Supporting Information

Bioinspired artificial small diametered vascular grafts with selective and rapid endothelialization based on amniotic membrane derived hydrogel

Xu Peng[a,b†], Xu Wang[a,c†], Can Cheng[a], Xiong Zhou[a], Zhipeng Gu[a], Li Li[d], Jun Liu[e] and Xixun Yu[a]*

a. College of Polymer Science and Engineering, Sichuan University, Chengdu 610065, China, E-mail: yuxixun@163.com

b. Laboratory animal center, Sichuan University, Chengdu 610065, China

c. College of Acupuncture and Massage College, Chengdu University of TCM, , Chengdu 610065, China

d. Department of Oncology, the 452 Hospital of Chinese PLA, Chengdu, Sichuan Province 610021, China

e. MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

† These authors contributed equally.

Pages: 8
Experimental method: 6
Tables: 1
Figures: 4

***Xixun Yu(Corresponding author)**
College of Polymer Science and Engineering, Sichuan University,
No.24 South Section 1, Yihuan Road, Chengdu , China, 610065, E-mail: yuxixun@163.com

Supplementary Experimental method

Decellularization process and ADA preparation

Briefly, fresh AM from new born rabbits were rapidly harvested and immersed into sterilized storage fluid at 4 °C for preservation (2 months at most). After a trypsin digested treatment at 37 °C for 4 h, the membranes were rinsed with PBS for several times. Then gentle scraping was put into effect to denude residual amniotic epithelial cells attached to AM. Light microscope and SEM were applied to appraise whether cellular components were completely removed. The AM (before & after decellular treatment) for scope experiments were fixed in 4% paraformaldehyde before observation.

Then, alginate dialdehyde (ADA) was employed as original crosslinking reagent for decellularized AM fixation. Followed by dissolving 5 g sodium alginate (Alg) in 250 mL ethanol-water (1:4, v/v) mixture, 5.7 g $NaIO_4$ was added and the selective oxidation was carried out at 37 °C in dark for 24 h. Subsequently, 10 ml ethylene glycol was applied as termination reagent for 2 h, and excess ethyl alcohol was added to precipitate ADA. To remove periodate, ADA was throughly purified using dialysis tube (MWCO, 3500) against distilled water, and obtained final product through lyophilizing dialysate.

Contact angle measurement

After 2×2 cm^2 membrane was glued to a glass slide, a drop of distilled water (5 μl) was added on their clean and dry surface and then evaluated the angle. In process, the measurement was performed on 5 randomly selected spots to get an average value for each analyzed membrane (n = 3), and the contact angle should be measured after 10s.

Thermal stability determination

Generally, when possessed a higher denaturation temperature (Td) and a stronger tensile strength, the materials tend to get a higher crosslinking degree and better crosslinking stability. In order to investigate whether the ADA fixation can form a stable crosslinked structure, or whether subsequent REDV grafting would destroy its stability, the Td and mechanical strength of various modified AM were measured as method in supplementary information. AM samples crosslinked by GA, a widely used crosslinking reagent in clinical application, were employed as control. As reported by Miles et al., the denaturation temperature (T_d) was determined using differential scanning calorimetry (DSC, DSC-200PC, Netzsch, Germany) to evaluate their thermal stability. Briefly, subsequent to various samples being weighted, they were

heated at a speed of 5 °C/min from 20 °C to 250 °C in respective aluminum pans and the temperature at endothermic peak was recorded as T_d.

Biomechanical strength test

After being cut into strips of 40 mm × 4 mm in size, the tensile strength of fresh, ADA-AM and further ADA/REDV-AM (n=6) were measured on an Instron material testing machine (Instron Co., USA) with an extension rate of 10 mm/min. In stretching process, their first decrease in load caused by the samples' fracture was recorded as the ultimate tensile stress. Then the E-modulus was calculated from the slope of stress-strain curve.

Description for cell culture process

Human umbilical vein endothelial cells (HUVECs, West China Hospital of Sichuan University) and human aortic smooth muscle cells (HASMCs, Jennio Biotech Co. Ltd, Guangzhou) were employed in these processes. HUVECs were cultured using Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, BI, Israel), 100U/ml penicillin and 100U/ml streptomycin (Sigma, USA) within a humidified atmosphere (37 ^{o}C, 5% CO_2 in 95% air). Meanwhile, HASMCs were cultured under similar situation, except using Minimum eagle medium (MEM) supplemented with 15% FBS. The culture medium was replaced every two days and cells from passage 3 were employed for further cytocompatibility measurement.

Description for operative process

(1) Subcutaneous implantation: Subsequent to isoflurane inhalation anesthesia, the rabbits were tightly bound and then shaved dorsal skin. On the back of both sides, a parallel lengthwise incision was performed symmetric to midline without rupturing peripheral microvascular, then the prepared samples were gently implanted. At predetermined time point (2w, 4w and 7w after operation), rabbits were sacrificed under anesthesia and their skin along with internally wrapped AM were harvested to evaluate in vivo tissue response reaction.

(2) Intramuscular implantation: After back muscles suffered from a blunt dissection, various AM tissues were implanted into corresponding intermuscular interlayer and sewed them up immediately. The rest steps were similar to subcutaneous implantation.

(3) Mesenteric implantation: Followed by shaving abdominal skin and iodophor disinfection, a 2-cm parallel incision was made at the backward of rabbit abdomen.

Once further cut through next muscle layer, the cavum abdominis was exposed and inner intestines were gently pulled out. Tear off the semitransparent membrane on mesenteric surface, then above modified AM was implanted inside and smoothly stitched using 5-0 nylon suture.

Table. S1 T_d (°C) of various AM after crosslinking or peptide-grafting

Samples	Fresh AM	ADA-AM	ADA/REDV-AM	GA-AM
T_d (°C)	79.6 ± 3.7	90.2 ± 1.7	109.1 ± 3.9	87.8 ± 3.5

To evaluate whether ADA fixation could form stable crosslinking structure or whether the further REDV modification would change their crosslinking stability, the Td and mechanical strength of various AM were measured. As presented in Table. S2, compared with fresh AM, the T_d values of all crosslinked samples were significantly increased. It was only 79.6 ± 3.7 °C of fresh AM, while that of ADA-AM reached 90.2 ± 1.7 °C, closing to 87.8 ± 3.5 °C of GA-AM. Meanwhile, T_d of ADA/REDV-AM was up to 109.1 ± 3.9 °C, which implied a significant increase of T_d after REDV grafting. Their increased thermal stability indicated that a stable effective crosslinking structure was formed between ADA and AM, moreover, subsequent REDV grafting did not destroy their stability, but rather enhanced them. As measured, T_d peak appeared in ADA/REDV-AM increased to 109.1 ± 3.9 °C. This phenomenon may be explained as below. Besides activated -COOH on ADA to accelerate their reactions with NH_2-terminated REDV peptide, EDC/NHS could also catalysis -NH2 and -COOH on original AM to generate double cross-linked network. The result of synchronous fixation is superior to that caused by ADA alone, thus ADA/REDV-AM obtained better thermal stability.

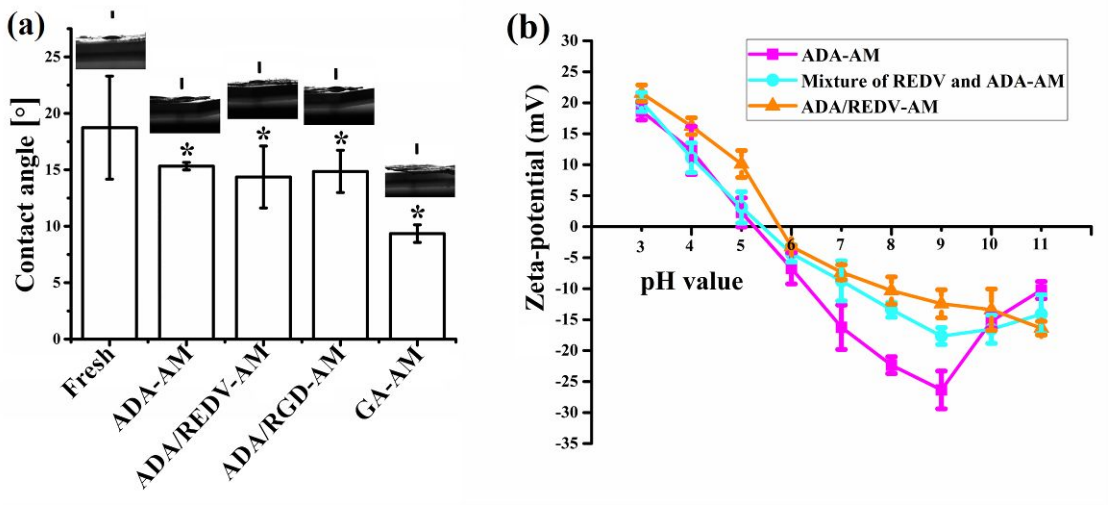

Figure.S1 CA value and zeta potential measurement

Generally, hydrophilic surface often implies better hemocompatibility through minimizing protein adsorption, inhibiting platelets activation and restrained their adhesion. It displayed CA values of various samples to evaluate their surface hydrophilicity. They slightly reduced from 18.53 ± 4.57° to 15.33 ± 0.33° and 14.52 ± 2.67° successively, indicating AM still maintained excellent surface hydrophilicity after ADA-crosslinking and REDV-grafting. Their inapparent changes in CA value also indicated success crosslinking and modification in each step.

ADA/REDV-AM have a comparable zeta potential (-6.5mV, figure S1) to fresh-AM at pH $\approx$ 7.2, implying the same charge distribution on their surface. The reconstructed framework and porous micro-architecture of AM would give a prominent influence on cells adhesion and proliferation. It may also function well in supplying physical supporting and inhibiting calcification because the damage of elastic fibers.

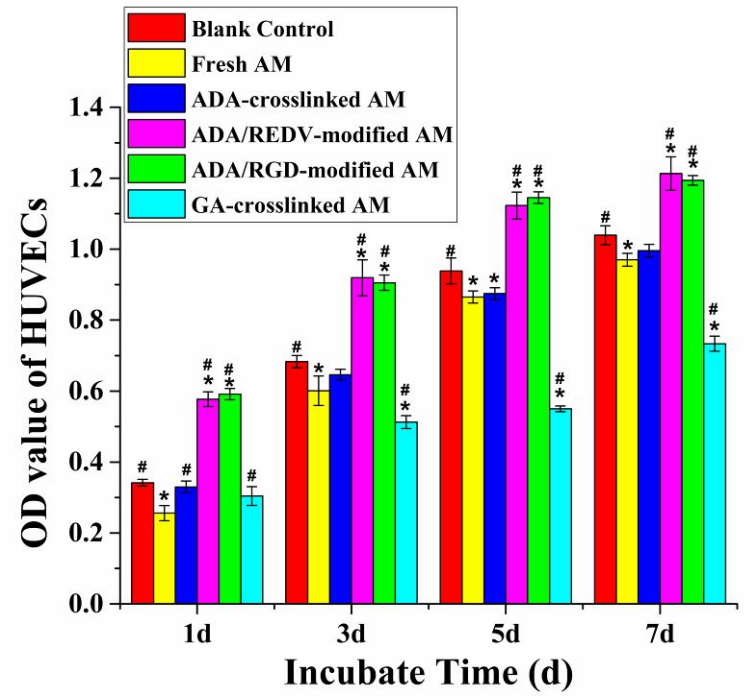

Figure.S2 HUVECs seeded on various AM tissues and their proliferation curve

HUVECs were seeded on various AM tissues and their proliferation curve was exhibited in Fig. S3. The CCK-8 assay results demonstrated that ADA-AM had no obvious cytotoxicity, and further REDV peptide-grafting effectively promote its biocompatibility. The proliferation rate of HUVECs co-cultured with ADA/REDV-AM is faster than any other samples.

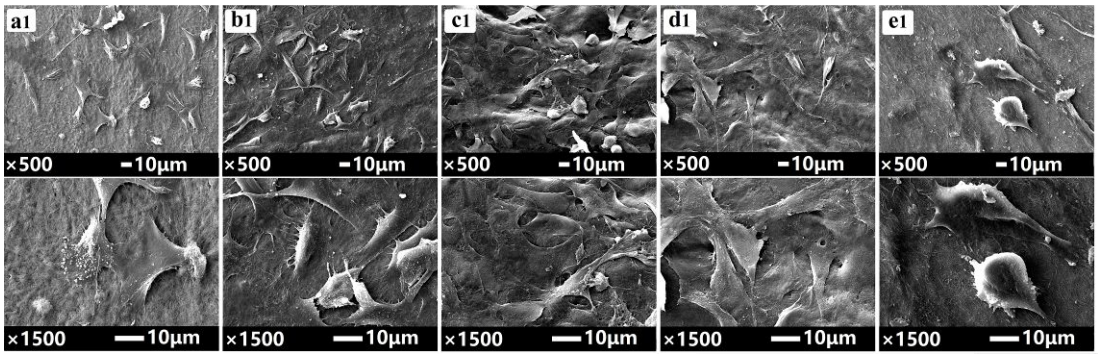

Figure.S3 SEM observation of HUVECs on various AM tissues
a1:Fresh-AM, b1:ADA-AM, c1:ADA/REDV-AM, d1:ADA/RGD-AM, e1: GA-AM

SEM images were obtained at higher magnification and lower cell density areas of various AM. Superior to others, the HUVECs on ADA/REDV-AM showed well spreading and tended to form a monolayer to cover surface. c) After 4d incubation,

the NO and PDGF secretion amount from HUVECs seeded on various treated AM (* means significant difference compared to fresh-AM).

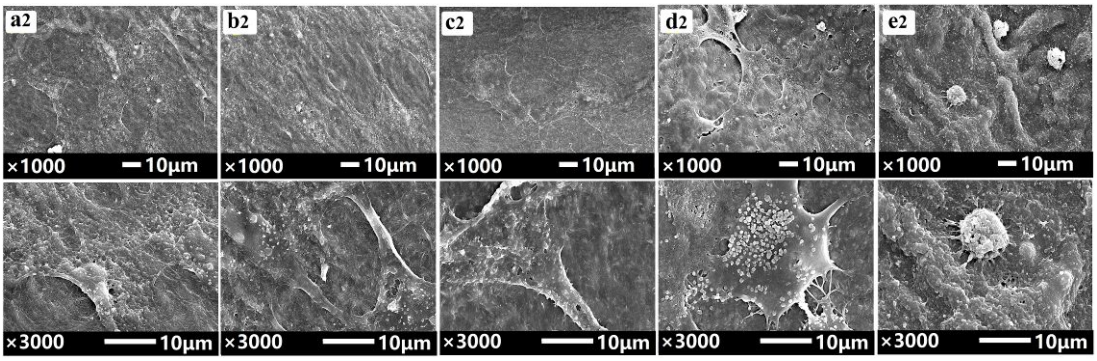

Figure.S4 SEM observation of HASMCs on various AM tissues
a2:Fresh-AM, b2:ADA-AM, c2:ADA/REDV-AM, d2:ADA/RGD-AM, e2: GA-AM

Most of HASMCs were shrank or even distorted on ADA/REDV-AM, exhibiting non-adhesive property. In contrast, HASMCs on ADA/RGD-AM formed a large area of attachment with their projected filopodium. A great deal of cell secretions (white spots) were also observed, suggesting high bioactivity of spread cells. Therefore, superior to undifferentiated promotion exhibited by ADA/RGD-AM, ADA/REDV-AM could selectively promote the adsorption and proliferation of ECs while impede the adhesion of SMCs.