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# *Bletilla striata* Polysaccharide Prevents Restenosis of Vein Graft Through Inhibiting Cell Proliferation in Rat Model

**Chun Dai[1], Yang Zhou[2], Bing Zhang[3], and Jianjun Ge[1]**

**Abstract**

Coronary artery bypass grafting (CABG) is still the most effective method for the treatment of coronary heart disease at present. However, the restenosis of vein grafts following surgery is an important complication of CABG. In this study, *Bletilla striata* polysaccharide (BSP), which has anti-inflammatory and antiproliferative properties, was used to prevent or delay the proliferation of venous bridge endothelial cells in a rat model. We transplanted the autogenous jugular vein to the rat carotid artery, and wrapped it with BSP. We carried out experiments in 4 groups (with 24 rats in each group): a high-BSP dose group (the HBG group, 10 mg), a low-BSP dose group (the LBG group, 3 mg), a pluronic gel group (the gel group), and a control group. Vein grafts were then harvested after 3, 14, and 28 days. Following transplantation, we used color Doppler ultrasound to assess the patency of the transplanted vein. The grafted veins were stained with hematoxylin and eosin (H&E) and Masson to measure the thickness of the intima and media of the blood vessels. Proliferating cell nuclear antigen (PCNA) and vascular cell adhesion molecule-I (VCAM-1) were assessed in vein grafts by immunohistochemistry and western blotting. We detected a significant reduction in the proliferation of endothelial cells in the BSP group compared with the control group ($P < 0.05$). H&E and Masson's trichrome staining showed that the extent of intimal hyperplasia in transplanted veins from the high BSP group (HBS) ($67.42 \pm 0.54$ μm) and low BSP group (LBS) ($120.83 \pm 1.87$ μm) groups was significantly lower than that in the control group ($257.03 \pm 2.74$ μm, $P < 0.05$), and that the extent of intimal hyperplasia in the HBS group was lower than that in the LBS group ($P < 0.05$). We found that the effect of BSP was dose-dependent, as high-dose BSP had a more significant inhibitory effect on cell proliferation than low-dose BSP ($P < 0.05$). The results of immunohistochemistry and western blotting showed that PCNA and VCAM-1 were significantly downregulated in the BSP treatment group on days 14 and 28 ($P < 0.05$). BSP inhibits the proliferation of vascular endothelial cells and reduces the expression of VCAM-1, thereby inhibiting the restenosis of graft veins.

**Keywords**

*Bletilla striata* polysaccharide, coronary artery bypass grafting, restenosis, endothelial cell

## Introduction

According to the 2019 Heart and Stroke Statistics compiled by the American Heart Association, the incidence of coronary atherosclerotic heart disease has been increasing steadily year by year; indeed, the mortality rate associated with coronary atherosclerotic heart disease now exceeds that of cancer[1].

Currently, there are two main clinical treatments for coronary heart disease, percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG)[2]. PCI is indicated for patients with less severe disease (one- or two-vessel disease), while CABG is mostly indicated for patients with severe coronary artery disease (CAD)[3]. Arterial grafts are preferred during surgery, but the great saphenous vein is

[1] Anhui Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, PR China
[2] Department of Cardiac Surgery, Anhui Provincial Hospital, Anhui Medical University, Hefei, PR China
[3] Division of Life Sciences and Medicine, The First Affiliated Hospital of USTC, University of Science and Technology of China, Hefei, Anhui, PR China

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**Corresponding Author:**
Jianjun-Ge, Department of Cardiac Surgery, Anhui Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, Shandong, 250021, PR China.
Email: zkdgjj@ustc.edu.cn

often used in patients with multiple vessel lesions; this is because this particular vein can be collected in a relatively simple and convenient manner and is long enough to provide good lengths of vein for grafting[4]. However, due to surgical trauma, changes in arterial blood pressure, and persistent inflammation, the smooth muscle cells of the graft bridge vessel migrate and the endothelial cells begin to proliferate, thus resulting in restenosis of the graft bridge vessel[5]. A previous study found that restenosis developed in more than 20% of grafts involving saphenous veins within 1 year of transplantation; the proportion of grafts showing restenosis increased to 40% in 2 years, and more than 60% of vein bridge vessels within 10 years of transplantation[6]. When the blood vessels that form the venous bridge undergo restenosis, the blood supply to the heart drops markedly, thus causing arrhythmia, myocardial infarction, and, in severe cases, death.

The initial factor responsible for inducing restenosis in vein grafts is injury to the vascular endothelium. Intimal hyperplasia, caused by the proliferation and migration of vascular smooth muscle cells (VSMCs), also represents one of the core mechanisms underlying restenosis[7]. Proliferating cell nuclear antigen (PCNA) is closely related to endothelial cell proliferation, while vascular cell adhesion molecule 1 (VCAM-1) is known to mediate endothelial cell adhesion[8]. During the process of restenosis, the release of inflammatory mediators plays an important role in the pathophysiological changes that take place in the venous bridge vessels. Early vasodilation following vein transplantation and vascular injury is known to activate the release of inflammatory factors, thus leading to the proliferation and remodeling of both vascular endothelial cells and VSMCs[9,10]. However, antiplatelet therapy and statin therapy are commonly administered after CABG at present[11,12]. However, these drugs have little effect in alleviating intimal hyperplasia of the grafts. Therefore, one of the most significant challenges in coronary heart surgery at present is the development of effective techniques to inhibit restenosis in the venous bridge.

*Bletilla striata* polysaccharide (BSP) is a traditional Chinese medicine (TCM) that is extracted from astragalus powder and has proven anti-inflammatory, hemostatic, and anti-edema effects[13]. Previous studies suggest that BSP has a particularly significant effect against inflammation and has the capability to inhibit the release of various inflammatory factors, particularly cytokines[14,15]. The biocompatibility of BSP was first described by Luo et al.[16].

Collectively, the existing literature supports the fact that the anti-inflammatory effect of BSP may play a role in inhibiting restenosis in vein bridges. We hypothesized that BSP could inhibit the proliferation and migration of both endothelial cells and smooth muscle cells and thus inhibit restenosis in vein bridges. We investigated this hypothesis by establishing a rat vein graft model and treating vein grafts with BSP. Intimal hyperplasia was detected by Masson's staining and correlated with protein expression by immunohistochemical and western blot analysis. Collectively, our research revealed that BSP can inhibit restenosis in vein bridges.

## Materials and Methods

### Extraction of Plant Material

BSP (95% *Bletilla striata* extract; 500 mg) was purchased from China Xi'an Ciyuan Biological Co., Ltd. The purity of this extract was 95% after identification. We then created two working concentrations of BSP by mixing 10 mg of BSP and 3 mg of BSP with 300 μl of pluronic-F127 gum.

### Animals and Diets

All animal experiments were approved by the Ethics Committee on Animal Experiments of the Anhui Animal Ethics Committee under the protocol number AH56743. Sprague-Dawley rats (male and female; age: 11 to 12 weeks; body weight: 275 to 325 g; $n = 96$) were purchased from the Animal Center of Anhui Medical University. Prior to experimentation, 100 male rats were acclimatized for 1 week at a temperature of 20 to 25 °C, a relative humidity of 40% t 70%, and a noise level <60 db. Rats were allowed access to standard rat food and water ad libitum.

The animal model used in this study was based on the vein bridge restenosis model described previously by Tianshu-Chu et al[17]. The procedure used for surgery was as follows. After the rats were anesthetized, heparin was injected into the tail vein to induce heparinization. A vertical incision (approximately 1 cm) was made in the middle of the neck and the veins were dissociated on one side. Next, we grafted the jugular vein to the carotid artery. The incision was sutured after we verified that the pulse of the grafted vein was normal and there was no bleeding. This technique results in vein bridge restenosis 14 to 28 days after surgery. Our experiment was divided into 4 groups (24 rats per group): a high BSP dose group (10 mg BSP/0.3 ml pluronic gum [the HBG group]), a low BSP dose group (3 mg BSP/0.3 ml pluronic gum [the LBG group]), a pluronic-F127 gel group (the GEL group), and a control group (bypass only). The doses of BSP used in this study were lower than the minimum dose for human–rat conversion as stipulated by the Chinese Pharmacopoeia 2015; this means that the dose of BSP used was safe for rats. All animals were only given water on the first day; they were allowed their normal diet on the second day after surgery. After surgery, rats were assessed by B-scan ultrasonography every 3 days. Grafted vessels were then harvested on days 3, 14, and 28, after surgery (8 rats at each time point). We cut vein graft segments from two sides of the cuff. One segment was treated with 10% formalin for hematoxylin and eosin (H&E) staining, Masson's trichrome staining, and immunohistochemistry. The other segments were placed at 80 °C to await western blot analysis. Rats were sacrificed by overdose of sodium pentobarbital.

### Histology Analysis

We investigated the histology of the grafted vessels by H&E staining and Masson's trichrome staining; Masson's staining was carried out using a Masson kit (M029) that we acquired from Shanghai Gefan Biological Co., Ltd. (Shanghai, China). The thicknesses of the intimal, medial, and adventitial tissues were then determined five times in each section by two independent researchers to obtain a mean value. The stained sections of vein graft were then observed using an Olympus-BX53 microscope (Olympus, Hefei, China) and data analyzed using Image-Pro Plus 6 software (Media Cybernetics, Rockville, MD, USA).

### Immunohistochemistry

Immunohistochemical staining was carried out in accordance with the manufacturer's instructions. The sections were first deparaffinized and rehydrated, then incubated with 30 g/l of 3% hydrogen peroxide solution at room temperature for 10 min and then washed 5 times in distilled water. After deparaffinization, we then performed antigen retrieval with citrate buffer for 20 min. We then deactivated endogenous peroxidase by incubating sections in 3% $H_2O_2$ for 20 min and blocked the section with 5% bovine serum albumin for 10 min. These sections were then incubated overnight at 4°C with two antibodies PCNA (D3H8P, #13110, 1:5,000; Cell Signaling Technology Companies, Shanghai, China) and VCAM-1 (EPR5047, AB134047, 1:500; Abcam Biological Companies, MA, USA). The next morning, sections were washed 3 times, and then incubated for 1 h in biotinylated secondary antibody (horseradish peroxidase [HRP] Affini-Pure Goat Anti-Rat IgG; BS10043, BS12478, Bioworld Technology Co., Ltd, Shanghai, China) working solution and then HRP-conjugated streptavidin working solution. The sections were then washed in phosphate-buffered saline (PBS; pH 7.2) for 5 min. Positive immunostaining was visualized by diaminobenzidine tetrahydrochloride with hematoxylin as a counterstain. Finally, the sections were dehydrated, mounted, and sealed. An image signal acquisition and analysis system (Olympus-BX53) was used for image acquisition. Positive cells were defined as those containing a brown deposit and were detected in random microscopic fields at high magnification (200×).

### Western Blot

Each tissue sample was mixed with 200 μl of protein lysate (RIPA P0013B) and homogenized. Samples were then lysed for 30 min, centrifuged for 10 min (4°C and 12,000 rpm), and the supernatants were collected. We then used the bicinchoninic acid protein concentration determination kit (PC0020, Solarbio Co., Beijing, China) in accordance with the manufacturer's guidelines. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was then used to separate the proteins in each lysed sample; for each sample, we loaded 20 μg of total protein. Electrophoresis was carried out at 80 V for 30 min or 120 V for 70 min, depending on the size of the proteins involved. After electrophoresis, we transferred the separated proteins from the gel to a polyvinylidene difluoride (PVDF) membrane in an ice bath (at 300 mA for 60 min). Next, we diluted the antibodies in PBS and incubated the membranes with the primary antibodies at 4°C overnight. Three antibodies were used: PCNA (1:1,000 dilution, D3H8P, CST, USA), anti-Vcam1 (1:3,000 dilution, Abcam, ab134047, USA), and anti-β-tubulin (1:2,000 dilution, GB11017, Servicebio, USA). The following morning, the membranes were washed in PBS and then incubated with a secondary (goat anti-mouse IgG-HRP; 1:3,000 dilution; SE131, Solarbio, USA) for 1 h at 20°C. The membranes were then washed 5 times with Tris Buffered Saline Tween (TBST); we then added electrochemiluminescence (ECL) coloring solution (PE0010, Solarbio Co., Beijing, China). We then exposed photo on the PVDF film (3300016-7Q, Hefei, China). Separated immunoreactive bands were then visualized and analyzed by Odyssey v1.2 software (LI-COR, USA).

### Statistical Analysis

Data are presented as mean ± standard deviation and were processed using SPSS version 21.0 software (Chicago, IL, USA). Because the data followed a normal distribution, comparisons among multiple groups were analyzed by one-way analysis of variance. Comparisons between two groups were analyzed by Fisher's least significant difference test. A $P$-value <0.05 was considered statistically significant.

## Results

### Cardiovascular Characteristics

Following vein transplantation, color Doppler ultrasound examination for all groups confirmed that all blood vessels were unobstructed, and that graft blood vessel pulse and blood flow were stable; blood flow was maintained at 60-170 cm/s, and the pulse was maintained at 270 to 420 bpm. After the rats were anesthetized and an incision made, we observed that the grafted veins were well filled and pulsating. After ligating the two ends of the blood vessels, we found that the veins of rats in the control group were significantly narrower than the BSP treatment group ($P < 0.05$). The transplanted blood vessels presented with differing degrees of stenosis when examined 14 and 28 days after surgery; the grafted veins in the control group at 28 days were significantly narrower than those of the rats in the HBG group (Fig. 1; $P < 0.05$).

### BSP Reduced Intimal and Medial Thickening in Vein Grafts, as Determined by Staining with H&E and Masson's Trichrome

In order to evaluate the effect of BSP on intimal and medial hyperplasia, we performed H&E staining and Masson's

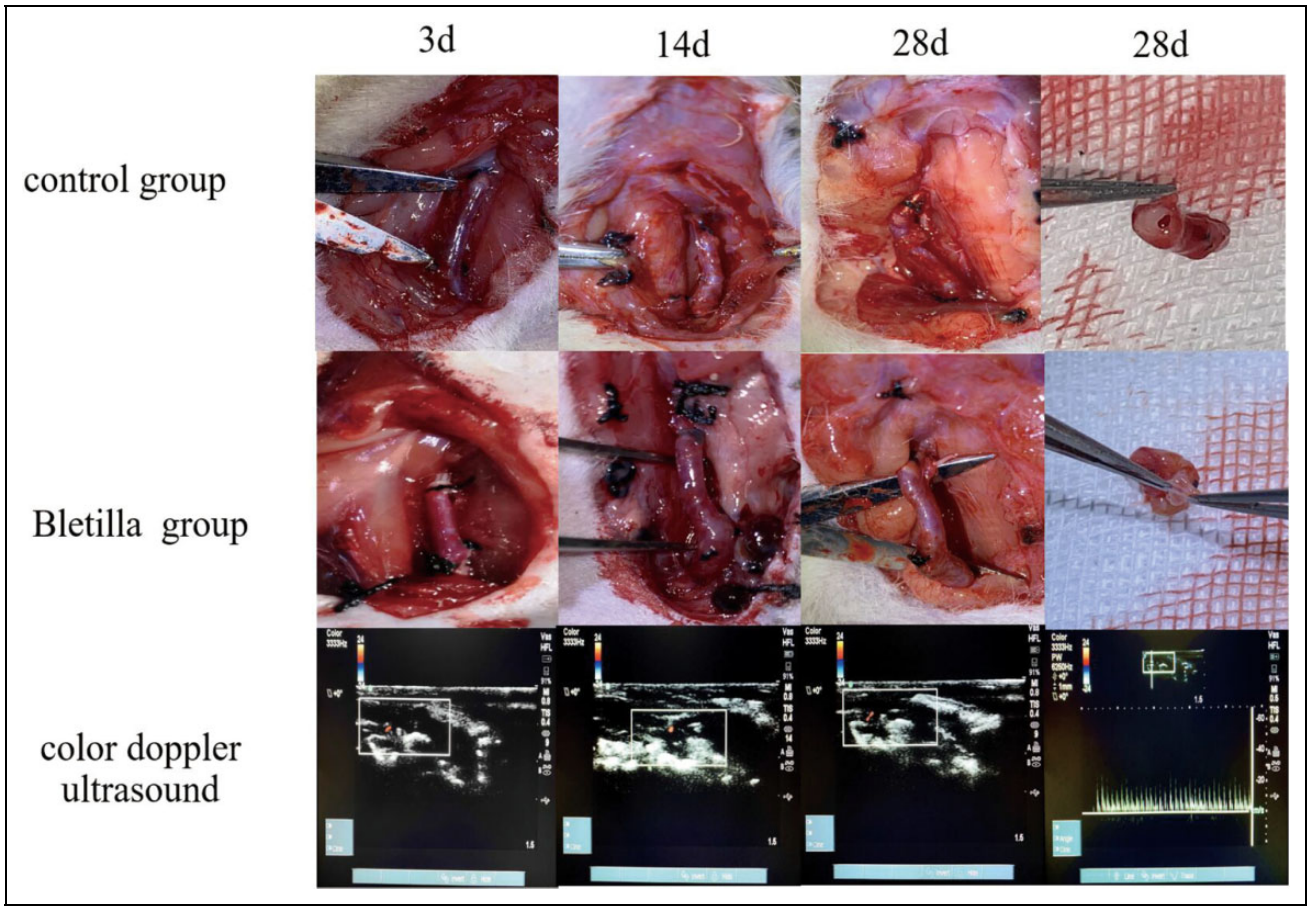


**Figure 1.** At 3, 14, and 28 days, we took out the graft vein and saw the graft vessels thickening and pulsing regularly. Color Doppler ultrasound was performed in rats after venous transplantation. The results indicated that all the transplanted vessels were unobstructed. d: days.

trichrome staining on days 3, 14, and 28 after surgery. There was no statistical difference between the gel group and the control group ($P > 0.05$). There were no significant differences between the control group and the gel group in terms of the thickness of the intima and media at 3, 14, and 28 days after grafting. Therefore, it was evident that the Pluronic F-127 gel had no effect on the thickness of the medial membrane and intima of the vein grafts.

Next, we measured the thickness of the intima; the intima in the control group was significantly thicker than in the HBS and LBS groups ($P < 0.01$). Results also showed that the intima of the LBS group was significantly thicker than that of the HBS group at 14 and 28 days (Fig. 2B; Table 1; $P < 0.05$).

Similarly, we also measured the thickness of the media, at 14 and 28 days, and the thickness of the media in the HBS and LBS groups was significantly reduced when compared with the control group ($P < 0.01$). Compared with the LBS group, the thickness of the media in the HBS group was significantly smaller (Fig. 2C; Table 1; $P < 0.05$).

In addition, the intima-to-media ratio in the HBS and LBS groups was significantly lower compared with that of the control group ($P < 0.01$). Compared with the LBS group, the intima-to-media ratio was significantly lower in the HBS group on days 14 and 28 (Fig. 2E; Table 1; $P < 0.05$).

## *BSP Reduced Cell Proliferation in Intimal and Medial of the Vein Graft, as Determined by Immunohistochemistry and Western Blotting*

The main components of the intima and media of vein graft are endothelial cells and smooth muscle cells, respectively[18]. We performed immunohistochemistry analysis and western blotting to determine the localization and levels of PCNA, an indicator of cell proliferation status, in order to explore the proliferation of endothelial cells and smooth muscle cells. There was no statistical difference between the gel group and the control group ($P > 0.05$); thus, the HBS and LBS groups were directly compared with the control group. With increasing time, the levels of PCNA in the transplanted veins gradually increased. BSP inhibited the production of PCNA, and this inhibitory effect increased as the dose of BSP increased (Fig. 3B, C; $P < 0.05$). These results indicated that BSP can inhibit the proliferation of both endothelial cells and VSMCs.

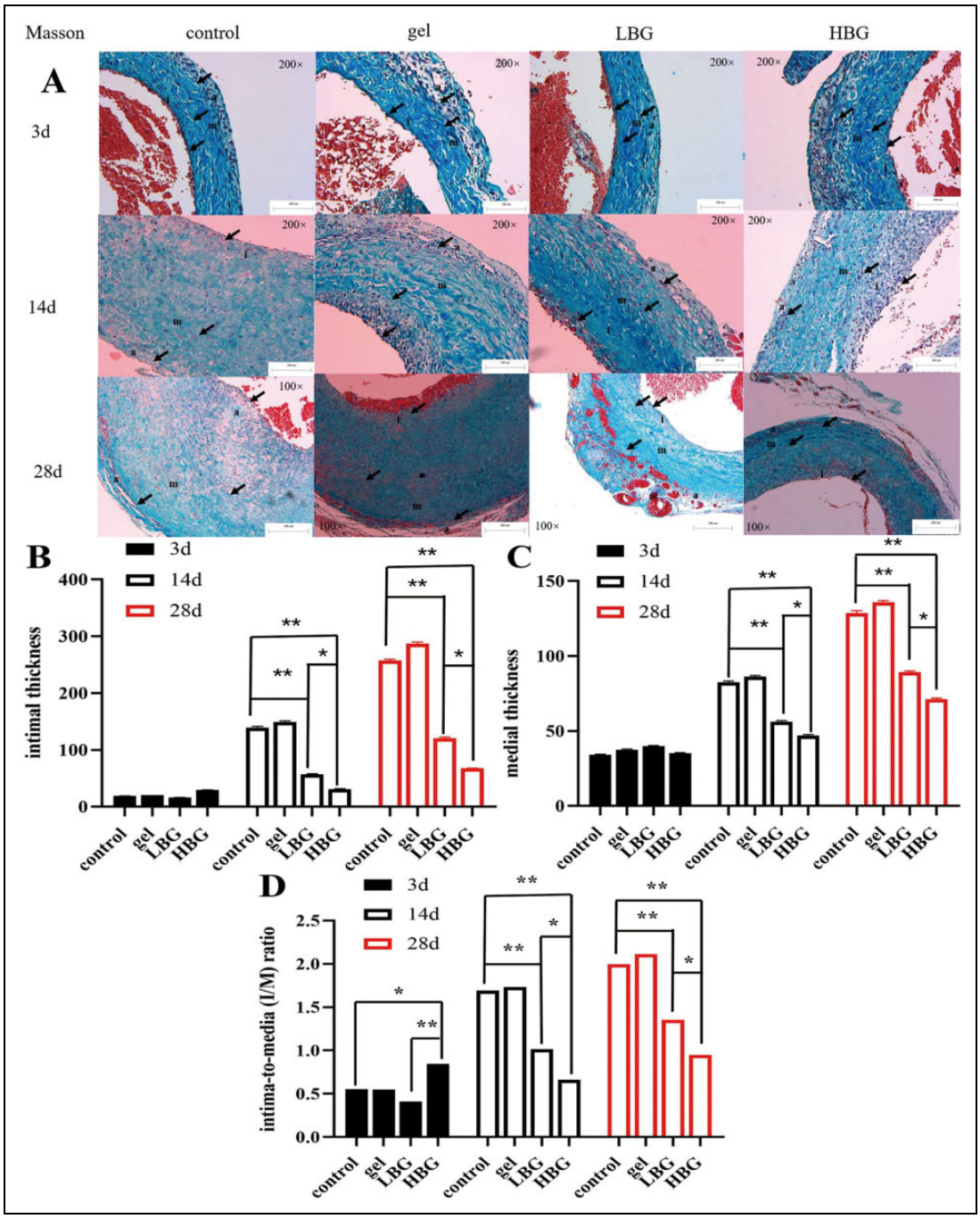


**Figure 2.** *Bletilla striata* polysaccharide inhibited the proliferation of the intima and medial membrane of the vein. (A) No significant difference was found in the four groups at 3-day (200×) time points, and the vessel wall thicknesses of HBG and LBG at 14-day (200×) and 28-day (100×) time points were decreased compared with the control group. The arrows indicate the intima, mesentery, and tunica adventitia of the blood vessels. (B) The change of intimal thickness, (C) the change of medial thickness, (D) the change of adventitia thickness, (E) intima-to-media (I/M) ratio. *$P < 0.05$, **$P < 0.01$.
a: adventitia of graft; i: intima of graft; m: middle venous membrane of graft.

## *BSP Inhibited the Expression of VCAM-1 Protein in vein Grafts, as Determined by Immunohistochemistry and Western Blotting*

Finally, we performed immunohistochemistry analysis and western blotting of VCAM-1. Compared with the control group, there was no inhibition of VCAM-1 secretion in response to BSP treatment when tested 3 days after surgery. However, on days 14 and 28, it was evident that BSP treatment had significantly inhibited the production of VCAM-1 ($P < 0.01$). Furthermore, compared with low-dose BSP, the

**Table 1.** The Thickness of Intimal, Medial, and Adventitia (μm).

| Group | Time (days) | Control | Gel | LBG | HBG | Analysis |
|---|---|---|---|---|---|---|
| Intima of graft | 3 | 18.90 ± 0.15 | 20.59 ± 0.07 | 16.33 ± 0.19 | 29.65 ± 0.23 | ANOVA |
| | 14 | 139.40 ± 1.93 | 149.58 ± 1.62 | 57.14 ± 0.94** | 31.08 ± 0.72** | ANOVA |
| | 28 | 257.03 ± 2.74 | 286.98 ± 2.92 | 120.83 ± 1.87** | 67.42 ± 0.54** | ANOVA |
| Middle of graft | 3 | 34.36 ± 0.19 | 37.56 ± 0.44 | 39.91 ± 0.41 | 35.14 ± 0.36 | ANOVA |
| | 14 | 82.50 ± 0.92 | 86.21 ± 0.87 | 56.34 ± 0.62** | 46.96 ± 0.70** | ANOVA |
| | 28 | 128.74 ± 1.53 | 135.75 ± 1.19 | 89.30 ± 0.77** | 71.21 ± 0.84** | ANOVA |

Note: The values of thickness ($n = 6$, mean ± SD). *$P < 0.05$, **$P < 0.01$ compared with the value of control group.
ANOVA: analysis of variance; SD: standard deviation.

inhibitory effect of high-dose BSP also increased (Fig. 4B, C; $P < 0.05$).

## Discussion

Despite advances in CAD therapies, CABG remains the main surgical treatment, and thus restenosis continues to plague the patency of vein grafts[19].

BSP is a TCM that is widely used for the regulation of hemostasis and inflammation[20]. BSP has a good anti-inflammatory effect and inhibits the release of various cytokines that are closely related to the progression of restenosis in venous bridge vessels[21]. Existing literature clearly shows that an inflammatory response plays an important role in the restenosis of venous bridge vessels[22]. Since BSP is known to exert anti-inflammatory properties, we hypothesized that this TCM may represent a potential treatment after CABG to prevent restenosis.

In the present study, we confirmed that the thickness of the intima and media of vein grafts significantly decreased following BSP treatment. In addition, we found that high-dose BSP inhibited vein graft vessel restenosis more significantly than low-dose BSP; thus, the effect of BSP on vein grafts was dose-dependent. Previous studies have shown that the process of restenosis in venous bridges created in the rat model normally occurs 2 to 4 weeks after surgery[23]. Consequently, we carried out postsurgical analysis on days 3, 14, and 28. Using a rat model, we found that BSP effectively prevented the development of restenosis in venous bridges. Pluronic-F127 gel is a commonly used drug-carrying gel that is inherently stable and has no inhibitory effect on restenosis in blood vessels[24,25]. Therefore, in this study, we used pluronic-F127 as a carrier for BSP in order to investigate the effect of BSP on restenosis in venous bridge vessels.

Intimal hyperplasia of vein grafts and the proliferation of medial smooth muscle cells are two of the most important factors underlying restenosis[26,27]. During surgery, endothelial cells in the venous bridge can be damaged, thus resulting in platelet adhesion, thrombosis, and vasospasm[28,29]. Simultaneously, these damaged endothelial cells can increase the sensitivity of cytokines and induce pathological changes in the VSMCs. This change eventually leads to dysplasia in the VSMC cells and their migration to the intima layer, thus leading to venous bridge stenosis and eventually the complete occlusion of the transplanted vessels[30,31]. PCNA is an important regulatory protein in the cell cycle, acts as a cofactor for DNA polymerase to assist in the synthesis of DNA, and facilitates the entry of cells into S-phase of the cell cycle. PCNA is also an established marker of cell proliferation and determines whether a cell will terminate differentiation or continue to proliferate[32–34]. Previous studies found that the blockade of PCNA expression led to an inhibition in the proliferation, migration, and secretion of the extracellular matrix of VSMCs, thus controlling the development of restenosis[35]. In our present experiment, we detected the localization and levels of PCNA by immunohistochemistry and western blotting and therefore demonstrated that BSP has an inhibitory effect on the cell proliferation of grafted veins. Similarly, we found that the inhibition of PCNA by BSP was dose-dependent.

Previous research has shown that VCAM-1 is closely related to restenosis in venous bridges[36,37]. VCAM-1 belongs to the immunoglobulin superfamily and can be expressed in endothelial cells, smooth muscle cells, and macrophages[38]. Some previous studies have observed an early increase in VCAM-1 levels within 6 h of injury, thus implying that VCAM-1 plays a role in early vascular inflammation and that the early application of a VCAM-1 antibody could inhibit the development of intimal hyperplasia[39]. Our results demonstrated that BSP inhibited early intimal hyperplasia and inflammation by inhibiting the production of VCAM-1. In addition, as the dose of BSP increased, its inhibitory effect on VCAM-1 also gradually increased.

Furthermore, although it has been confirmed in recent studies that the restenosis of a vein bridge is caused by the proliferation of endothelial cells and the migration of smooth muscle cells, there is still no perfect drug to inhibit this pathology[40]. In recent years, researchers have a number of drugs, such as paeonol[26], rapamycin[41], and atorvastatin[42]; however, none of these drugs are satisfactory. Our study confirmed the effect of BSP on inhibiting the restenosis of vein grafts in a rat model for the first time. This study provides a new treatment method for the restenosis of vein grafts after CABG.

In summary, our current data demonstrate the biological efficacy of BSP in a rat model of vein graft hyperplasia. Collectively, our analyses showed that BSP inhibited

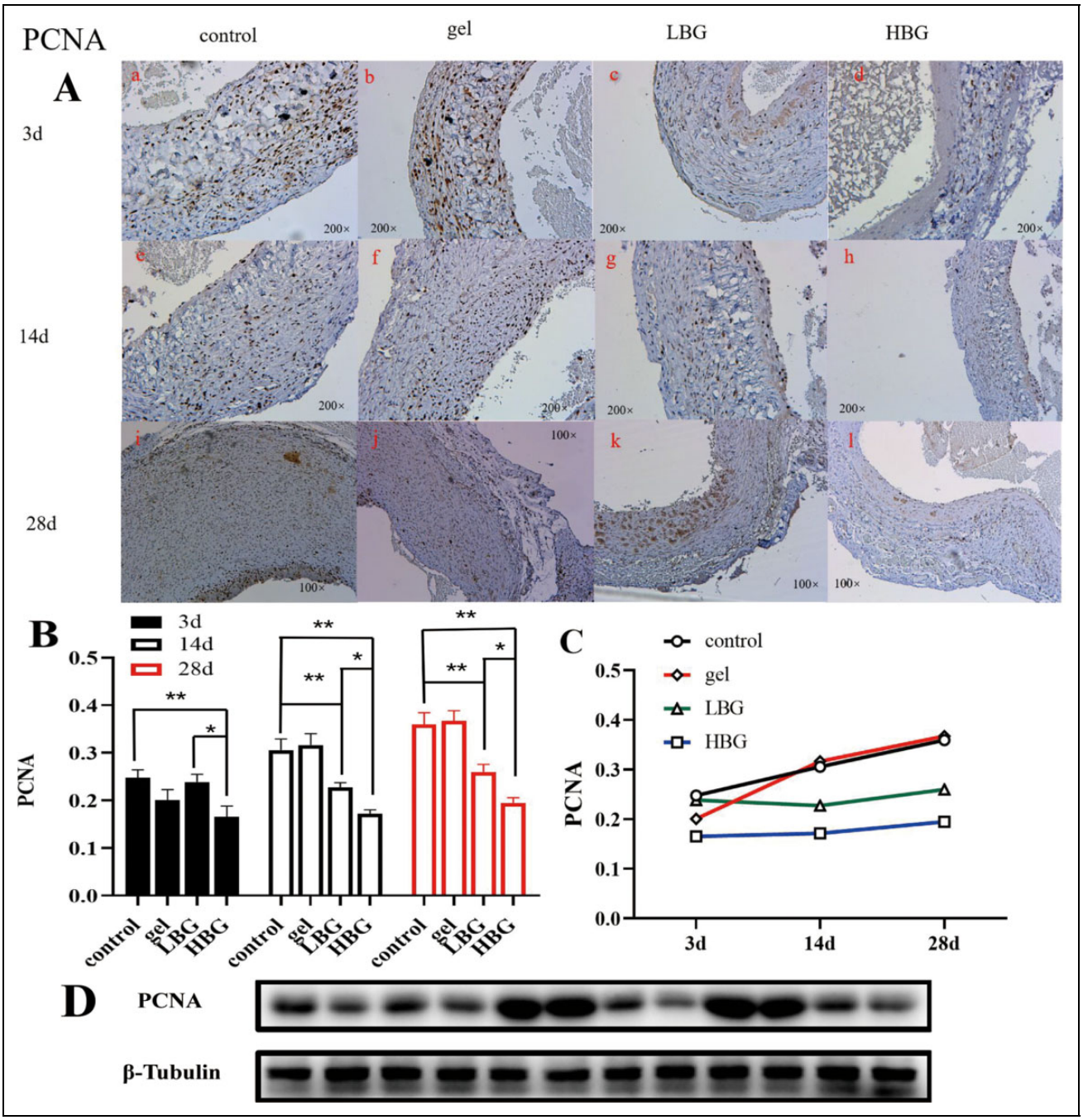


**Figure 3.** BSP inhibits the expression of PCNA in vein grafts. (A) There was no significant difference in the expression of PCNA in the 4 experimental groups at the 3-day (200×) time point, and the expression of PCNA in HBG and LBG at the 14-day (200×) and 28-day (100×) time points was lower than in the control group. The decrease of PCNA in HBG was more than in LBG, and the difference was statistically significant ($P < 0.05$). (B) The expression of PCNA by immunohistochemistry. (C) The expression of PCNA changed with time. (D) Expression of PCNA in western blotting (*$P < 0.05$, **$P < 0.01$).
D: days; PCNA: proliferating cell nuclear antigen.

restenosis in the grafted veins and that a high dose of BSP has a stronger inhibitory effect on restenosis than a low dose of BSP. However, the specific mechanism of action and pharmacokinetics associated with the effect of BSP on vascular endothelial cells and smooth muscle cells have yet to be elucidated. In future, we plan to conduct further research to identify the specific mechanisms responsible for how BSP can inhibit restenosis in vein grafts. In this study, high-dose BSP had a stronger inhibitory effect on restenosis than low-dose BSP, although the dosage remained small. Further

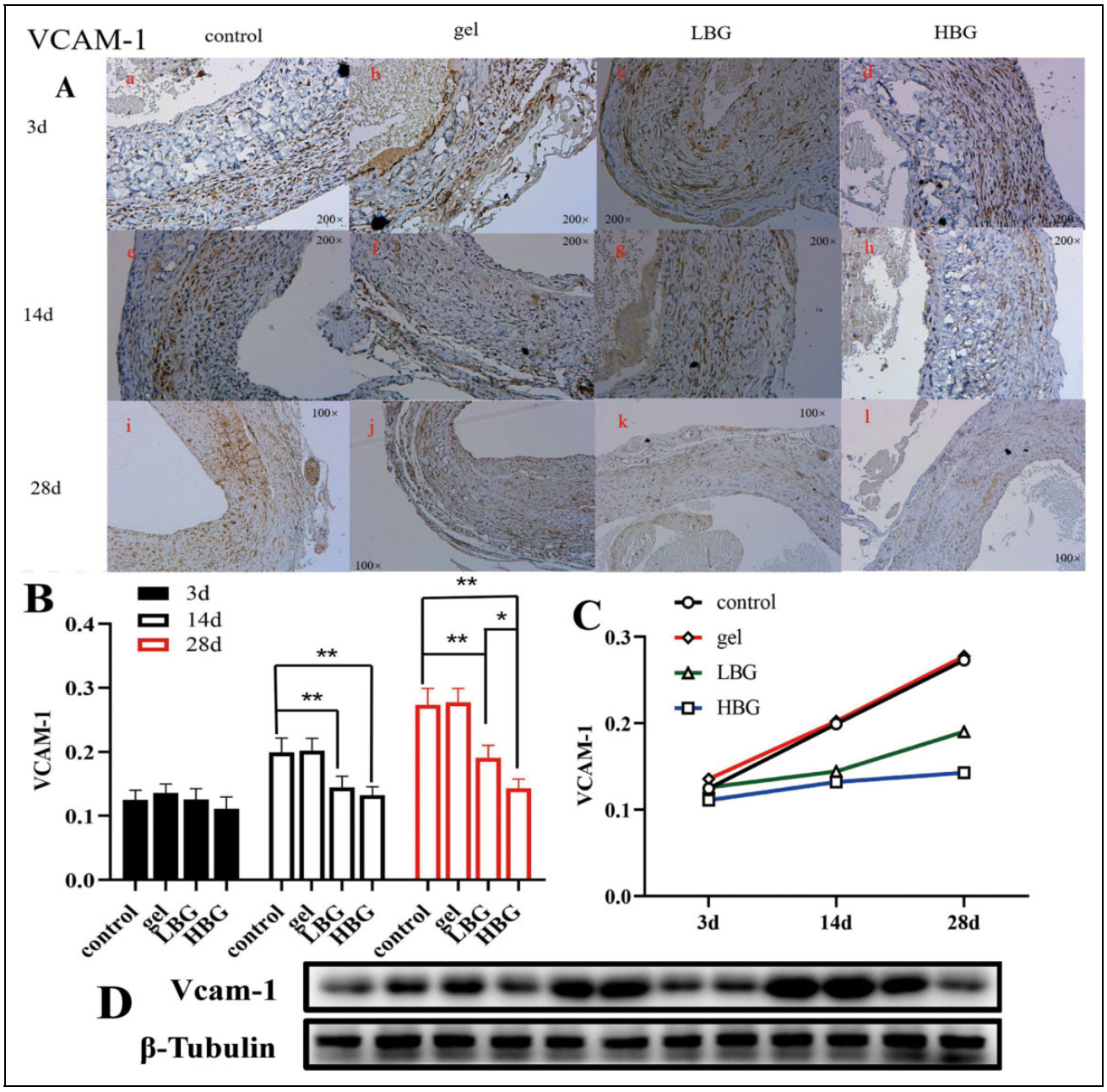


**Figure 4.** BSP inhibits the expression of VCAM-1 in vein grafts. (A) VCAM-1-positive cells were stained brown by immunohistochemical staining. The decrease of VCAM-1 in HBG is more than LBG, and the difference is statistically significant ($P < 0.05$). (B) The expression of VCAM-1 by immunohistochemistry. (C) The expression of VCAM-1 changed with time. (D) Expression of VCAM-1 in western (*$P < 0.05$, **$P < 0.01$).
d: days; VCAM-1: vascular cell adhesion molecule 1.

studies are now needed to define the optimal therapeutic dosage of BSP. In the present study, we used rats as experimental animals, which cannot fully represent human reactions and cannot be observed over long periods of time. The porcine model of artery bypass grafting would be more clinically relevant and appropriate for long-term observations than the rat model because of the close resemblance between pigs and humans with regard to the coronary circulation. Our team will conduct further research on intimal hyperplasia pathophysiological processes in pigs after CABG and the impacts of related interventions on grafted veins.

## Conclusion

Collectively, our present results confirmed that BSP inhibited intimal hyperplasia and promoted vascular patency in a

rat model. These positive effects appeared to be associated with reductions in intimal thickening, cell proliferation in the vein graft, and the expression levels of VCAM-1. It is clear that BSP can prevent restenosis in venous bridges, at least to some extent, thus providing a new treatment option for the prevention and treatment of restenosis in venous bridges following CABG surgery. However, while the effects of BSP seem promising, the long-term effects and clinical significance of BSP for CABG surgery still need to be investigated.

## Author Contributions

Chun-Dai contributed to conceptualization, methodology, resources, and writing the original draft; Yang-Zhou to formal analysis and investigation; Bing-Zhang to visualization and supervision; and Jianjun-Ge to conceptualization, writing—review and editing, project administration, and funding acquisition.

## Ethical Approval

This study was approved by the Ethics Committee on Animal Experiments of the Anhui Animal Ethics Committee, Anhui Province, China (Protocol number AH56743).

## Statement of Human and Animal Rights

All of the experimental procedures used in this study were conducted in accordance with the Institutional Animal Care Guidelines of Anhui Medical University, China and were approved by the Ethics Committee on Animal Experiments of the Anhui Animal Ethics Committee, Anhui Province, China.

## Statement of Informed Consent

There are no human subjects in this article. Consequently, informed consent is not applicable.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## ORCID iD

Chun Dai https://orcid.org/0000-0001-8765-3351

## Study Association

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## References

1. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, et al. Heart disease and stroke statistics-2019 update: a report from the american heart association. Circulation. 2019;139(10):e56–e528.
2. Thuijs D, Kappetein AP, Serruys PW, Mohr FW, Morice MC, Mack MJ, Holmes DR Jr, Curzen N, Davierwala P, Noack T, Milojevic M, et al. Percutaneous coronary intervention versus coronary artery bypass grafting in patients with three-vessel or left main coronary artery disease: 10-year follow-up of the multicentre randomised controlled SYNTAX trial. Lancet. 2019;394(10206):1325–1334.
3. Watanabe H, Shiomi H, Morimoto T, Furukawa Y, Nakagawa Y, Ando K, Kadota K, Tazaki J, Watanabe H, Natsuaki M, Minatoya K, et al. Percutaneous coronary intervention versus coronary arterial bypass grafting in patients with multi-vessel coronary revascularization (from the CREDO-Kyoto PCI/CABG registry/cohort-2). Catheter Cardiovasc Interv. 2020;96(1):42–51.
4. Janiec M, Friberg Ö, Thelin S. Long-term clinical outcomes after coronary artery bypass grafting with pedicled saphenous vein grafts. J Cardiothorac Surg. 2018;13(1):122.
5. Schröder J, Vogt F, Burgmaier M, Reith S, Almalla M. Long-term clinical outcomes after treatment of stent restenosis with two drug-coated balloons. Coron Artery Dis. 2018;29(8):632–637.
6. de Vries MR, Simons KH, Jukema JW, Braun J, Quax PH. Vein graft failure: from pathophysiology to clinical outcomes. Nat Rev Cardiol. 2016;13(8):451–470.
7. Guida G, Ward AO, Bruno VD, George SJ, Caputo M, Angelini GD, Zakkar M. Saphenous vein graft disease, pathophysiology, prevention, and treatment. a review of the literature. J Card Surg. 2020;35(6):1314–1321.
8. Chumachenko PV, Ivanova AG, Belokon EV, Akchurin RS. [Adhesion molecules and mononuclear cell subpopulations in the coronary and pulmonary arteries of patients with coronary heart disease]. Arkh Patol. 2015;77(6):9–14.
9. Huang Z, Liu Y, Liang L, Liu W, Sooranna SR, Mo J, Liu L, Li Z, Li K, Guo J. Correlation between coronary stenosis and Toll-like receptors 2 and 4 levels in chinese zhuang patients with coronary heart disease. Exp Ther Med. 2019;18(3):2346–2352.
10. Simons KH, de Vries MR, Peters HAB, Hamming JF, Jukema JW, Quax PHA. The protective role of Toll-like receptor 3 and type-I interferons in the pathophysiology of vein graft disease. J Mol Cell Cardiol 2018;121:16–24.
11. Korotaeva ES, Koroleva LY, Kovaleva GV, Kuzmenko EA, Nosov VP. Double antiplatelet therapy in patients with acute coronary syndrome after percutaneous coronary intervention: individual efficacy and hemorrhagic safety of P2Y12 blockers of ticagrelor and clopidogrel in actual clinical practice [in Russian]. Kardiologiia. 2019;59(suppl 5):27–36.
12. Yagi S, Kondo D, Ise T, Fukuda D, Yamaguchi K, Wakatsuki T, Kawabata Y, Ito H, Saijo Y, Seno H, Todoroki T, et al. Association of decreased docosahexaenoic acid level after statin therapy and low eicosapentaenoic acid level with in-stent restenosis in patients with acute coronary Syndrome. J Atheroscler Thromb. 2019;26(3):272–281.
13. Chen Z, Cheng L, He Y, Wei X. Extraction, characterization, utilization as wound dressing and drug delivery of bletilla

striata polysaccharide: a review. Int J Biol Macromol. 2018; 120(Pt B):2076–2085.
14. Jiang F, Li M, Wang H, Ding B, Zhang C, Ding Z, Yu X, Lv G. Coelonin, an anti-inflammation active component of bletilla striata and its potential mechanism. Int J Mol Sci. 2019; 20(18):4422
15. Li JY, Kuang MT, Yang L, Kong QH, Hou B, Liu ZH, Chi XQ, Yuan MY, Hu JM, Zhou J. Stilbenes with anti-inflammatory and cytotoxic activity from the rhizomes of bletilla ochracea schltr. Fitoterapia. 2018;127:74–80.
16. Luo S, Song S, Zheng C, Wang Y, Xia X, Liang B, Feng G. Biocompatibility of bletilla striata microspheres as a novel embolic agent. Evid Based Complement Alternat Med. 2015; 2015:840896.
17. Tianshu C, Congrong G, Zhiwei Z, Fei L, Ayu S, Yuanbiao Z, Jing C, Ge J. Rapamycin combined with α-cyanoacrylate contributes to inhibiting intimal hyperplasia in rat models. Arq Bras Cardiol. 2019;112(1):3–10.
18. Klein B, Destephens A, Dumeny L, Hu Q, He Y, O'Malley K, Jiang Z, Tran-Son-Tay R, Berceli S. Hemodynamic influence on smooth muscle cell kinetics and phenotype during early vein graft adaptation. Ann Biomed Eng. 2017;45(3): 644–655.
19. Caliskan E, de Souza DR, Böning A, Liakopoulos OJ, Choi YH, Pepper J, Gibson CM, Perrault LP, Wolf RK, Kim KB, Emmert MY. Saphenous vein grafts in contemporary coronary artery bypass graft surgery. Nat Rev Cardiol. 2020;17(3): 155–169.
20. He X, Wang X, Fang J, Zhao Z, Huang L, Guo H, Zheng X. Bletilla striata: medicinal uses, phytochemistry and pharmacological activities. J Ethnopharmacol. 2017;195:20–38.
21. Liu B, Zhang Q, Wu X, Fu Y, Wang H, Guan Y, Yang S, Liu Y, Cao W, Wang J. Effect of Bletilla striata on the Prevention of Postoperative Peritoneal Adhesions in Abrasion-Induced Rat Model. Evid Based Complement Alternat Med 2019;2019: 9148754.
22. Han S, Xu S, Zhou J, Qiao A, Boriboun C, Ma W, Li H, Biyashev D, Yang L, Zhang E, Liu Q, et al. Sam68 impedes the recovery of arterial injury by augmenting inflammatory response. J Mol Cell Cardiol. 2019;137:82–92.
23. Wang D, Tediashvili G, Pecha S, Reichenspurner H, Deuse T, Schrepfer S. Vein interposition model: a suitable model to study bypass graft patency. J Vis Exp 2017;15(119): 54839.
24. Dung TH, Huong LT, Yoo H. Morphological feature of pluronic f127 and its application in burn treatment. J Nanosci Nanotechnol. 2018;18(2):829–832.
25. Wang H, Williams GR, Wu J, Wu J, Niu S, Xie X, Li S, Zhu LM. Pluronic F127-based micelles for tumor-targeted bufalin delivery. Int J Pharm. 2019;559:289–298.
26. Zhang JY, Lei L, Shang J, Huo TM, Zhang B, Chen G, Zeng ZY, Li SK. Local application of paeonol prevents early restenosis: a study with a rabbit vein graft model. J Surg Res. 2017; 212:278–287.
27. Kim FY, Marhefka G, Ruggiero NJ, Adams S, Whellan DJ. Saphenous vein graft disease: review of pathophysiology, prevention, and treatment. Cardiol Rev. 2013;21(2):101–109.
28. Zhang SZ, Wang GX, Zhou XT. The clinical application of microincision vein harvesting of the great saphenous vein in coronary artery bypass grafting. BMC Cardiovasc Disord. 2020;20(1):297.
29. Ren W, Liang L, Li Y, Wei FY, Mu N, Zhang L, He W, Cao Y, Xiong D, Li H. Upregulation of miR-423 improves autologous vein graft restenosis via targeting ADAMTS-7. Int J Mol Med. 2020;45(2):532–542.
30. Wu W, Zhang W, Choi M, Zhao J, Gao P, Xue M, Singer HA, Jourd'heuil D, Long X. Vascular smooth muscle-MAPK14 is required for neointimal hyperplasia by suppressing VSMC differentiation and inducing proliferation and inflammation. Redox Biol. 2019;22:101137.
31. Li G, Chen SJ, Oparil S, Chen YF, Thompson JA. Direct *in vivo* evidence demonstrating neointimal migration of adventitial fibroblasts after balloon injury of rat carotid arteries. Circulation. 2000;101(12):1362–1365.
32. Kang HJ, Park H, Yoo EJ, Lee JH, Choi SY, Lee-Kwon W, Lee KY, Hur JH, Seo JK, Ra JS, Lee EA, et al. TonEBP Regulates PCNA Polyubiquitination in Response to DNA Damage through Interaction with SHPRH and USP1. Science. 2019; 19:177–190.
33. Sheng C, Mendler IH, Rieke S, Snyder P, Jentsch M, Friedrich D, Drossel B, Loewer A. PCNA-mediated degradation of p21 coordinates the dna damage response and cell cycle regulation in individual cells. Cell Rep. 2019;27(1):48–58.
34. Strzalka W, Ziemienowicz A. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. Ann Bot 2011;107(7):1127–1140.
35. Huang B, Hu P, Hu A, Li Y, Shi W, Huang J, Jiang Q, Xu S, Li L, Wu Q. Naringenin attenuates carotid restenosis in rats after balloon injury through its anti-inflammation and anti-oxidative effects via the RIP1-RIP3-MLKL signaling pathway. Eur J Pharmacol. 2019;855:167–174.
36. Liu X, Qu C, Zhang Y, Fang J, Teng L, Zhang R, Zhang X, Shen C. Chemokine-like factor 1 (CKLF1) aggravates neointimal hyperplasia through activating the NF-κB /VCAM-1 pathway. FEBS Open Bio. 2020;10(9):1880–1890.
37. Li H, Han Y, Qi R, Wang Y, Zhang X, Yu M, Tang Y, Wang M, Shu YN, Huang W, Liu X, et al. Aggravated restenosis and atherogenesis in ApoCIII transgenic mice but lack of protection in ApoCIII knockouts: the effect of authentic triglyceride-rich lipoproteins with and without ApoCIII. Cardiovasc Res. 2015;107(4):579–589.
38. Radecke CE, Warrick AE, Singh GD, Rogers JH, Simon SI, Armstrong EJ. Coronary artery endothelial cells and microparticles increase expression of VCAM-1 in myocardial infarction. Thromb Haemost. 2015;113(3):605–616.
39. Edlinger C, Lichtenauer M, Wernly B, Pistulli R, Paar V, Prodinger C, Krizanic F, Thieme M, Kammler J, Jung C, Hoppe UC, et al. Disease-specific characteristics of vascular cell

adhesion molecule-1 levels in patients with peripheral artery disease. Heart Vessels. 2019;34(6):976–983.
40. Montenegro FS, Correia M, Muccillo F, Souza ESCG, De Lorenzo A. Associations between endothelial progenitor cells, clinical characteristics and coronary restenosis in patients undergoing percutaneous coronary artery intervention. BMC Res Notes. 2018;11(1):278.
41. Liu Y, Yang F, Zou S, Qu L. Rapamycin: a bacteria-derived immunosuppressant that has anti-atherosclerotic effects and its clinical application. Front Pharmacol. 2018;9:1520.
42. Roopmani P, Krishnan UM. Harnessing the pleiotropic effects of atorvastatin-fenofibrate combination for cardiovascular stents. Mater Sci Eng C Mater Biol Appl. 2018;92:875–891.