




A Novel Method to Improve the Physical Property and Biocompatibility of Decellularized Heart Valve Scaffold with Sericin and Polydopamine

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Abstract

Cardiac valve replacement is an effective method to treat valvular heart disease. Artificial valves used routinely in clinic still have defects. In our study, we explored a novel method to modify the performance of Decellularized Heart Valve (DHV) scaffold. The decellularized porcine aortic valve was prepared using sequential hydrophile and lipophile solubilization method. The sericin was extracted from silk fibroin-deficient silkworm cocoon by lithium bromide method. First, DHV was immersed in sericin solution to produce the sericin–DHV composite scaffold. Then, we modified the DHV by making a Polydopamine (PDA) coating on the DHV first and then binding the sericin. The physical properties and biological compatibility of our composite scaffold were assessed *in vitro* and *in vivo*. Sericin were successfully prepared, combined to DHV and improved its biocompatibility. PDA coating further promoted the combination of sericin on DHV and improved the physical properties of scaffolds. The decay rate of our modified valve scaffold was decreased *in vivo* and it showed good compatibility with blood. In conclusion, our modification improved the physical properties and biocompatibility of the valve scaffold. The combination of PDA and sericin promoted the recellularization of decellularized valves, showing great potential to be a novel artificial valve.

Keywords Decellularized heart valve · Sericin · Polydopamine · Biocompatibility

1 Introduction

Valvular heart disease is one of the diseases that seriously threaten human health. Cardiac valve replacement has been widely used in clinical practice for many years and it becomes an important method for the treatment of heart valve disease [1]. Currently, the artificial valves, including mechanical valves and biological valves, that are routinely used in clinic still have defects [2]. Although mechanical heart valves have good durability and can be used for more than 30 years, the risk of bleeding and thrombosis is still high [3]. Patients treated with mechanical valve replacement

require life-long anticoagulant therapy. Biological valves have lower risk of thrombosis, but they are prone to decay with time going by [2, 4]. Recently, many studies focus on tissue engineered heart valves to make implanted valves achieve self-growth and damage repair in the body [5].

Decellularized scaffold has great potential to be used in tissue engineering [6]. Through various physical and chemical methods, the original cell components of allogeneic or heterogeneous heart valve are removed, and the Extracellular Matrix (ECM) remains intact [7]. DHV retains a relatively complete natural structure and has good affinity for cells to seed on scaffold. Some attempts to implant allogeneic DHV into human have achieved good therapeutic effect; however, heterogeneous DHV have experienced severe rejection after implantation [8, 9]. This may be caused by the insufficient of recellularization of valve scaffold [10]. The recellularization of DHV is essential for the valve to achieve tissue regeneration, damage repair, anti-decay, and physiological function [11, 12]. During the process of decellularization, the damage of ECM is unavoidable. Many studies have tried to modify DHV to improve its biocompatibility by combining

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cytokines, antibodies, peptides, or high molecular polymer [13, 14].

Sericin is a natural protein, which can be extracted from silkworm cocoons [15]. The source of sericin is abundant and it has arisen attention to be applied in the field of biomedical engineering [16]. The amino acids of sericin have many high reactive groups on their side chains, which provide binding sites for cross-linking and polymerization. Studies also have found that sericin has anti-oxidation, promoting healing, and antibacterial activities, showing good biocompatibility [17]. Sericin has been used in clinical medicine, such as cartilage damage repair, treatment of myocardial infarction and neuron regeneration, but its application in the modification of DHV needs to be further investigated [18–20].

PDA has been widely used as a coating material [21]. The main advantage of PDA is that it can attach to almost all material surfaces, including metals, oxides, ceramics, and even anti-adhesion polytetrafluoroethylene, etc. [22]. In addition, PDA also has good biocompatibility and is widely used in the preparation and modification of biomedical materials [23]. Considering this, PDA has great potential to improve the performance of DHV.

In our study, we prepared sericin combined DHV and explored the effects of sericin on the structure and biological compatibility of valve scaffold. Furthermore, we tried to use PDA to increase the combination of sericin and DHV and we performed *in vivo* experiments to assess the biocompatibility of our modified DHV. Results showed that the physical property and biocompatibility of DHV were improved with sericin and PDA modification.

2 Materials and Methods

2.1 Preparation of DHV

Hearts of adult pigs (weight ranging from 120 to 140 kg) were purchased from local abattoir. The hearts were immersed in cold isotonic saline containing heparin (100 U/ml) and transferred to the laboratory. Aortic valve leaflets were dissected and rinsed with cold isotonic saline containing heparin until the blood on surface was removed completely. Then, the valve leaflets were stored in DMEM (Hyclone) medium containing 100 U/ml penicillin (Gibco) and 100 mg/ml streptomycin (Gibco) at 4°C.

The decellularized porcine aortic valve was prepared according to the Sequential Hydrophile and Lipophile Solubilization (SHLS) method as previously described with modifications [7]. Briefly, transfer the valve leaflets to sterile six-well plates and all steps were performed under aseptic condition. All reagents were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) unless otherwise indicated.

Each well containing three valve leaflets was filled with 4 ml working solution for decellularization. First, leaflets were decellularized with TRIS-HCL buffer (40mM, PH7.8) containing 2 mM Tributylphosphine (TnBP) and 2% (w/v) CHAPS for 24 h at room temperature with shaking. Second, leaflets were treated with TRIS-HCL buffer (40mM, PH7.8) containing 2mM TnBP, 2% (w/v) 3-[(3-Cholamidopropyl) Dimethylammonio]-Propanesulfonate (CHAPS), 1% (w/v) Amidosulfobetaine 14 (ASB-14) and 2% (w/v) Sulfobetaine 3–10 (SB 3–10) for 24 h at room temperature with shaking. Third, wash leaflets with sterile PBS 4 times for 24 h at room temperature with shaking, replace PBS every 6 h. Fourth, leaflets were treated with nuclease solution containing 100 U/ml Benzonase® nuclease for 24 h at 37°C with shaking. And then, wash leaflets with PBS 4 times for 24 h to remove the reagents. Finally, DHV were stored in PBS containing 100 U/ml penicillin (Gibco) and 100 mg/ml streptomycin (Gibco) at 4°C.

2.2 Cell Preparation

Human Umbilical Vein Endothelial Cells (HUVEC) (ECV304) were purchased from China center for type culture collection. Valvular Interstitial Cells (VIC) were isolated from porcine aortic valve. The valve leaflets were digest with DMEM medium containing 0.2% collagenase I at 37°C for 12 h. Then, centrifuge at 1000 rpm for 5 min and remove supernatant. Resuspend cells with DMEM medium containing 10% fetal calf serum and plant cells into T25 flask. Change medium 24 h later and remove suspended cells. Passage cells for 2–5 times and VIC could be used for experiments. All steps were performed under aseptic condition.

2.3 DHV Treated with PDA

Transfer the DHV to sterile six-well plates and each well contains three valve leaflets. The leaflets were incubated with 4 ml 10 mM Tris-HCL solution containing 1mg/ml or 2 mg/ml PDA at room temperature for 12 h. Then, wash leaflet with PBS for 3 times. The leaflets were stored in PBS at 4°C before use.

2.4 Extraction of Sericin Protein and the Combination of Sericin to Decellularized Leaflet

A silk fibroin-deficient mutant silkworm cocoon (140-Nds) was obtained from the Sericultural Research Institute (China Academy of Agricultural Sciences, Zhenjiang, Jiangsu, China). The sericin was isolated as previously described with modifications [24]. Briefly, cut cocoon pieces (1.0 g) were immersed in 55ml 6 mM Lithium Bromide (LiBr)

solution at 35°C for 24 h. The residue was removed by centrifugation and filtration. Add ¼ (v/v) 1 M Tris-HCL buffer (PH9.0) to the supernatant. Then, this solution was added to cellulose dialysis membranes (MWCO 3500 Da) and dialyzed against water (1L ddH₂O and 1ml Tris-HCL buffer) for 2 h at room temperature. Transfer the solution to tubes and centrifuge at 3500 r/min for 5 min. Collect the supernatant and repeat dialyze step for 24 h. Replace water every 2 h. Concentrate the obtained supernatant using polyethylene glycol (PEG-6000) solution. The sericin solution was stored at 4°C before use.

Transfer the decellularized valve leaflets (PDA-coated DHV or pure DHV) to sterile six-well plates and each well contains three valve leaflets. The leaflets were incubated with 1 ml sericin solution at 37°C for 1 h. Then, remove sericin solution and wash leaflet with PBS for 3 times. The decellularized leaflets combined with sericin were stored at 4°C before use.

2.5 Detection of Combined Sericin on Decellularized Leaflets

When incubate the leaflet in sericin solution, detect the concentration of sericin protein in solution at 0, 1 and 2 h using Bicinchoninic Acid (BCA) method according to manufacturers' instruction. The photoluminescent image of decellularized leaflet was taken using In-Vivo FX Pro imaging system (Bruker, USA). Place leaflet on observation plate. With excitation light at 430 nm, detect the emission light of leaflet at 535 nm and record the image.

2.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectra of leaflet scaffold combined with or without sericin were obtained using iS50 FTIR system (Nexus, Thermal Nicolet, USA) for the spectral ranging from 4000 to 100 cm⁻¹.

2.7 Assessment of Physical Properties of Valve Scaffolds

Fix leaflets with 2.5% glutaraldehyde for 24 h and wash with PBS three times for 15 min each. Then, dehydrate tissues by sequential immersion in 30, 50, 70, 90% ethanol for 15 min each. Immerse tissues in 100% ethanol for 15 min and repeat this step 3 times. Next, transfer the dehydrated leaflets to tertiary butanol for 15 min and repeat this step. Dry specimens in lyophilizer. Finally, dried specimens were sputtered with gold and observed with Scanning Electron Microscope (SEM) (EDAX, USA).

The structure of modified DHV was analyzed using atomic force microscopy (Solver Nano, NT-MDT)

according to manufacturers' instruction. Dried leaflets were cut into slices and were settled on microscope stage. The scan area was chosen as 10 × 10 μm². Select non-contact mode for detection. Calculate the root mean square standardized error (Rq). Generate surface topography image and record.

The mechanical tests were performed using a uniaxial material testing system (Instron Model 5848, Boston, Massachusetts, USA) as previously described.

2.8 Cell Proliferation and Viability Assay

Sericin solution was treated under high temperature (120°C) and high pressure for 30 min to obtain sericin degradation products. VIC were seeded on 96-well plates and cultured overnight. Then, replace medium with DMEM medium containing 0 μg/μl, 0.1 μg/μl and 0.5 μg/μl sericin degradation products. Cell counts were recorded after 0, 48 and 96 h. Cell viability was detected using CCK-8 kit (CA1210, Solarbio) according to manufacturers' instruction after 24 h and 96 h.

2.9 Cell Migration, Apoptosis, and Compatibility Assay

VIC were seeded on 6-well plates and cultured overnight. Then, replace medium with DMEM medium containing 0 μg/μl and 0.5 μg/μl sericin degradation products and incubate in 37°C, 5% CO₂ chamber overnight. To perform cell migration assay, a scratch was generated using a 10 μl pipette tip and wash cells with PBS. Add the same culture medium and culture cell in 37°C, 5% CO₂ chamber. Images were recorded after 0 and 36 h later. To perform cell apoptosis assay, add 600 ul H₂O₂ or DMEM medium to each well and total protein was extract from cells. Whole cell lysates were subjected to immunoblot assay using standard protocol.

HUVEC were seeded on valve leaflets and incubate in 37°C, 5% CO₂ chamber. At day 1 and day 4, the concentration of NO in supernatant was detected using nitric oxide assay kit (S0021, Beyotime) according to manufacturers' instruction. At day 19, wash with PBS and fix with 4% paraformaldehyde. Then, mount with DAPI and HUVEC on leaflet were observed under laser confocal fluorescence microscopy.

2.10 Laser Confocal Fluorescence Microscopy

Plant VIC on valve leaflet and incubate in 37°C, 5% CO₂ chamber for 24 h. Wash with PBS and fix with 4% paraformaldehyde at room temperature for 30 min. Cell membrane was stained with 10 μM DIO solution, or cell was treated with 0.1% Triton X-100 and then F-actin was stained with 5 μg/ml rhodamine phalloidin (PHDR1, Cytoskeleton Inc.).

Finally, mount with DAPI and VIC on leaflet were observed under laser confocal fluorescence microscopy.

2.11 Animal Studies

Sprague–Dawley (SD) rats at age of 8 weeks and New Zealand white rabbits at age of 3–4 months were purchased from laboratory animal center, Tongji Medical College of Huazhong University of Science and Technology. All animals were maintained in specific pathogen free facility. All animal experiments in this study were approved by Laboratory Animal Ethics Committee of Union Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology.

Embed valve subcutaneously into rat to study its degradation in vivo. Valve leaflets were cut along the middle line. Half of valve was stored in PBS at 4°C, and the other half of valve was embedded subcutaneously under abdominal skin. Embedded valves were excavated half a month later for further investigation.

Transplant valve into the rabbit carotid artery to study its compatibility with blood. The valve was sutured into a tubular shape and then valve tube was transplanted into the rabbit carotid artery. The rabbits were injected with antibiotics daily for 3-day post-operation to prevent infection. Add aspirin to diet to prevent the formation of thrombus.

2.12 Histologic Staining

Fix tissues in 4% paraformaldehyde for 24 h. The tissues were dehydrated and embedded in paraffin. Then, the paraffin-embedded blocks were cut into 8 µm slices. Haematoxylin and eosin (H&E), Masson trichrome and Verhoeff Van Gieson (EVG) staining were used for histological analysis of leaflet tissue.

2.13 Immunofluorescent Staining

VIC were digested and seeded on the coverslip in the well of 6-well plate. When the cells were adherent to coverslip, wash with PBS and fix with 4% paraformaldehyde at room temperature for 30 min. Wash with PBS for 10 min and repeat wash step 2 times. Next, immerse cells with 0.1% Triton X-100 for 5 min and wash with PBS 3 times. Then, block cell with 5% BSA at room temperature for 30 min and incubate with primary antibody at 4°C overnight. Rinse with PBS for 3 times and incubate with second antibody at room temperature for 30 min in the dark. Wash with PBS for 3 times and stain with DAPI at room temperature for 15 min in the dark. Finally, seal coverslip with glycerinum and record data using fluorescence microscope (Nikon, Japan).

2.14 Statistical Analysis

Student's *t* test was used for comparison between two groups and one-way ANOVA was used for the comparison of more than three groups. Data were expressed as mean ± SE. *P* value < 0.05 was considered to be statistically significant.

3 Results

3.1 The Physical Structure and Histological Characteristics of Sericin-Modified DHV

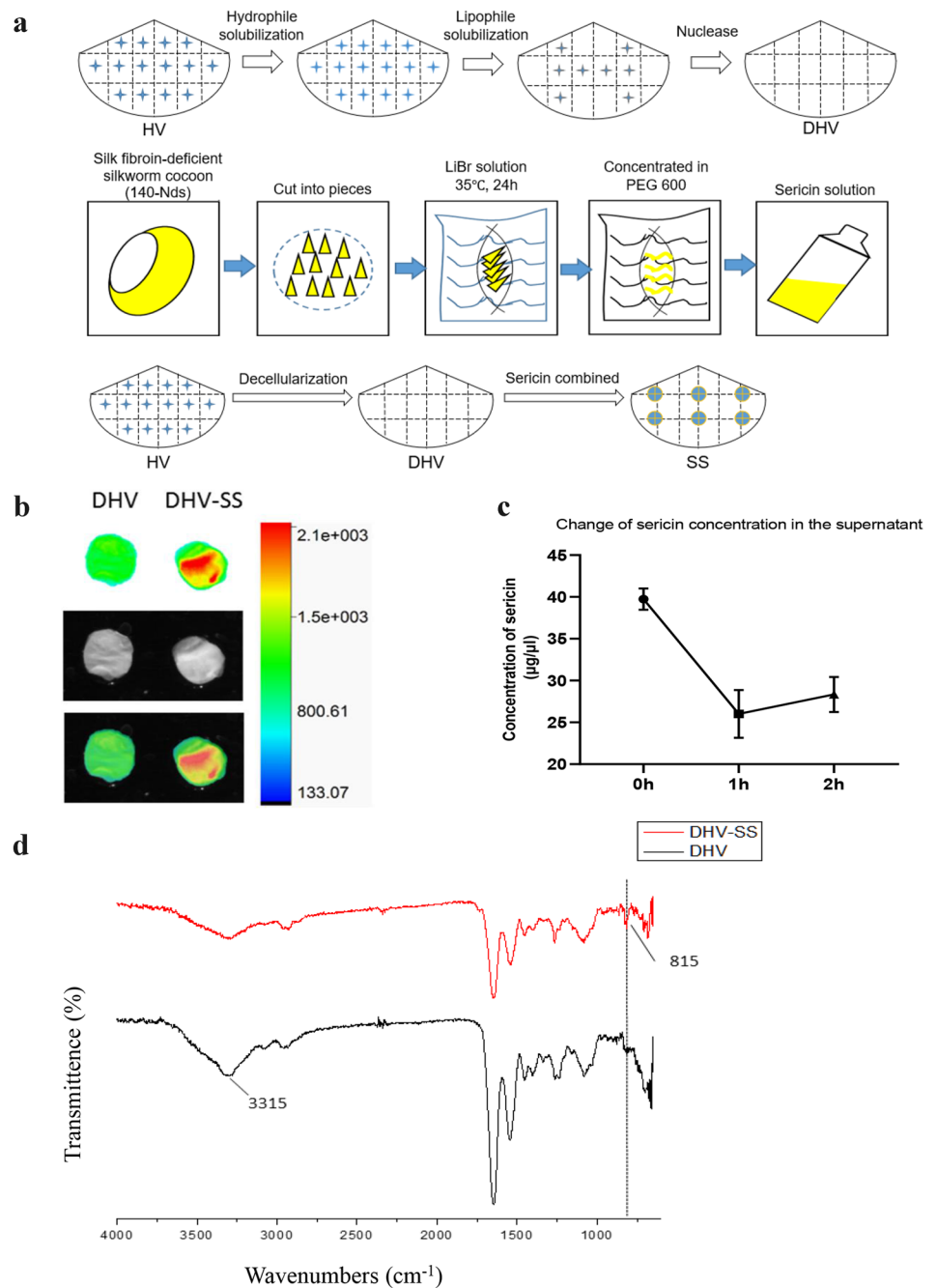
The diagram shows the workflow to prepare the sericin combined DHV (DHV-SS) (Fig. 1a). DHV and sericin solution were successfully prepared following our protocol. Then, we assessed the combination between DHV and sericin from multiple aspects. Sericin has the effect of autofluorescence, and we could detect stronger emission light from the DHV-SS at 535nm⁻¹ (Fig. 1b). After we immersed leaflets in sericin solution, we detected the remaining sericin concentration in the supernatant at different timepoints. The baseline, 1- and 2-h concentration were 39.74 ± 0.63 µg/µl, 26.84 ± 0.95µg/µl, and 28.34 ± 1.04µg/µl, respectively, showing the sericin combined to DHV rapidly within the first hour (Fig. 1c). FTIR image showed that there was a new peak at 815cm⁻¹ after the combination of sericin, while characteristic peak of collagen in DHV at 3315 cm⁻¹, 1645 cm⁻¹, and 1541 cm⁻¹ remained unchanged (Fig. 1d). These results indicated that sericin could bind to DHV successfully using our protocol.

After the preparation of DHV-SS, we further investigated its physical properties and histological characteristics. Sericin did not change the general view of DHV. We performed tissue staining and found that SHLS method could remove all cells completely, leaving intact original structure and ECM compositions. In addition, the binding of sericin had no significant influence on the histological structure of DHV (Fig. 2a). Then, we observed the microstructure of valve using SEM. After valve decellularization, the surface of DHV became smoother and its internal pore size was smaller. In addition, we found protein particles on the surface of DHV-SS, further confirming the combination of sericin on DHV (Fig. 2b). The diameters of the internal pores of the natural valves and DHV were 101.3 ± 7.450µm and 41.95 ± 2.767µm, respectively. After sericin modification, the cross section had no significant change compared with the DHV.

3.2 The Physical Structure and Histological Characteristics of PDA-Modified DHV-SS

PDA has great potential to be used as a biomedical material. Next, we used PDA to further improve the physicochemical

Fig. 1 Preparation of sericin-DHV scaffolds. **a** Diagram represents the protocol of how to prepare sericin-DHV scaffold. **b** Intensity of autofluorescence from DHV or DHV-SS. **c** Change of sericin concentration in the supernatant after the immersion of DHV ($n = 4$; *NS*: not significant; $***P < 0.001$; Student's *t* test). **d** Infrared spectrum detection of the protein peak of DHV or DHV-SS



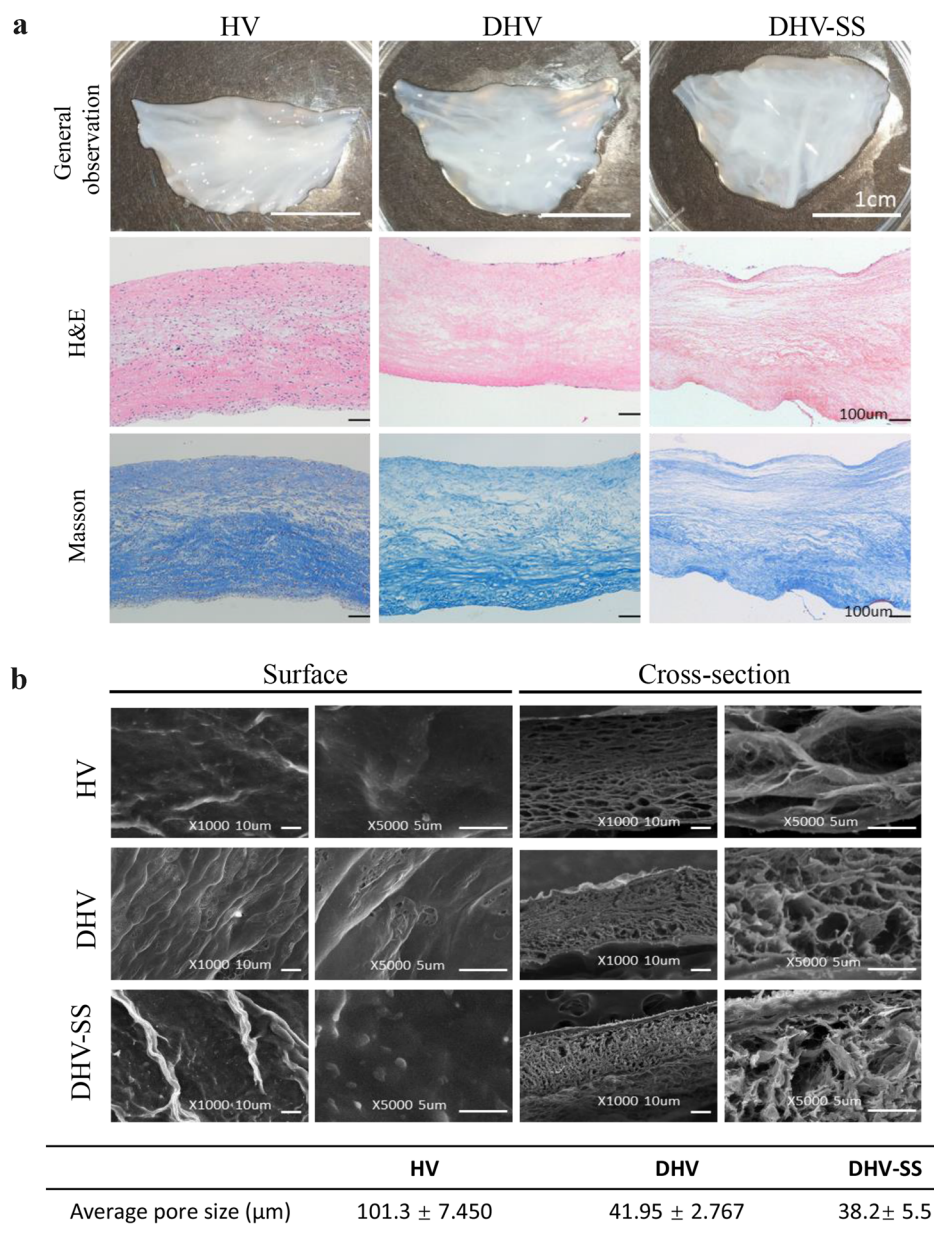
property of valve scaffold. The diagram shows the workflow to further modify DHV-SS with PDA (Fig. 3a). PDA with super adhesive properties formed a layer of nanometer-sized black particles on the surface of the valve. The color of DHV combined with PDA appeared black brown and it became deeper with the increase of PDA concentration (Fig. 3b). We observed the microstructure of PDA-modified DHV using SEM and AFM. The surface of PDA-modified DHV had many particles at nano scale and the surface became rougher with the increase of PDA concentration (Fig. 3b). Furthermore, histological analysis showed

that PDA modification had no obvious effect on the internal fiber structure of the valve (Fig. 3c).

3.3 Sericin Degradation Products Have Good Biocompatibility with VIC

The primary porcine VIC was successfully isolated and cultured. The cells were spindle-shaped under light microscope. Immunofluorescence staining showed positive expression of Smooth Muscle Actin (α -SMA) and vimentin (Fig. 4a). When we cultured VIC with sericin

Fig. 2 Influence of sericin on DHV structure. **a** General view and histological analysis of heart valve scaffold. **b** Structure of heart valve scaffold observed by scanning electron microscope. Data is shown as mean \pm SE (25 random pores per sample)

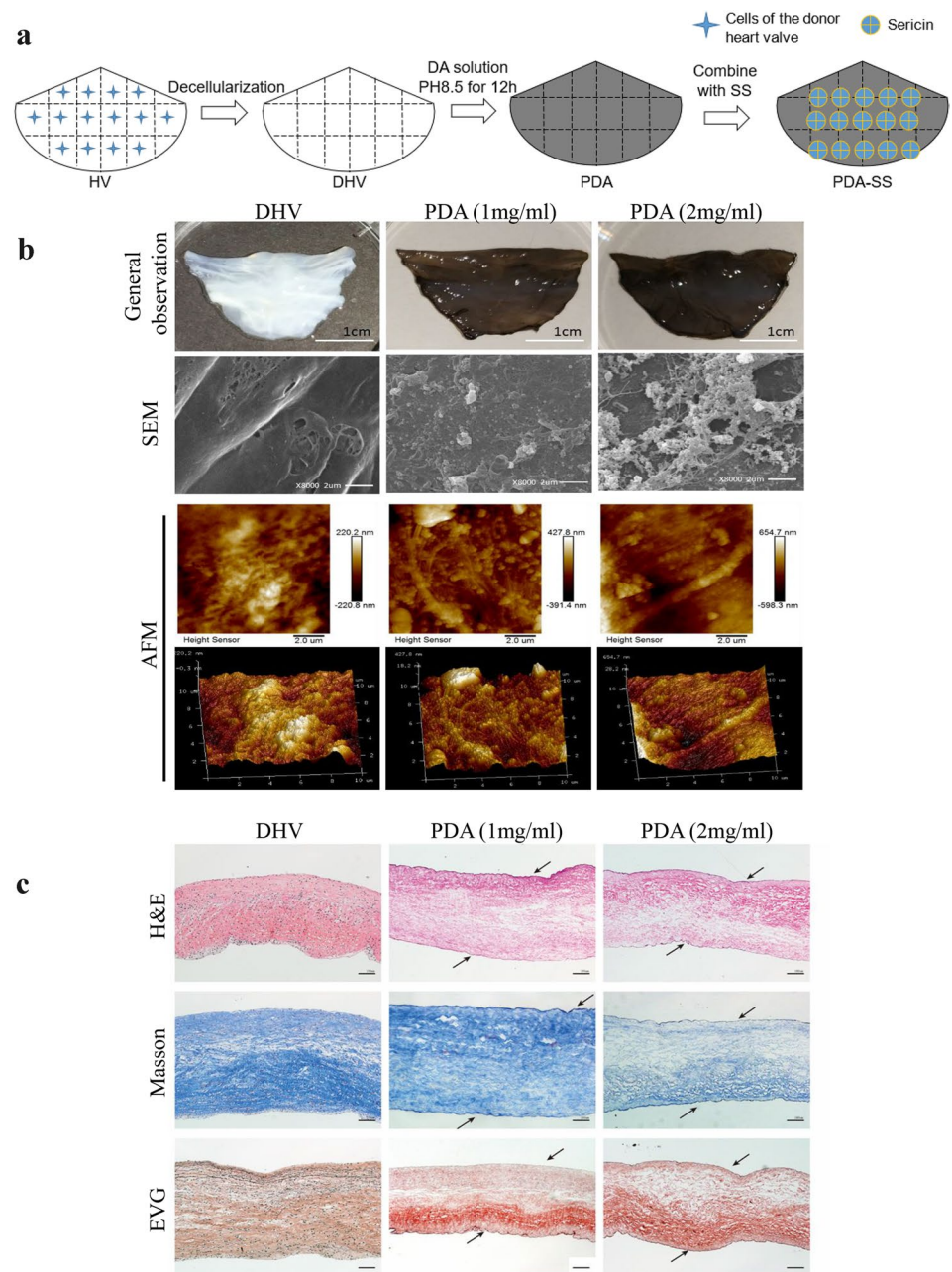


degradation products, the migration distance of VIC was about 1.3 times longer than that in control group (Fig. 4b). The viability of VIC cells was also significantly improved with the treatment of sericin degradation products (Fig. 4c). We counted the cell number at 48th and 96th hour and result showed that sericin promoted the proliferation of VIC (Fig. 4d). When we cultured VIC under hypoxia condition and treated with H_2O_2 , we detected the expression level of apoptosis marker PDCD4 in VIC. The relative expression level of PDCD4 was significantly lower in sericin treated group (Fig. 4e). These data suggested that sericin degradation products could promote VIC migration, proliferation, viability and inhibit VIC apoptosis.

3.4 VIC Expands Better on Sericin-Modified DHV

Then we investigated the adherence and proliferation of VIC on sericin-modified DHV. We planted VIC on valve leaflet and cultured for 24 h. We stained cell membrane with DIO. The results showed that DHV-SS had more adherent VIC and VIC spread better on sericin-modified leaflets (Fig. 5a). Cell skeleton was stained with phalloidin, and we observed more VIC on DHV-SS at day 1 and day 4 after plantation. Cell expanded well with spindle shape on DHV-SS (Fig. 5b). In conclusion, adhesion, proliferation, and viability of VIC on DHV-SS scaffold were improved.

Fig. 3 Morphological characteristics of PDA-modified DHV-SS scaffold. **a** Diagram shows the protocol of how to further modify DHV-SS with PDA. **b** Observe and analyze the structure of PDA-modified DHV-SS scaffold. **c** Histological characteristics of heart valve scaffold modified with PDA



3.5 PDA Further Improves the Performance of Sericin Combined DHV

We planted HUVEC on PDA-modified DHV to study the influence of PDA on biocompatibility of DHV. Cells could adhere to valve leaflet and grow normally (Fig. 6a). There were no significant differences of NO secretion from HUVEC at day 1 and day 4 after plantation, indicating PDA-modified DHV had good biocompatibility (Fig. 6b). Then, we investigated the effect of PDA on physicochemical properties of DHV. We detected the water contact angle on leaflet, PDA could make the static contact angle of the valve

smaller, which means increasing the hydrophilicity of the valve (Fig. 6c). When sericin was combined, a new peak at 815cm⁻¹ could be detected with FTIR, and the characteristic peak of the valve itself did not change significantly (Fig. 6d). Sericin concentration in the supernatant decreased faster in the PDA-modified valves than DHV group, indicating PDA increased the binding capacity of DHV (Fig. 6e). In addition, we performed uniaxial tensile test on valve scaffold. Sericin modification didn't change mechanical property of DHV, but PDA modification could improve the ultimate tensile stress of scaffold from 4.7 MPa to 9.7 MPa (Fig. 6f). Above all, PDA further improved the performance of valve scaffold.

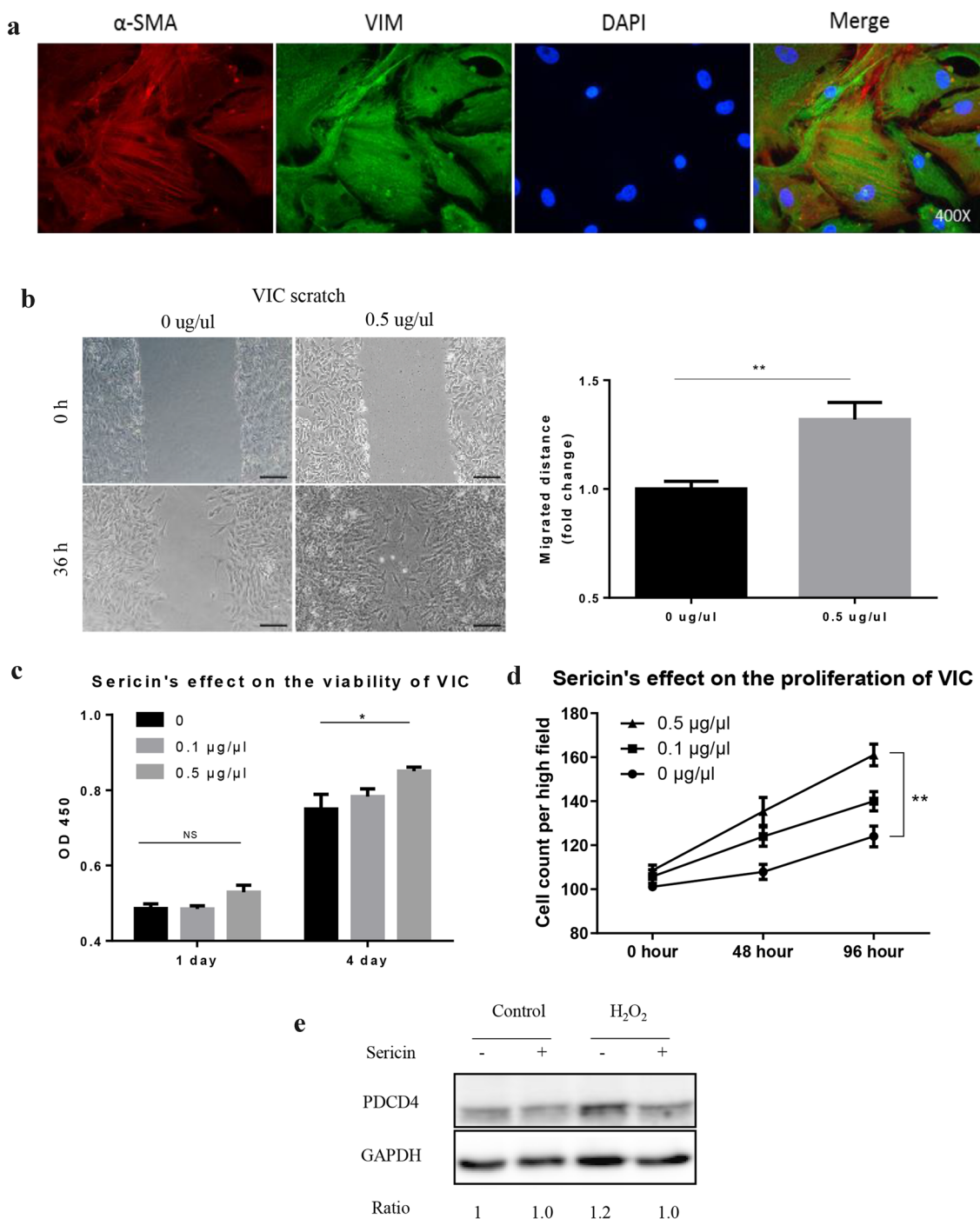


Fig. 4 Influence of sericin-DHV scaffolds on VIC. **a** Immunofluorescence staining shows the positive expression of α -SMA and VIM in VIC. **b** Migration of VIC with or without sericin degradation products ($n = 4$, $**P < 0.01$, Student's t test). **c** Effect of sericin on VIC

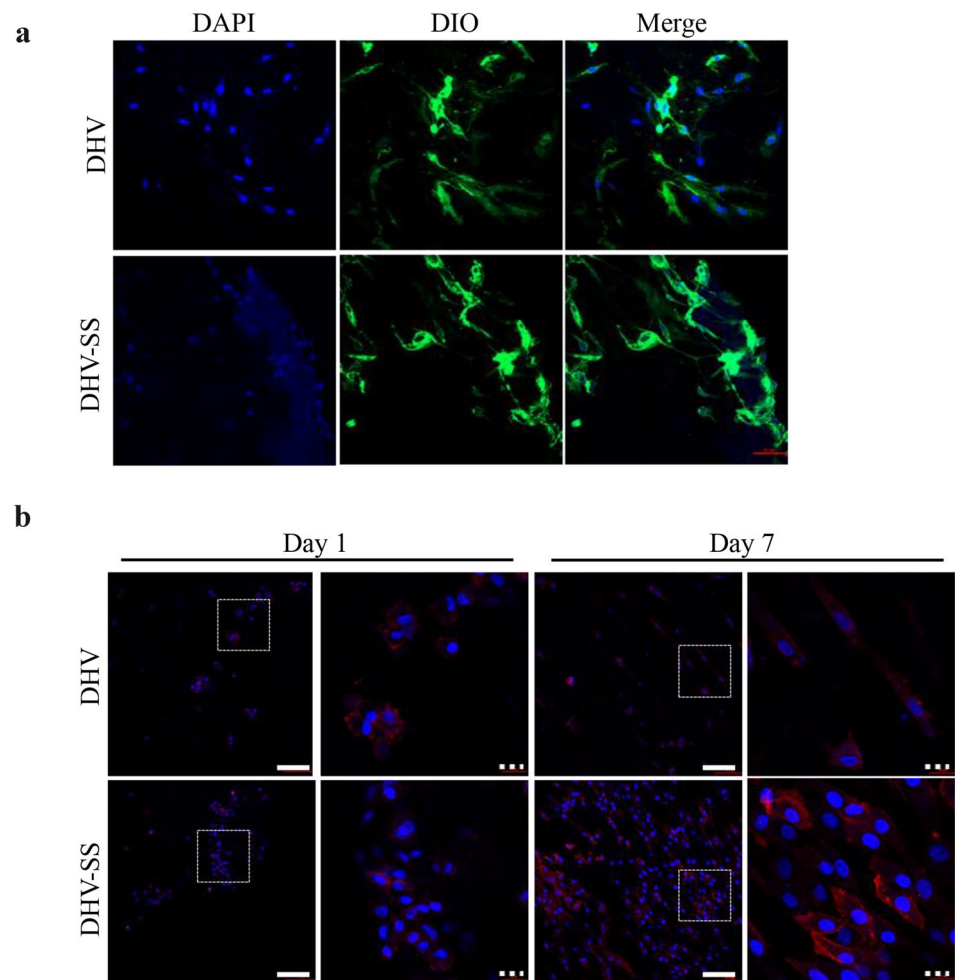
viability via the detection of CCK8 ($n=5$; NS: not significant; $*P < 0.05$; Student's t test). **d** Proliferation of VIC with different concentration of sericin ($n = 15$, $**P < 0.01$, Student's t test). **e** Immunoblot detection of PDCD4 from VIC with the stimulation of H₂O₂

3.6 Sericin and PDA-Modified DHV Has Good Biological Compatibility In Vivo

We used two kinds of animal model to assess the biological compatibility of PDA-modified DHV-SS in vivo. First, we

embedded the valve leaflet subcutaneously and the valves were excavated half a month later. Compared with valves in vitro, the whole structure of the PDA and PDA-SS groups was relatively intact, while the remaining two groups were significantly degraded. H&E staining of tissue sections

Fig. 5 Growth of VIC on sericin-DHV scaffolds. **a** Plant VIC on DHV or DHV-SS scaffold and observe the adherence and the morphological characteristics of cell on valve scaffold 24 h later. **b** At day 1 and day 7 after planting VIC on scaffold, phalloidin staining shows the number and morphology of VIC



showed that all groups had different degrees of cellular infiltration, in which the SS group had less cell infiltration than the DHV group. CD68 staining showed that the infiltrating cells were mainly macrophages. Masson staining showed valve collagen fibers in the PDA and PDA-SS groups were kept more complete (Fig. 7a). Second, after the valve was sutured into a tubular shape, we transplanted the valve into the rabbit carotid artery (Fig. 7b). Ultrasound results showed that the PDA-SS composite stent remained unobstructed 1 month after transplantation, indicating PDA-SS-modified DHV had good compatibility with blood (Fig. 7c).

4 Discussion

Decellularization methods may cause damage to the structure of the valve, and the ideal decellularization method aims to remove cells completely and reduce the negative effects caused by reagents [25]. In our study, we adopted a SHLS method which was reported by our group as a modified decellularization method [7]. Compared with traditional method using chemical reagents to remove cells on valve,

our protocol could keep the physical property of leaflet and clear the heterogenous antigen that cause immune rejection. Histological analysis showed that our method removed cell components completely and didn't change the collagen fiber skeleton of valve scaffold.

The common method to obtain sericin from silkworm cocoon includes high temperature hydrolysis, acid, alkali, and organic solvent methods [15, 26]. These traditional methods have some limitations, such as low output, affecting sericin structure and biological characteristics. In our research, the LiBr extraction method was used to dissolve sericin at a relatively low temperature [24, 27]. In addition, the sericin was obtained from silk fibroin deletion cocoons, reducing the dissolution of sericin in LiBr solution. This method can keep the structure and biological activity of sericin for the following combination to DHV.

Sericin is a biological material with the effect of autofluorescence, which may be related to its composition including tryptophan, phenylalanine, and other aromatic amino acids [28]. Under the activation of excitation light in the wavelength ranging from 280 to 300 nm, the emission spectrum of sericin is in the range of 280–300 nm and 340–600 nm.

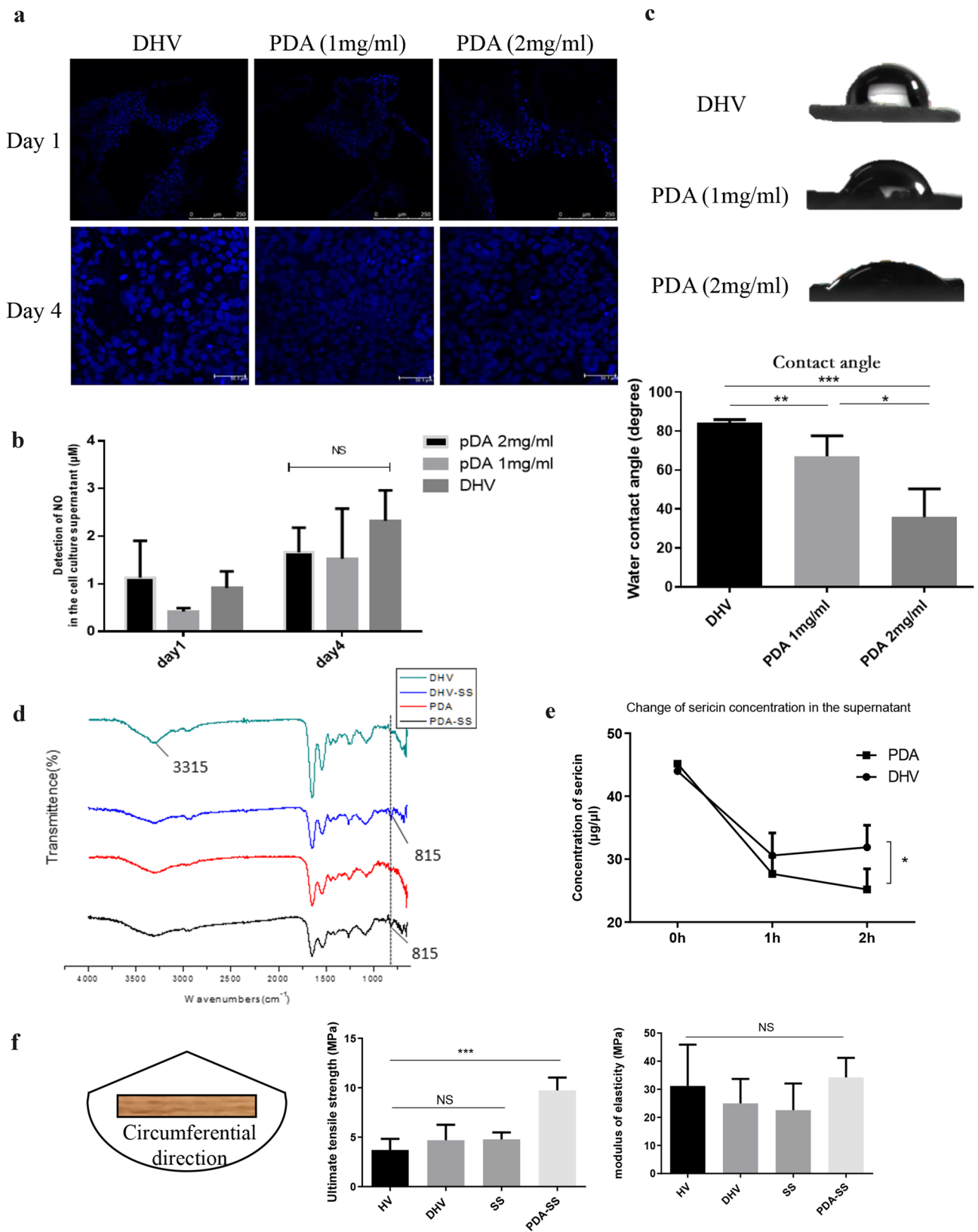


Fig. 6 PDA further modifies DHV-SS scaffold. **a** Plant HUVEC on valve surface, the observation of cell at day 1 and day 4 with or without the treatment of PDA. **b** Detect the NO secretion by HUVEC at day 1 and day 4 ($n = 4$; NS, not significant; one-way ANOVA). **c** DHV-SS treated with different concentration of PDA and observe the water contact angle on scaffold surface ($n = 4$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, one-way ANOVA). **d** Compare the protein peak of valve scaffold with or without PDA modification by infrared spectrum. **e** Change of sericin concentration in the supernatant after the immersion of DHV with or without the treatment of PDA ($n = 4$, $*P < 0.05$, Student's t test). **f** Compare the mechanical properties of valve scaffold with different modification ($n = 4$; NS: not significant; $***P < 0.001$; one-way ANOVA)

We detected the emission light signal from sericin-modified DHV and the concentration of sericin in solution decreased rapidly within first hour of incubation. In addition, FTIR image showed that the characteristic peak of collagen didn't change and there was a new protein peak at 825 cm^{-1} after the modification of sericin. These results confirmed that sericin could bind to DHV successfully using our protocol.

Dopamine can be polymerized into PDA in weak alkaline solution and the size of the formed nanoparticles can be deposited on the surface of organic or inorganic materials forming an adhesive coating [29, 30]. The coating can also react with other molecules to further change the size and properties of material [31]. We further modified DHV with PDA and observed its structure using SEM and AFM. The surface of DHV was smooth, after PDA modification, a layer of relatively uniform nanoparticles polymer could be formed on the surface of DHV. These nanoparticles increased the roughness of DHV and provided more sites for cell adherence. Histological analysis showed that PDA was concentrated on the surface of DHV, and the polymerization reaction had little impact on the structure of collagen fibers.

The adhesion, migration, proliferation, and cell viability of seed cells on the surface of scaffold are important indicators for the evaluation of biomedical materials [32]. We had performed several experiments to assess the influence of sericin-modified DHV on VIC. Studies have found that sericin could promote the migration of HUVEC in a dose dependent manner [19]. The similar phenomenon was also observed in our research. We found sericin could promote the migration of VIC, which was important for the recellularization of DHV. Besides, we also found that sericin could increase the viability of VIC cells. Cell viability is determined by many factors, among which the balance between oxidants and antioxidants plays a key role [33, 34]. Studies have shown that sericin has antioxidant activity. The specific amino acid sequence of sericin contributes to its ability to scavenge reactive oxygen species and increase the activity of antioxidant enzymes [35–37]. In addition, several studies have verified that sericin can promote cell proliferation [38]. Sericin has a mitogenic effect on a variety of mammalian cells, such effect has been tested in several cell lines, such

as the culture of Hela cells, HEK293 cells, HepG2 cells, etc. [38]. In summary, sericin promotes VIC adhesion, migration, and proliferation, enhances its viability, which will promote the recellularization of decellularized valves.

A certain degree of increased hydrophilicity of DHV can provide more favorable conditions for cell adhesion and growth. PDA contains profound amino groups and hydroxyl groups, which can provide hydrophilic groups on the surface of hydrophobic materials, so that the hydrophilic properties of hydrophobic biomaterials can be improved [39]. We have found that the hydrophilicity of PDA-modified DHV was significantly increased by testing the static contact angle. After modification with PDA, the ultimate tensile stress of DHV had been significantly improved, which was conducive to maintaining the durability of the valve in vivo. The PDA-coated surface is highly reactive and can react with a variety of molecules via Schiff-base and Michael addition chemistries. Sericin is rich in hydrophilic groups, such as amino groups, which can be molecularly cross-linked with PDA through the above reaction. In our research, PDA, as a bridging medium, improved the binding efficiency and stability of sericin on the surface of decellularized valves. Taken together, PDA modification further improved the performance of DHV.

Finally, we performed in vivo experiments to investigate the biocompatibility of our PDA-modified DHV-SS. The decay rate of PDA-modified DHV-SS was significantly decreased compared with other group and sericin could reduce the infiltration of inflammatory cells to valve scaffold, which might be related to the anti-inflammatory function of sericin [40]. The immune cells that infiltrated into the embedded DHV were mainly macrophages, which could secrete inflammatory cytokines, participate in the phagocytosis of graft, and affect tissue remodeling [41, 42]. The mechanism of how macrophage affect implanted valve need further investigation. Considering that the physiological conditions of valve are in the continuous scouring of blood flow, we transplanted the valve into the rabbit carotid artery which allowed the valve to be contact with blood directly. The PDA-modified DHV-SS could maintain patency in vivo for 1 month, showing good compatibility with blood.

5 Conclusions

In this study, two natural biological material, sericin and PDA, were used to modify the traditional DHV. We found that sericin improved the biocompatibility of DHV, with no significant influence on the internal structure of the scaffold. In addition, PDA further promoted the combination of sericin on DHV and modified the physical properties of valve scaffolds. The decay rate of our modified valve scaffold was significantly decreased in vivo and it showed good

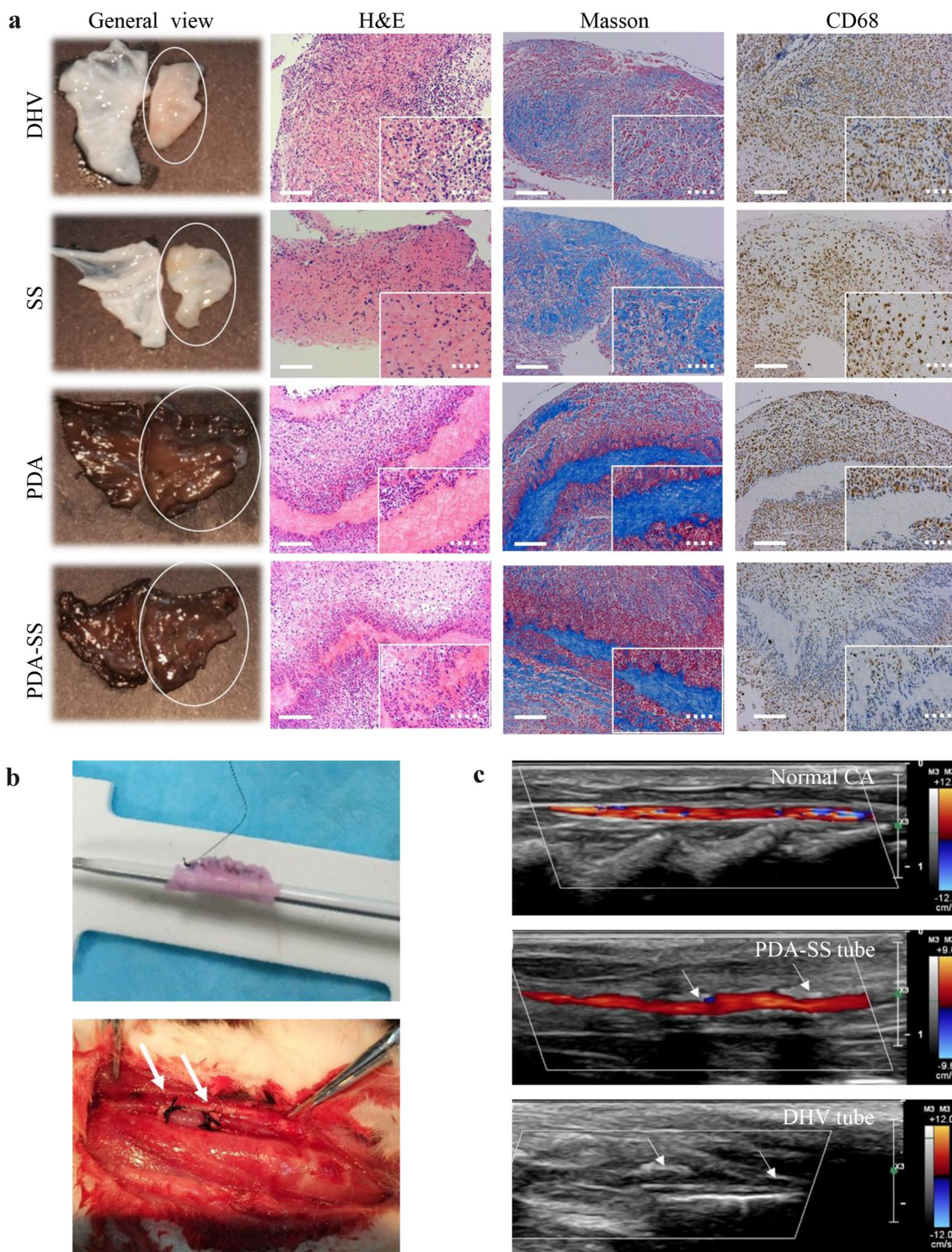


Fig. 7 Compatibility tests of composite scaffold in vivo. **a** We subcutaneously embedded the valve scaffold under the abdominal skin of rat. The general view and slice staining of embedded valve scaffold. **b** Pictures show how to make valve scaffold match the carotid artery

of rabbit, and the surgery field of transplanting valve scaffold to rabbit carotid artery. **c** Carotid ultrasound doppler images of transplanted valve scaffold 1 month after transplantation

compatibility when contacted with blood. This study demonstrates that modification with sericin and PDA to DHV improved the physical properties and biocompatibility of valve scaffold, which can be an efficient approach to develop functional DHV scaffolds for clinical applications. In future large animal in vivo studies, we anticipate showing the modified scaffolds will provide both the proper mechanical support and the appropriate biochemical environment for cell seeding and growth.

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Data Availability Statement All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare that they have no conflicts of interest to this work.

Ethics Approval The study was approved by Laboratory Animal Ethics Committee of Union Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology.

Consent to Participate Written informed consent was obtained from each study participant.


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