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A three-layered hydrogel patch with hierarchy releasing of PLGA nanoparticle drugs decrease neointimal hyperplasia

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ABSTRACT

Hydrogel is a nature scaffold that can degraded in animal body and can be used as a drug delivery system, we hypothesized that patch made of three layers of hydrogel with different PLGA nanoparticle drugs (bio-patch) can be used to decrease venous neointimal hyperplasia. Rat inferior vena cava (IVC) patch venoplasty model was used. Samples from rat IVC direct suture (DS), decellularized thoracic artery patch (TA) venoplasty and bovine pericardial patch (BPP) venoplasty were examined at day 14 after implantation. Sodium alginate and hyaluronic acid (SA/HA) hydrogel was used, three layers hydrogel patch (control) and three layers hydrogel patch with PLGA nanoparticle drugs (bio-patch) were used in rat IVC venoplasty. Patches were harvested at day 14 and analyzed. In rats, TA and BPP patch showed a thicker neointima and adventitia compared to the DS, there were larger numbers of CD68 and PCNA positive cells in both groups. The control hydrogel patch showed much thinner neointima and adventitia compared to TA and BPP patches. In both of the neointima and peri-patch area, bio-patch showed significantly fewer smooth muscle cells, fewer CD68, fewer PCNA positive cells, fewer collagen-1 positive cells, fewer p-smad2 positive cells, fewer TNF-α positive cells compared to control hydrogel patch. Bio-patch made of hydrogel and PLGA nanoparticle drugs showed a thinner neointimal thickness, and is biocompatible to the animal body. These results showed the potential application of hydrogel patch in vascular surgery.

1. Introduction

Prosthetic vascular grafts like Dacron and expanded poly tetra fluoroethylene (ePTFE) have a lower patency rate in peripheral bypass surgeries [1,2]. Both grafts showed poor long-term patency rate when the diameter is smaller than 6 mm [3]. In addition, biological graft like cryopreserved allografts, bovine or porcine pericardium, are also commonly developed as vascular grafts [4–6]. However, these materials have a risk of infection, foreign body reaction as non-autologous materials, meanwhile they cannot be absorbed by the body, which limit their application [7]. Along with the advancement of material science and technologies, various of novel materials are emerging and under investigation in vascular surgery [8–10]. Decellularized fish swim bladder also showed potential application as tube and patch grafts [3], plant leaf can also be used a patch to repair rat inferior vena cava (IVC) [11,12]. Great progress has been made in the field of vascular patches recently, however, the big challenge of neointima formation still not be solved [13,14].

In clinical and basic researches, various modifications of these materials have been made to enhance the performance of the grafts. Heparin coated grafts and stents have been used in patients to prohibit acute thrombosis formation [15,16], paclitaxel-or rapamycin-coated balloons and stents have been widely used to decrease neointimal hyperplasia by inhibiting neointimal smooth muscle cell proliferation [17]. We

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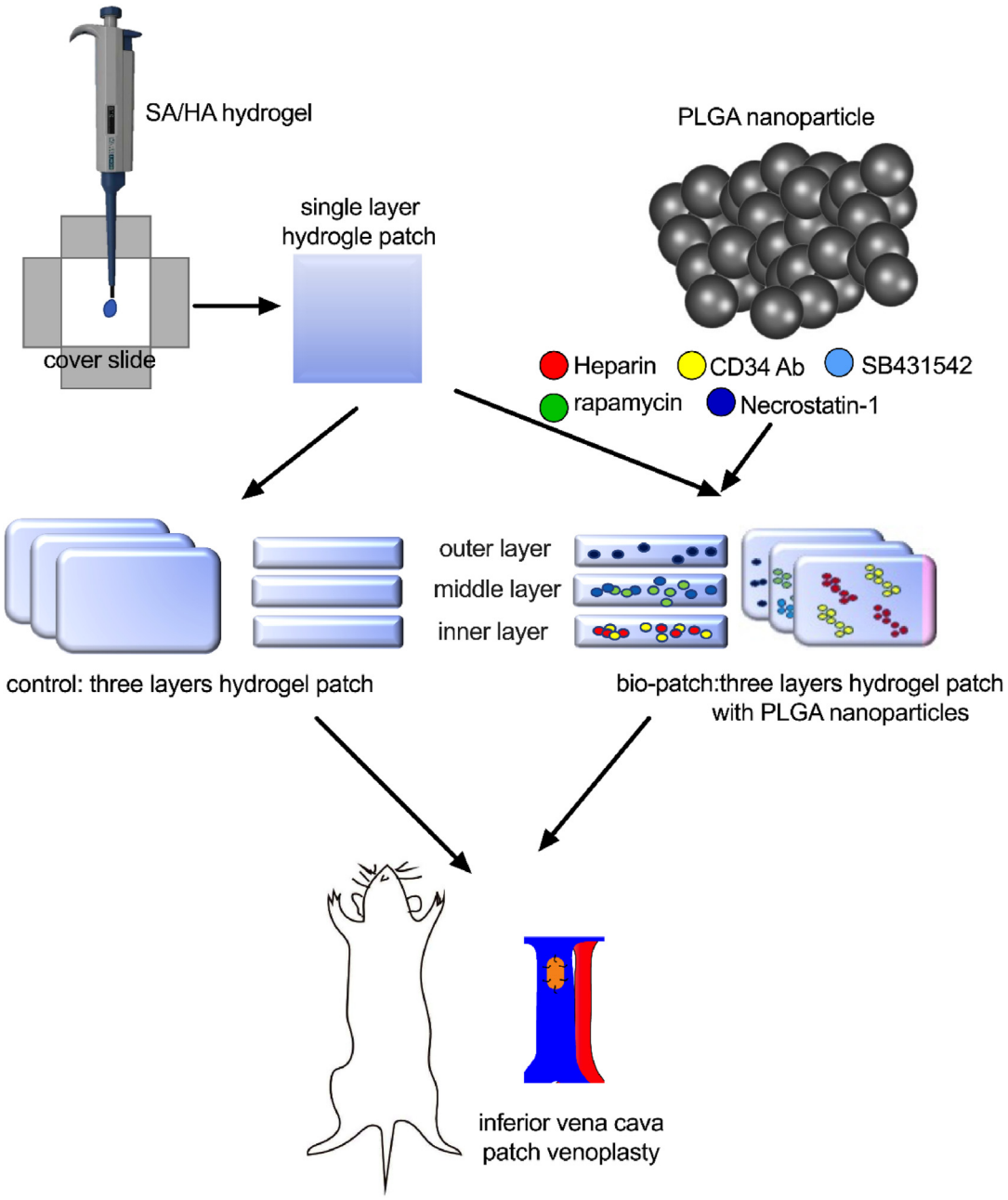

Fig. 1. Illustration showing the study design. (This illustration was designed by Professor. Hualong Bai).

previously showed heparin coated human great saphenous vein patch was with a decreased neointima thickness in rats [5], poly (lactic-co--glycolic acid) (PLGA) nanoparticle rapamycin conjugated pericardial patch can decrease neointimal thickness in a rat venoplasty model [13]. Cluster of differentiation 34 (CD34) antibody and heparin conjugated alloy can promote reendothelialization and inhibiting platelet and macrophage adhesion [18]. Beyond the modification of the grafts, novel biomaterials were also developed and showed excellent results in clinical trials [8,10,19]. Recently, wang et al. develop a self-adaptive liquid gating membrane-based catheter with anticoagulation and positionally drug release properties, they showed the multifunctional liquid gating membrane-based catheter significantly attenuates blood clot formation and can be used as a general catheter design strategy to offer various drugs positionally releasing applications [20].

The healing process of vascular graft after implantation is a complex event. After the implantation of artificial blood vessels or patches, inflammation is first induced, and since artificial blood vessels are not covered by endothelial cell layer, platelet adhesion, aggregation and thrombosis will be triggered, and some cytokines will be released due to activated platelets. Moreover, the activation of inflammatory cells and platelet leads to the release of cytokines, such as TNF-a and PDGF-BB, which further activate the surrounding smooth muscle cells to promote their proliferation and migration. Meanwhile, the activation of fibroblasts in the adventitia participates in the process of intimal hyperplasia [21]. But traditional and current modifications of the grafts, treatment protocols are only focused on one step in the process of neointimal hyperplasia, and there is still no experiment on vascular graft that can release different drugs hierarchically; so a hierarchy treatment protocol is needed to effectively inhibit neointimal hyperplasia.

Sodium alginate and hyaluronic acid (SA/HA) hydrogel has good hydrophilicity and biocompatibility, it is a nature scaffold that can degenerated in animal body and can be used as a drug delivery system

Table 1
Antibodies used in this experiment.

antibody	vendor	Lot number	concentration
	Primary antibody		
α-actin	abcam	ab5694	IF/IHC:1:100
CD68	abcam	ab31360	IF/IHC:1:100
CD3	Santa Cruz	Sc-20047	IHC:1:200
COUP-TFII	Abconal	A10251	IHC:1:200
Eph-B4	Abconal	A3293	IHC:1:200
PCNA	Abcam	ab29	IF/IHC:1:100
Cleaved caspase-3	Cell Signaling	9,661	IHC:1:100
dll-4	Abclone	A12943	IHC:1:100
Ephrin-B2	Abclone	A12961	IHC:1:100
CD34	abcam	ab81289	IF:1:100
nestin	abcam	ab 11306	IF:1:100
vWF	abcam	ab11713	IF:1:100
Collagen-1	Abclonal	A5786	IHC:1:200
p-smad2	Cell Signaling	CS18338	IHC:1:200
TNFα	Abcam	Ab6671	IHC:1:200
	Secondary antibody		
HRP Goat anti-Rabbit	Beyotime	A0208	1:100
HRP Goat anti-Mouse	Beyotime	A0216	1:100
488 Goat anti-Mouse	ABclonal	AS073	1:200
CY3 Goat anti-Rabbit	ABclonal	AS007	1:200
488 Donkey anti-Rabbit	ABclonal	AS035	1:200
Rhodamine Donkey anti-goat	ABclonal	AS069	1:200

[22]. Injectable hydrogel can be loaded with mesenchymal stem cells for the treatment of traumatic brain injury [23], this hydrogel also showed potential application in the repairing of cardiovascular diseases [24]. We previously showed the hydrogel can deliver therapeutic agent to the needle puncture site in rat artery and vein [25,26]. PLGA nanoparticles is better than direct drug delivery due to cell response to nano effect, PLGA rapamycin nanoparticle can release the drugs gradually and inhibit neointimal hyperplasia more effectively than rapamycin alone [27]; hydrogel can also be used to deliver PLGA nanoparticle rapamycin in plant derived patches [11].

Inspired by these previously researches, we hypothesized that patch made of three layers of hydrogel with different PLGA nanoparticle drugs (bio-patch) can hierarchically release drugs to decrease venous neointimal hyperplasia in a rat inferior vena cava patch venoplasty model. The inner hydrogel layer with nanoparticle heparin and CD34 can prevent acute thrombosis and attract endothelial progenitor cell (EPC), the middle hydrogel layer with PLGA rapamycin and SB431542 can effectively reduce proliferation and collagenization, and the outer hydrogel layer with PLGA necrostain-1 can inhibit the effect of TNF-α.

2. Experimental section

2.1. Animal care

The study was approved by the First Affiliated Hospital of Zhengzhou University, Animal Care and Use Committee. All animal care complied with the Guide for the Care and Use of Laboratory Animals. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were observed. Male Sprague Dawley (SD) rats (6 to 8 weeks old) were used for all the animal experiments, rats were anesthetized with 10% chloral hydrate (intraperitoneal injection), and adequate anesthesia was confirmed through a lack of reaction to a toe and tail pinch; ointment on the eyes was placed to prevent dryness while the animals were under anesthesia, and the ventral abdomen hair was removed using a hair remover while wearing sterile gloves. For post-operative analgesia, buprenorphine was given at 0.1 mg/kg intramuscularly no less than every 12 h for 24 h following the surgical procedures. The status of the animal was checked every day in the animal room, ensuring proper recovery from the peri-operative period as well as adequate treatment of post-surgical pain.

2.2. Three layers of hydrogel patch with PLGA nanoparticle drugs (bio-patch) fabrication

The PLGA nanoparticle was made as previously described [11,28]. Briefly, 100 mg PLGA were added into 1 ml ethyl acetate (EtAc), and let the polymer dissolve overnight, 2 ml of 0.3% w/v D-α-Tocopherol polyethylene glycol 1000 succinate (Vitamin E-TPGS) and 1 ml polymer solution was mixed. Then the hardened nanoparticles were collected and

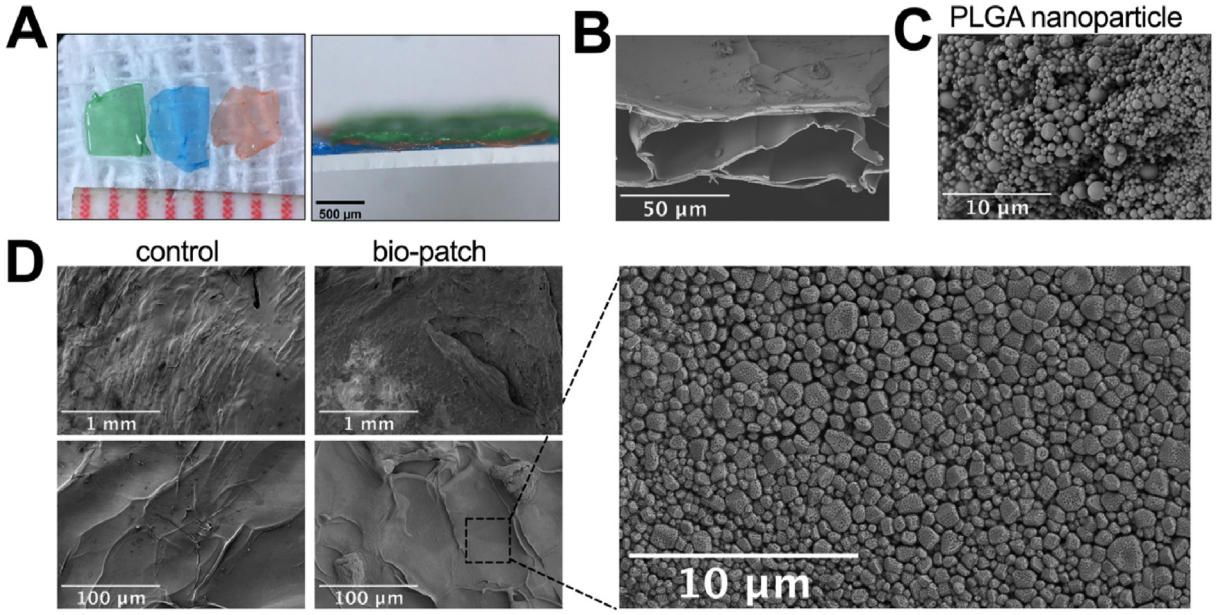

Fig. 2. Fabrication and structure of control patch and bio-patch. **A)** Hydrogel with green, blue, and red color. Three layers of hydrogel with green, blue, and red color compared with cover slide. **B)** Scanning electro-microscope (SEM) photograph showing the cross section of the three layers of hydrogel patch, scale bar, 50 μm; n = 3. **C)** SEM photograph showing the poly (lactic-co-glycolic acid) (PLGA) nanoparticles. **D)** SEM photographs showing the control and bio-patch, upper row, low power; lower row, high power; note the zoomed in photographs showing particles on the surface of the bio-patch.

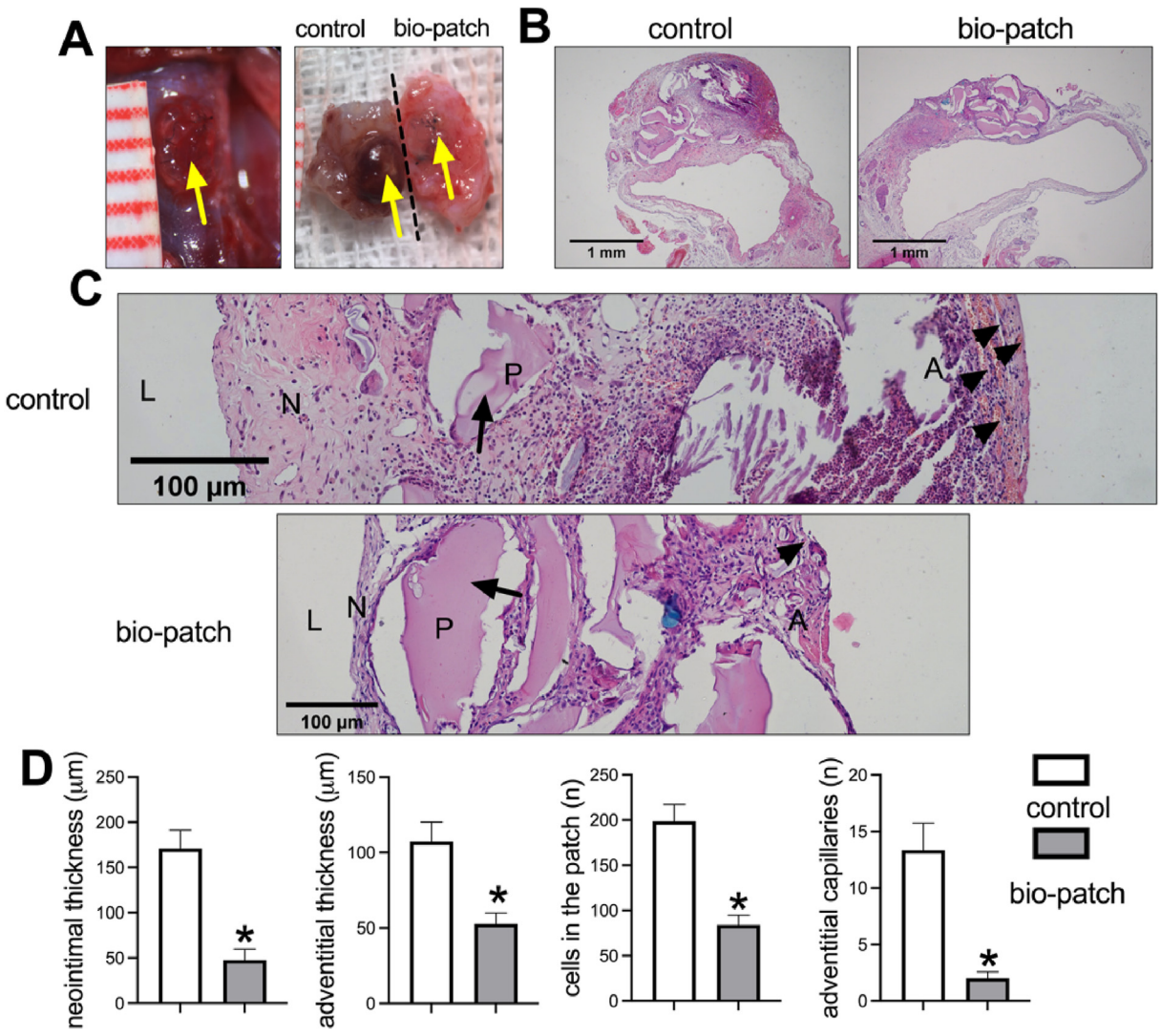

Fig. 3. Bio-patch decreases neointimal thickness compared to control three layers hydrogel patch. **A)** Photographs of three layers of hydrogel patch in IVC after completion of the anastomoses; ruler marks 1 mm, yellow arrow showing the patch, black dotted line separating the control and bio-patch sample. Photographs of control patch and bio-patch harvested at day 14, yellow arrow showing the patch, note the dark red color of the control patch compared to the transparent nano-patch, ruler marks 1 mm. **B)** Hematoxylin & eosin (H&E) staining of the control patch and nano-patch at day 14, scale bar, 1 mm; n = 3. **C)** High power photographs showing the neointima (N), patch (P) and adventitia (A); L, lumen; arrows showing the hydrogel; arrow head showing the adventitial new formed capillaries; scale bar, 100 μm; n = 3. **D)** Bar graphs showing the neointimal thickness (*, p = 0.0065, *t*-test), adventitia thickness (*, p = 0.0205, *t*-test), cells infiltrated into the patch (*, p = 0.0058, *t*-test), adventitial new formed capillaries (*, p = 0.0058, *t*-test); n = 3.

lyophilized for 72 h. Scanning electronic microscopy (SEM) was taken as previously described [29]. CD34 antibody, heparin, rapamycin, SB431542, Necrostation-1 were added in the process to make different PLGA nanoparticles. The final concentrations of different drugs are: rapamycin, 45 ug/mg PLGA; heparin, 45 ug/mg PLGA; CD34, 60 ug/mg PLGA; SB431542, 60 ug/mg PLGA; Necrostation-1 60 ug/mg PLGA.

The hydrogel was made as we previously described [23,25]. Briefly, sodium alginate and hyaluronic acid (SA/HA) were used to make a 1.5 wt % SA/HA composite solution, and $CaCl_2$ (10 wt%) was added to the above solution to make hydrogel. The gel as trimmed into 3 mmx3 mm patches, three layers of hydrogel was piled up together to make control patch. For the bio-patch, the inner layer of hydrogel was mixed with PLGA nanoparticle heparin (1 mg) and CD34 antibody (1 mg), the middle layer hydrogel was mixed with PLGA nanoparticle rapamycin (1 mg) and SB431542 (1 mg); the outer layer was mixed with PLGA nanoparticle Necrostation-1 (1 mg)(Fig. 1); scanning electronic microscopy (SEM) was carried out as previously described [29]. For the hydrogel with colors, green, blue and red color was added to the hydrogel.

2.3. Rat IVC direct suture and patch venoplasty model

The rat IVC was exposed and a 3-mm venotomy was made [30], then the IVC was closed directly using running 11-0 nylon sutures; the abdomen was closed. The IVC was explanted at day 14 for analysis as described below.

Rat IVC venoplasty model was used as previously described [13]. Briefly, the rat IVC was exposed and a 3-mm venotomy was made, patches (3 mm x 3 mm) were sewn to the IVC using running 11-0 nylon sutures; the patches include pericardial patch (BPP), decellularized thoracic artery (d-TA) patch, three layers hydrogel patch (control), bio-patch. Then the clamps were removed and the abdomen was closed [13]. The patch was explanted at day 14 for analysis as described below.

2.4. Histology analysis

The rats were anesthetized, and tissues were fixed by transcardial perfusion of PBS followed by 10% formalin. The samples were fixed,

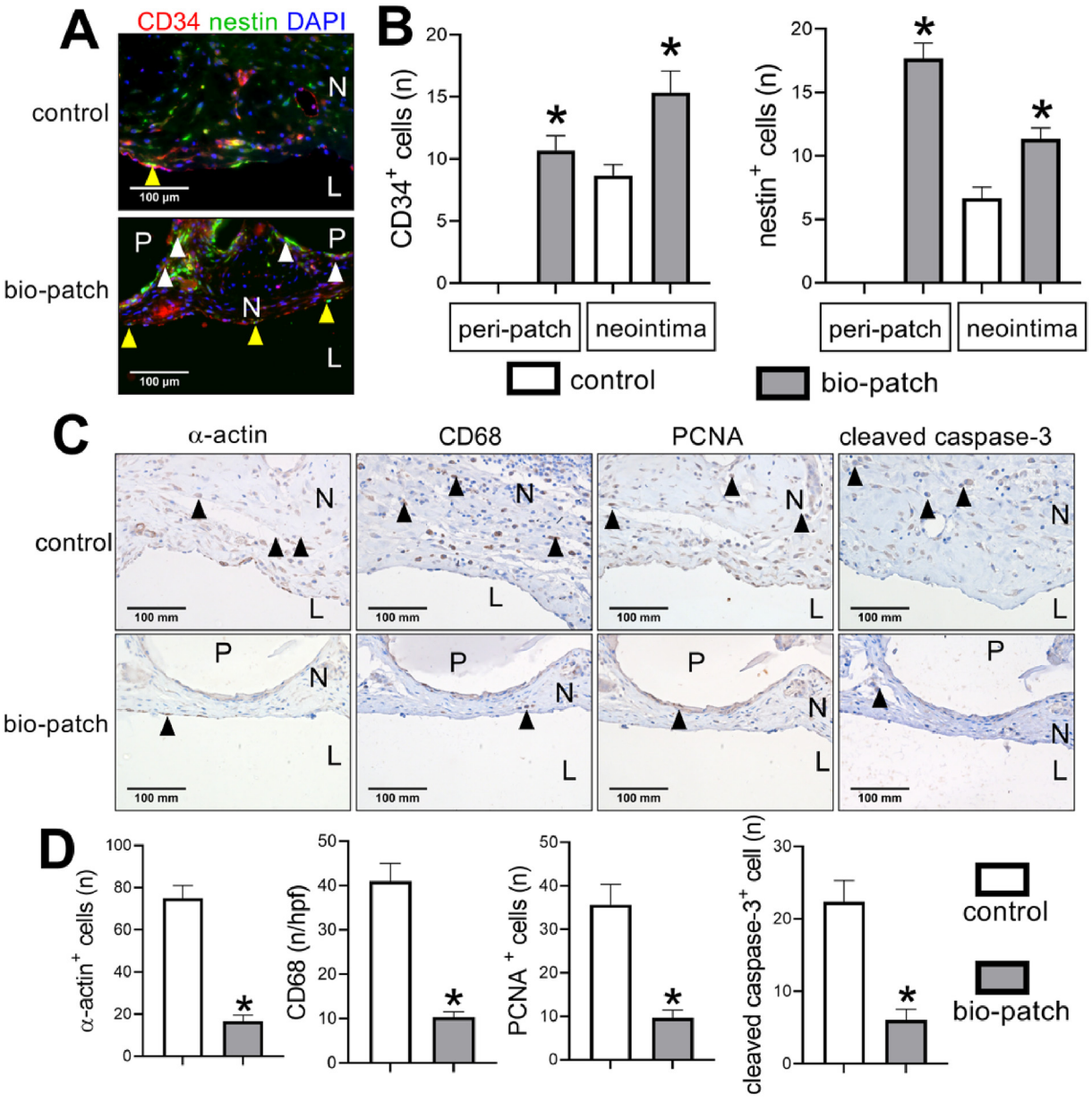

Fig. 4. Bio-patch decreases neointimal thickness through the functions of the PLGA nanoparticle CD34 antibody, heparin, and rapamycin. **A)** Immunofluorescence photograph stained for CD34 (red), nestin (green) and DAPI (blue) in the neointima and in the peri-patch area harvested at day 14; white arrowhead showing the CD34 and nestin positive cells around the hydrogel in the bio-patch group, yellow arrowhead showing the CD34 and nestin positive neointimal endothelial cells in the bio-patch group; N, neointima; L, luman; P, patch; scale bar, 100 μm; n = 3. **B)** Bar graph showing the CD34 positive cells in the peri-patch area (*, *t*-test, p = 0.0009) and in the neointima (*, *t*-test, p = 0.0278); bar graph showing the nestin positive cells in the peri-patch area (*, *t*-test, p = 0.0001) and in the neointima (*, *t*-test, p = 0.0201). n = 3. **C)** Immunohistochemistry photographs showing the neointimal stained for α-actin, CD68, PCNA and cleaved caspase-3; N, neointima; L, lumen; P, patch; scale bar, 100 μm, n = 3; black arrowhead showing the positive cells. **D)** Bar graphs showing the α-actin positive cells (*, p = 0.0010, *t*-test), CD68 positive cells (*, p = 0.0019, *t*-test), PCNA positive cells (*, p = 0.0066, *t*-test) and cleaved caspase-3 positive cells (*, p = 0.0080, *t*-test) in the control patch and bio-patch; n = 3.

embedded in paraffin, sectioned (4-μm thickness). The tissue sections were deparaffinized and stained with hematoxylin and eosin (H&E; Baso, Zhuhai, China) according to the manufacturer's recommendations.

2.5. Immunohistochemistry

The sections were heated in citric acid buffer (pH 6.0, Beyotime, Shanghai, China) at 100 °C for 10 min for antigen retrieval and then treated with 0.3% hydrogen peroxide for 30 min. Sections were incubated overnight at 4 °C with primary antibodies (Table 1). After overnight incubation, the sections were incubated with appropriate secondary antibodies (Table 1) for 1 h at room temperature and then treated with a 3,3N-diaminobenzidine tetrahydrochloride (DAB) horseradish peroxidase color development kit (Beyotime, Shanghai, China) to detect the reaction products. Finally, the sections were counterstained with hematoxylin (Baso, Zhuhai, China). Positive cell numbers were counted and blindly reviewed by three professional pathologists.

2.6. Immunofluorescence analysis

The sections were incubated overnight at 4 °C with primary antibodies (Table 1) diluted in dilution buffer (Beyotime, Shanghai, China).

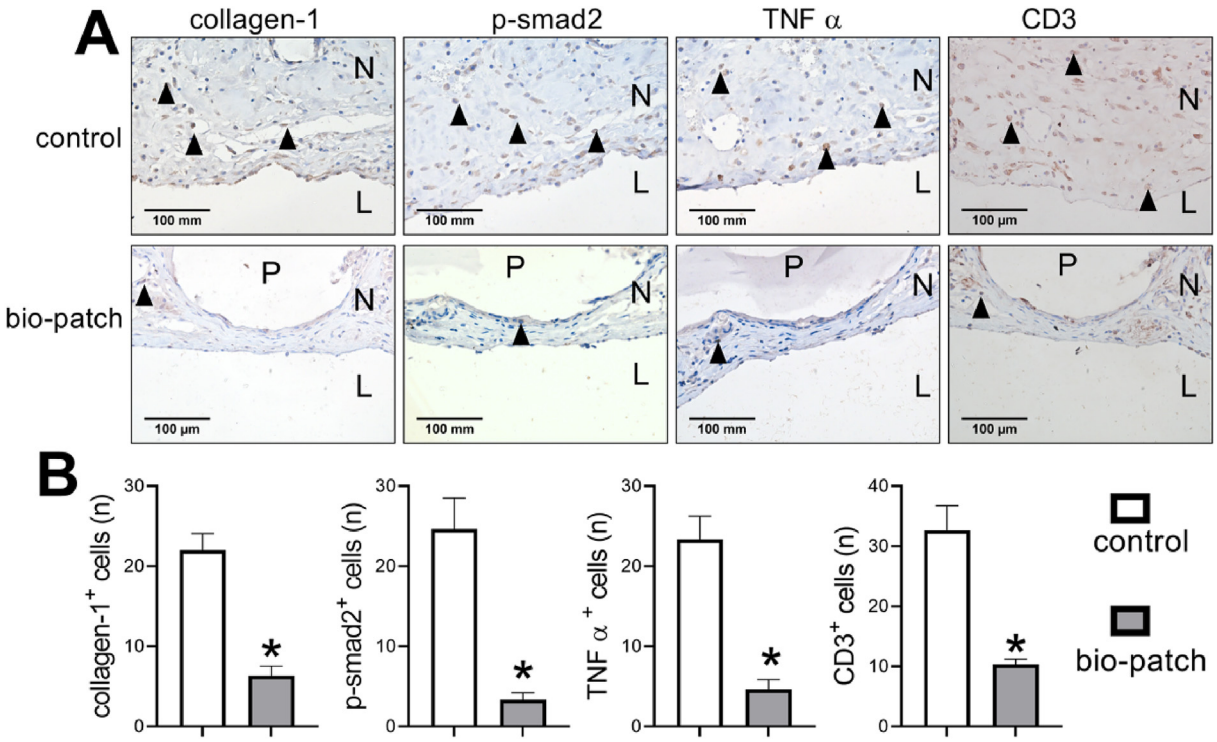

Fig. 5. Bio-patch decreases neointimal thickness through the functions of the PLGA nanoparticle SB431542 and necrostatin-1. **A)** Immunohistochemistry photographs showing the neointimal stained for collagen-1, p-smad2, TNF α and CD3; N, neointima; L, lumen; P, patch; scale bar, 100 μm, n = 3; black arrowhead showing the positive cells. **B)** Bar graphs showing the collagen-1 positive cells (*, p = 0.0029, *t*-test), p-smad2 positive cells (*, p = 0.0057, *t*-test), TNF α positive cells (*, p = 0.0040, *t*-test) and CD3 positive cells (*, p = 0.0060, *t*-test) in the control patch and bio-patch; n = 3.

The sections were incubated with secondary antibodies (Table 1) for 1 h at room temperature, after which the sections were stained with the fluorescent dye DAPI (Solarbio, Beijing, China) to mark cellular nuclei. Positive cell numbers were counted and blindly reviewed by three professional pathologists.

2.7. Statistical analysis

The data are expressed as the mean ± SEM. Statistical significance was determined by ANOVA and t-tests. P-values less than 0.05 were considered significant. The data were analyzed using Prism 6.0 software (GraphPad Software; La Jolla, CA, USA).

3. Result

We firstly compared the difference of the healing process of direct suture (DS) of rat IVC, decellularized thoracic aorta (TA) patch venoplasty and bovine pericardial (BPP) patch venoplasty in rat, there was a very thin neointima and adventitia in the DS group (Supplementary Figs. 1A and 1B); in the TA and BPP patch venoplasty groups, there were a thick neointima and adventitia compared to the DS (Supplementary Figs. 1A and 1B); there were also more α-actin positive cell, CD68 positive cells, PCNA positive cells in the neointima, inside the patch and in the adventitia compared to DS group (Supplementary Fig. 1C). There were few cells were cleaved caspase-3 positive in the neointima, inside the patch and in the new formed adventitia in these three groups (Supplementary Fig. 1C). These results showed that patch material can influence the neointimal thickness after patch venoplasty.

Hydrogel has multifunctional roles in biomedical research, when combined with other materials, they have been used as vascular graft [31,32]; we also showed a biomimetic elastin fiber hydrogel patch can be used to repair rat aorta [33]. But there was still no research on whether the pure SA/HA hydrogel can be used as a vascular patch. So we constructed three layers hydrogel patches to see if they can be loaded with PLGA nanoparticle drugs in different layers and implanted as a patch (Fig. 1). Firstly, we stained the hydrogel with green, blue, and red colors, there was no mix of colors between two layers (Fig. 2A). The size of the patch was 3x3mm, the thickness of three layers patch was 150 μm (Fig. 2A). SEM photograph showed that the three layers hydrogel patch was smooth with interspace between layers (Fig. 2B). The PLGA nanoparticles were with similar diameters (Fig. 2C). We then fabricated bio-patch with PLGA nanoparticle heparin and CD34 antibodies in the inner layer, PLGA nanoparticle rapamycin and SB431542 in the middle layer, PLGA nanoparticle necrostation-1 in the outer layer (Fig. 1). SEM photographs showed the smooth surface of the control patches, but there were particles on the surface of the bio-patch surface (Fig. 2D).

Then we implanted the patches to the rat IVC, both control patch and bio-patch were transparent after implantation. The bio-patch showed much more transparent with fewer mural thrombus than the control hydrogel patch at day 14 (Fig. 3A). In the control patch, H&E staining showed almost half of hydrogel was absorbed (Fig. 3B, 3C). There was a significantly thinner neointima and adventitia in the bio-patch group compared to the control group (Fig. 3C, 3D). There were fewer cells migrated into the interspaces (Fig. 3C, 3D), and fewer newly formed capillaries in the bio-patch compared to the control patch in the adventitia (Fig. 3C, 3D). The newly formed tissue kept the original shape and did not collapse even after the hydrogel degraded (Fig. 3).

In both the control and bio-patch groups, there were CD34 and nestin positive cells in the peri-patch area and neointima, but there were more CD34 and nestin positive cells in the bio-patch group compared to the control group (Fig. 4A, 4B). In the neointima, there were fewer α-actin positive cells, fewer CD68 positive cells, fewer PCNA positive cells, fewer cleaved caspase-3 positive cells in the bio-patch group compared to the control group (Fig. 4C, 4D). There were fewer collagen-1 positive cells, fewer p-smad2 positive cells, fewer TNF α positive cells, fewer CD3 positive cells in the bio-patch group compared to the control patch group (Fig. 5A, 5B). Immunohistochemistry showed the neointimal endothelial

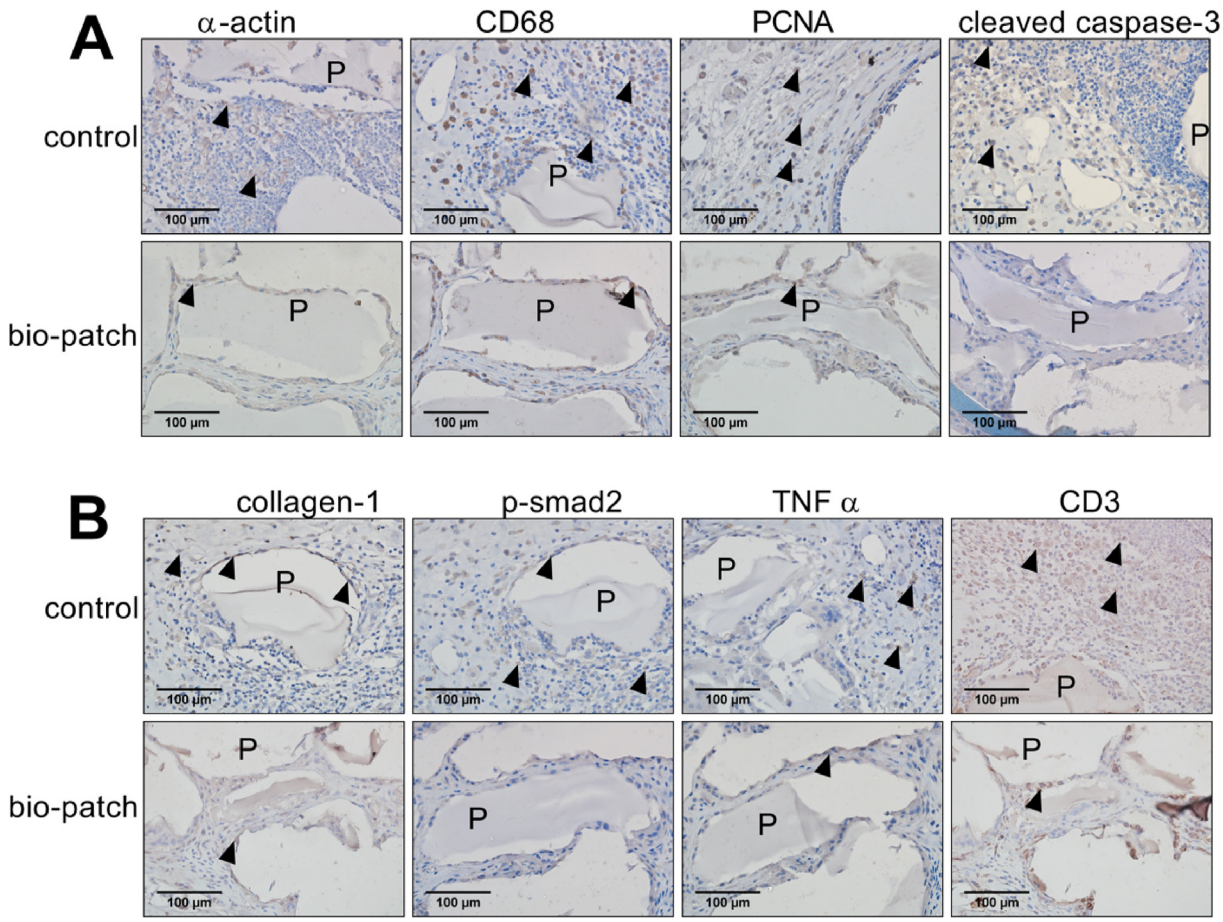

Fig. 6. Bio-patch functions on the peri-patch area. **A)** Immunohistochemistry photographs showing the neointimal stained for α-actin, CD68, PCNA and cleaved caspase-3; P, patch; scale bar, 100 μm, n = 3; black arrowhead showing the positive cells. **B)** Immunohistochemistry photographs showing the neointimal stained for collagen-1, p-smad2, TNF α and CD3; P, patch; scale bar, 100 μm, n = 3; black arrowhead showing the positive cells.

cells expressed venous identity markers Eph-B4 and COUP-TF II in both groups (Supplementary Fig. 2).

In the peri-patch area, there were also fewer α-actin positive cells and CD68 positive cells, fewer PCNA positive cells and cleaved caspase-3 positive cells, fewer collagen-1 positive cells and p-smad2 positive cells, fewer TNF α positive cells and CD3 positive cells in the bio-patch group compared to the control patch group (Fig. 6A, 6B). In the newly formed adventitia, there were also fewer α-actin positive cells and CD68 positive cells, fewer PCNA positive cells and cleaved caspase-3 positive cells, fewer collagen-1 positive cells and p-smad2 positive cells, fewer TNF α positive cells and CD3 positive cells in the bio-patch group compared to the control patch group (Supplementary Fig. 3).

4. Discussion

In this research, we showed that SA/HA hydrogel can be used as a patch in a rat inferior vena cava (IVC) venoplasty model, three layers of hydrogel patch with PLGA nanoparticle drugs (bio-patch) can release drugs in a hierarchy fashion and inhibit venous neointima hyperplasia, this research showed the potential clinical application of bio-patch in vascular surgery in the future.

Because of the slow blood flow in the venous system, acute thrombosis and lumen occlusion occur from 45% to 100% in 24 to 72 h after venous interventions in clinic [34,35], there is still no perfect solution to solve these complications. We previously showed mural thrombus, discontinuous of neointimal endothelial cells, disorganized smooth muscle cells, macrophage cells in the neointima of human spiral saphenous vein graft implanted into popliteal vein [36]; together with our experience of healing process of rat IVC patch venoplasty [5,13,37]; we found that acute mural thrombus formation, incomplete of neointimal endothelial progenitor cell accumulation, proliferation of neointimal cells, macrophages migration all contributed to the venous neointimal formation and hyperplasia. However, current commonly used treatment methods are always focusing on anticoagulation and antiproliferation, although these techniques contributed a better clinical outcome, but long-term results are still not satisfactory [38]. Therefore, a comprehensive method to decrease venous neointimal hyperplasia is needed.

The healing process after patch venoplasty is similar to other vein interventions [13,39]. Neointimal rapid reendothelialization is critical to prohibit further thrombus formation and decrease neointimal hyperplasia, so to decrease the acute thrombus formation and promote early reendothelialization are the first step to enhance graft patency rate. We showed PLGA nanoparticle heparin in the inner layer of the patch can decrease acute thrombus accumulation like we showed before [5], the CD34 antibody nanoparticles in the inner layer can also attract more endothelial progenitor cells to the luminal neointimal surface and peri-patch area; similar results have been showed in other researches [40,41]. Smooth muscle cells and macrophages play important roles in neointimal hyperplasia, our group found one third smooth muscle cells (SMC) and half macrophages were proliferating cell nuclear antigen (PCNA) or Ki67 positive at day 7 after rat IVC patch venoplasty [13]. which means SMCs and macrophages were in a proliferation state and should be the target of treatment [13,37]. We also found collagens deposited in the neointima and adventitia at day 30, this collagen deposition also contributed to the thick neointima and adventitia [13]; so inhibiting neointimal collagen deposition is also a target to decrease the

neointimal hyperplasia [42,43]. The finding of macrophages in the hydrogel patch is like other patches [44,45]. Macrophages play an important role in the foreign body reaction [46], depletion of macrophages can decrease neointimal thickness [47,48]. TNF-α excreted from macrophages is an important factor to induce foreign body reaction [49,50], we used TNF α inhibitor necrostatin-1 to alleviate the effect of TNF α and showed a much thinner adventitia and weaker foreign body reaction. Macrophages may also play a role in the hydrogel absorption and degradation.

To find better vascular grafts is the dream of both the vascular surgeons and scientists. Nature vessels have three layers of structures, intima, medium and adventitia; so fabrication of different layers vascular graft has attracted more and more attention [51–53]. Hu et al. designed and fabricated a triple-layered vascular scaffold, the results demonstrated that human umbilical vein endothelial cells can successfully attach to the surface of the graft and maintain high viability [54]. We previously showed hydrogel can be used to deliver cells, antibody and drugs [11,24,25]. This bio-patch not only has three layers, but also has different PLGA nanoparticle drugs in each layer to inhibit neointimal hyperplasia, the different drugs can release in a hierarchy fashion along with the degradation process, and the PLGA nanoparticle can also make the drug delivery more effective. There are still no well-defined sequential phases in the neointimal formation and hyperplasia, different phases are always overlapped, the hierarchy release of different drugs in the bio-patch is also not in a strictly sequential fashion; future researches on the different sequential phases in the neointimal hyperplasia is needed. Recent researches on drug release have developed rapidly, gold nanoparticles (AuNPs) have been widely applied in the biomedical field due to their tunable localized surface plasmon resonance (LSPR) properties, versatile surface modifiability, and favorable biocompatibility [55]. Future research using other drug carriers should also be tested in this bio-patch.

5. Conclusion

In conclusion, we showed a novel idea to fabricate a three layers vascular patch with different PLGA nanoparticle drugs (bio-patch), this bio-patch can effectively decrease neointimal hyperplasia in a rat IVC venoplasty model; this research showed that the bio-patch may be a potential substitute of other prosthetic patches in vascular surgery.

CRediT authorship contribution statement

Shunbo Wei: Data curation. **Jing'an Li:** Methodology, Data curation, Writing – review & editing. **Hao He:** Data curation. **Chang Shu:** Data curation. **Alan Dardik:** Methodology, Supervision. **Hualong Bai:** Conceptualization, Methodology, Data curation, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.smaim.2021.12.005.

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