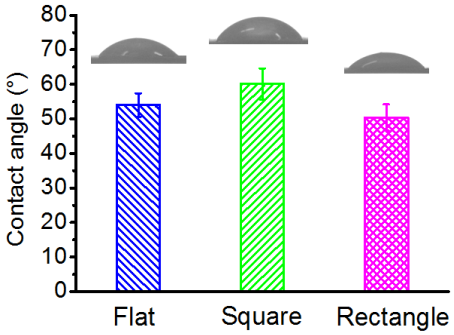

Figure S5. Water contact angles of c-6a-PEG-PCL membrane with square, rectangle pattern and flat.

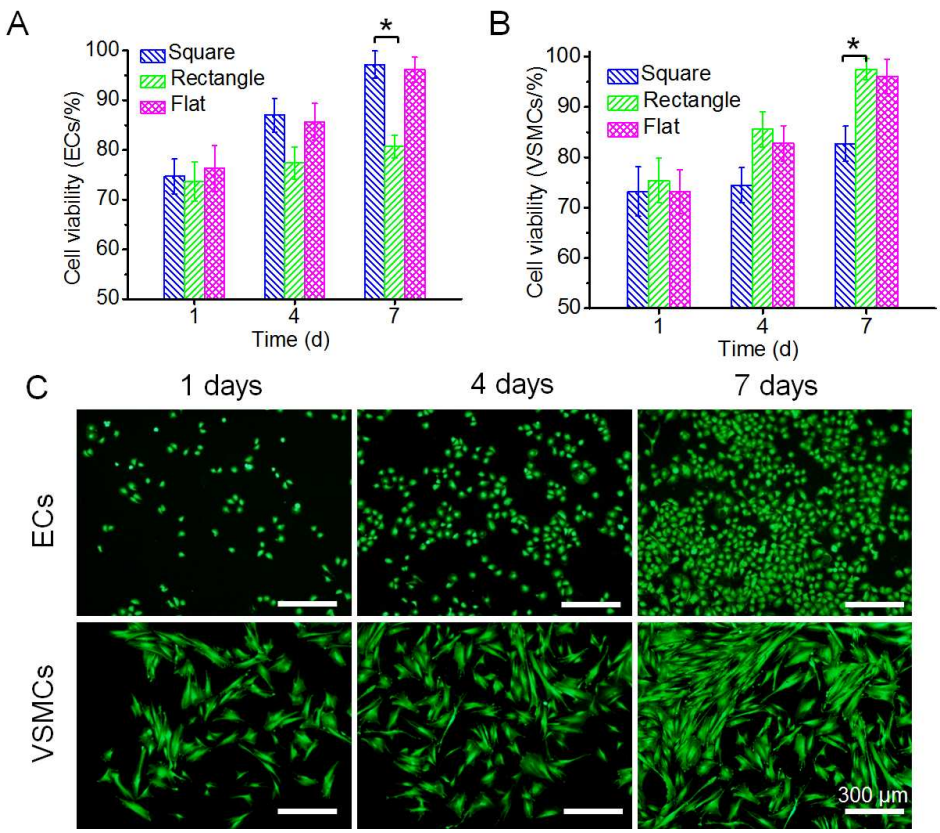

Figure S6. The ECs and VSMCs proliferation on different surface topological structures. (A, B) The proliferation of EC and SMC on the different topological structures (square, rectangle, flat) at 1, 4 and 7 days. (C) Live-dead fluorescence images showing the viability of ECs and VSMCs cultured on flat c-6a PEG-PCL membrane for 1, 4 and 7 days. The live cells were stained green. (*P<0.05)

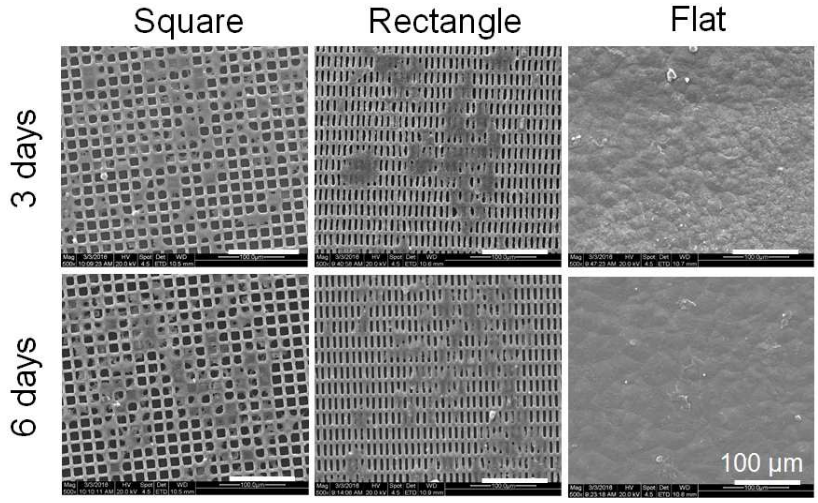

Figure S7. SEM images of ECs morphology and distribution on different surface topology at 3 and 6 days.

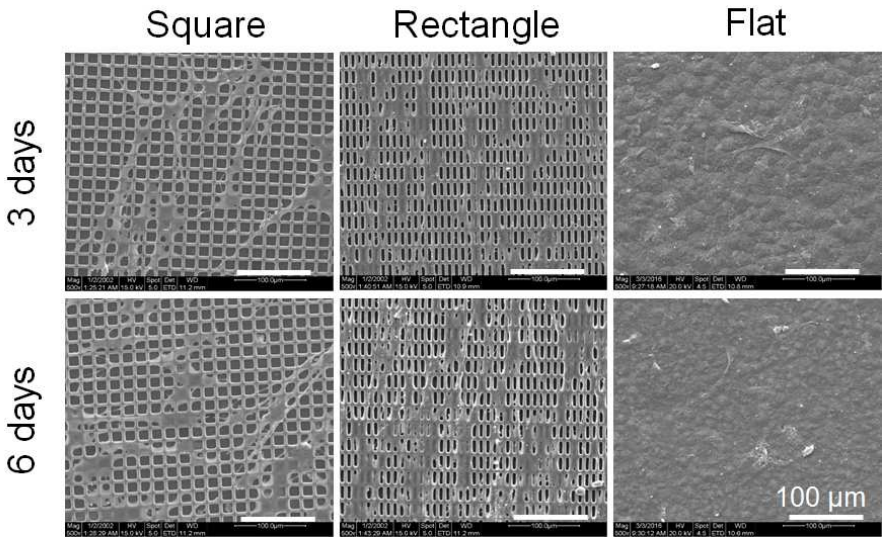

Figure S8. SEM images of VSMCs morphology and distribution on different surface topology at 3 and 6 days

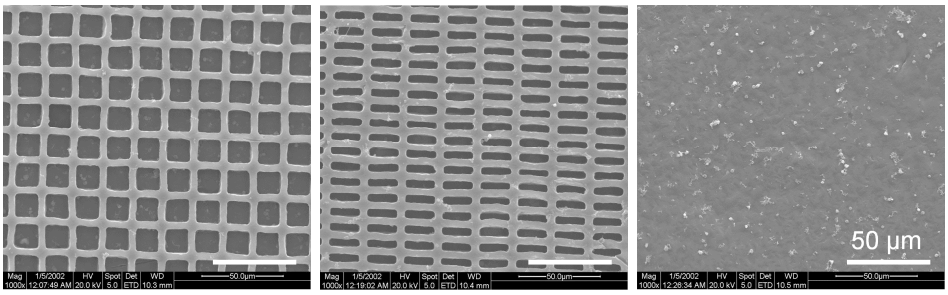

Figure S9. SEM images showing the platelet adhesion on the square, rectangle patterned and flat surfaces.

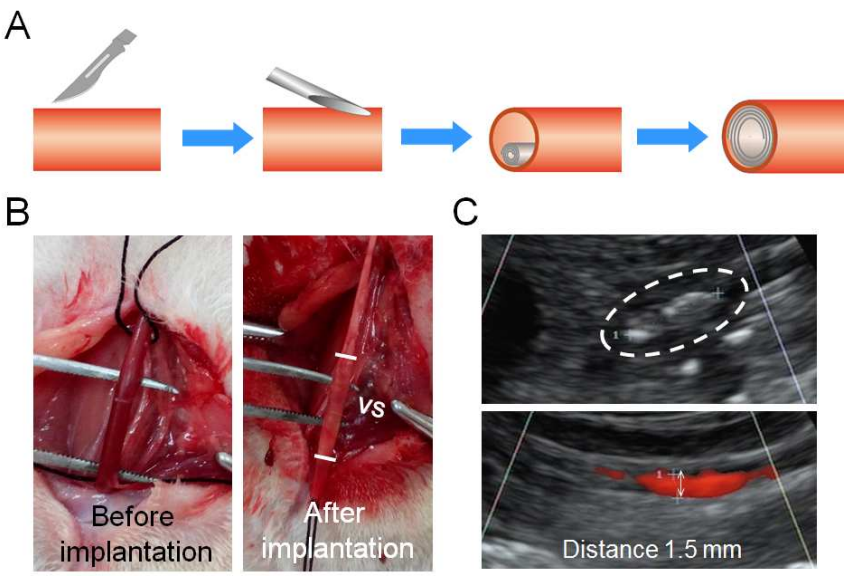

Figure S10. Implantation and patency of the multilayered shape memory scaffolds. (A) Schematic of *in vivo* implantation and shape recover of the scaffolds. (B) Digital photograph of cervical artery before and after implantation. (C) The patency of vascular graft was assessed by color Doppler ultrasound imaging.

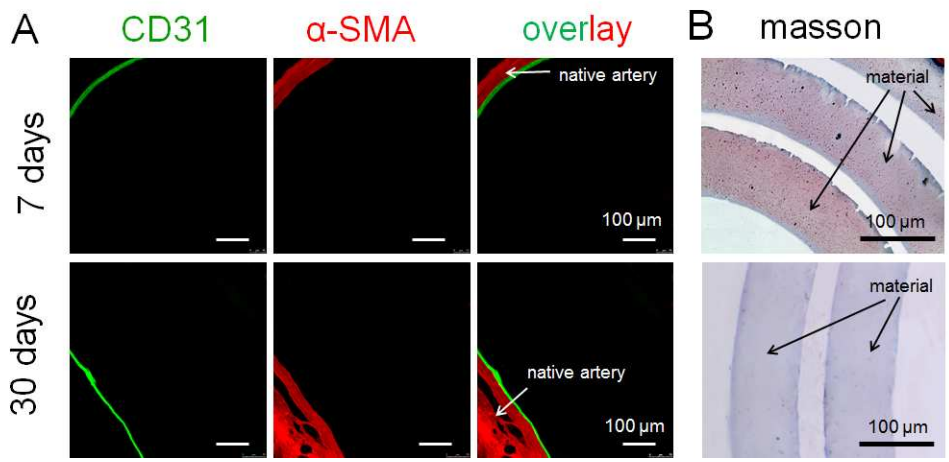

Figure S11. *In vivo* histological staining of the vascular grafts with flat surface. (A) Immunofluorescent staining for endothelial cells (CD31, green) and smooth muscle cells (α-SMA, red) after 7, 30 days implanted. (B) Masson's Trichrome staining of the grafts implanted at 7, 30 days.

References:

(1) Zhou, S.; Gong, T.; Zhao, K.; Wang, W. X.; Chen, H.; Wang, L. Thermally Activated Reversible Shape Switch of Polymer Particles. *J. Mater. Chem. B* **2014,** *2*, 6855-6866