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Polyester vascular patches acquire arterial or venous identity depending on their environment

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Abstract

Polyester is commonly used in vascular surgery for patch angioplasty and grafts. We hypothesized that polyester patches heal by infiltration of arterial or venous progenitor cells depending on the site of implantation. Polyester patches were implanted into the Wistar rat aorta or inferior vena cava and explanted on day 7 or 30. Neointima that formed on polyester patches was thicker in the venous environment compared to the amount that formed on patches in the arterial environment. Venous patches had more cell proliferation and greater numbers of VCAM-positive and CD68-positive cells, whereas arterial patches had greater numbers of vimentin-positive and alpha-actin-positive cells. Although there were similar numbers of endothelial progenitor cells in the neointimal endothelium, cells in the arterial patch were Ephrin-B2- and notch-4-positive while those in the venous patch were Eph-B4- and COUP-TFII-positive. Venous patches treated with an arteriovenous fistula had decreased neointimal thickness; neointimal endothelial cells expressed Ephrin-B2 and notch-4 in addition to Eph-B4 and COUP-TFII. Polyester patches in the venous environment acquire venous identity, whereas patches in the arterial environment acquire arterial identity; patches in the fistula environment acquire dual arterial-venous identity. These data suggest that synthetic patches heal by acquisition of identity of their environment.

Keywords

polyester patch; neointimal hyperplasia; arteriovenous fistula; endothelial cell identity; patch angioplasty

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Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

It has been almost 60 years since polyester was introduced for use in cardiovascular surgery.¹ Polyester vascular grafts are still widely used in vascular procedures today,^{2,3} including reconstruction of the aorta^{4,5} and peripheral arteries.^{6,7} Polyester-covered stents are also used for endovascular reconstruction.^{8,9} Polyester patches are used clinically, especially in carotid artery reconstruction, despite some studies reporting increased stenosis in polyester patches compared to vein patches.^{10–12} However, while the application of polyester in the arterial system is very common, polyester is infrequently used in the venous system, primarily attributed to the lower blood flow and higher chance of thrombosis.^{13–17} In the venous system, since there is also a high incidence of anastomotic neointimal hyperplasia and stenosis, a distal arteriovenous fistula (AVF) is typically created to maintain the patency of the polyester graft, both in human and animal models.^{18–20}

The importance of vascular identity to healing of vascular devices and interventions is beginning to be understood. Vascular endothelial cell identity is genetically predetermined; Eph-B4 is a determinant of venous identity, whereas Ephrin-B2 is a determinant of arterial identity.²¹ In a rat pericardial patch angioplasty model,²² CD34/VEGFR2 dual-positive cells, that is, endothelial progenitor cells (EPC), contribute to neointimal endothelialization of patches, with acquisition of cell identity dependent on the particular vascular environment into which the patch was implanted; endothelial cells in the neointima that formed on pericardial patches implanted into the aorta acquired arterial identity whereas cells in patches implanted into the inferior vena cava (IVC) acquired venous identity.^{23–25} Of note, the thickness of the venous neointima was dependent on venous flow and could be manipulated via distal AVF creation and ligation.²⁴

The response to implantation of polyester grafts has been studied, with accumulation of smooth muscle cells, myofibroblasts, and macrophages mainly at the anastomoses, and various degrees of neointimal and midbody endothelialization.^{26–30} However, despite these data, it is not clear whether polyester patches heal with neointimal endothelial cells acquiring endothelial cell identity; it is also not clear whether polyester heals differently in the venous and arterial environments. We hypothesized that polyester patches acquire the identity of their environment, have increased neointimal thickness in the venous environment compared to the arterial environment, and that increased flow reduces the neointimal thickness that forms on the polyester surface.

MATERIALS AND METHODS

Animal model

All experiments were approved by the Institutional Animal Care and Use Committee at the Yale University School of Medicine; NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. Male Wistar rats (6–8-week old) were used for patch implantation ($n = 28$) as previously described.^{22–24,31} Microsurgical procedures were performed aseptically using a dissecting microscope (Leica MZ 95, Germany). Polyester patches (collagen impregnated knitted polyethylene terephthalate fabric, approximately 3 mm × 1.5 mm × 0.6 mm; LeMaitre Vascular,

Burlington, MA) were implanted into the rat aorta or IVC using running 10–0 nylon sutures. In some rats, an aortocaval fistula was created immediately after patch implantation as previously described.²⁴ Rats were sacrificed on postoperative days 7, 14, or 30 and the patches were explanted for analysis as described below. No immunosuppressive agents, antibiotics, antiplatelet agents, or heparin were given at any time.

Histology and EVG staining

Rats were anesthetized with isoflurane inhalation, and tissues were fixed by transcardial perfusion of phosphate buffered saline (PBS) followed by 10% formalin. Tissue was removed and fixed overnight in 10% formalin followed by a 24-h immersion in 70% alcohol. Tissue was then embedded in paraffin and sectioned (4 μ m thickness). Tissue sections were deparaffinized and stained with hematoxylin and eosin or EVG staining kit (Artisan Elastic Stain Kit, Dako). Neointimal and adventitial thickness were the mean of measurements from the surface edge to the edge of the patch in three independent areas.

Immunohistochemistry

Tissue sections were deparaffinized and then incubated using primary antibodies overnight at 4°C. After overnight incubation, the sections were incubated with EnVision reagents for 1 h at room temperature and treated with Liquid DAB Substrate Chromogen System (Dako). Finally, the sections were counterstained with Mayer's Hematoxylin (Dako).

Immunofluorescence

Tissue sections were deparaffinized and then incubated with primary antibodies overnight at 4°C. The sections were incubated with secondary antibodies for 1 h at room temperature, after which sections were stained with the fluorescent dye 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) to mark cellular nuclei.

Primary and secondary antibodies

Primary antibodies included: anti- α -actin (Abcam, ab5694; IHC and IF, 1:200); anti-cleaved Caspase-3 (Cell Signaling #9661; IHC, 1:50); anti-CD34 (R&D, AF4117; IF, 1:100); anti-CD68 (ED1; Abcam, ab31630; IF, 1:50); anti-COUP-TFII (Novus Biologicals, NBP1–67885; IF, 1:100); anti-Eph-B4 (Santa Cruz, SC5536; IF, 1:50); anti-eNOS (phospho S1177) antibody (Abcam, ab184154; IF, 1:50); anti-non-muscle Myosin IIB antibody (Abcam, ab684; IF, 1:50); anti-notch-4 antibody (Santa Cruz, SC5594; IF, 1:50); antiproliferating cell nuclear antigen (PCNA) (Dako, monoclonal mouse Anti-PCNA; IF, 1:100); and anti-VEGFR2 (Abcam, ab2349; IF, 1:100). Anti-Von Willebrand Factor antibody (Abcam, ab11713; IF, 1:100).

Secondary antibodies used for IF were donkey anti-goat Alexa-Fluor-488, donkey anti-rabbit Alexa-Fluor-568, and donkey anti-mouse Alexa-Fluor-568-conjugated antibodies (Invitrogen, 1:500).

Statistical analysis

Data are expressed as the mean $\pm$ SEM. Statistical significance for these analyses was determined by *t* test or ANOVA (Prism 6; GraphPad Software, La Jolla, CA). *p*-values <0.05 were considered significant.

RESULTS

Thicker neointimal thickness in polyester patch venoplasty than angioplasty

To compare the response to polyester patch angioplasty in a small diameter artery and vein, we used the patch angioplasty model to repair the rat aorta and IVC. Polyester patches were sewn into the rat aorta and IVC as previously described.^{23,31} Prior to implantation, there were no cells in the polyester patch (Fig. 1A,F). After implantation, polyester patches were incorporated into the rat aorta and IVC wall without any thrombus (Fig. 1B,C). At day 7, there was neointima formation on the patch luminal side in both the aorta and IVC angioplasty; however, the neointima of the IVC patch angioplasty was much thicker compared to the neointima of the aorta patch angioplasty (Fig. 1C,D). There was no difference in “adventitia” thickness formed on the outer (peritoneal) surface of the patch (Fig. 1C,E). A similar number of cells infiltrated into the patch body both in the aorta and IVC angioplasty (Fig. 1C,F). At day 30, the neointima was thicker than at day 7 in both of the aorta and IVC angioplasty, and the neointima was still thicker in the IVC angioplasty compared to aorta angioplasty (Fig. 1C,D). More cells infiltrated into the patch body compared to day 7, with a similar number of cells in both the aorta and IVC angioplasty (Fig. 1C,F). The outer “adventitia” also became thicker compared to day 7, with a similar thickness in both of the aorta and IVC angioplasty (Fig. 1C,E).

Cell composition and turnover of the patch neointima

Since venous polyester patches have thicker neointima than arterial patches, we examined the cell composition in the neointima. A thicker layer of smooth muscle cells covered the luminal side of the polyester patch in the IVC patch angioplasty compared to a much thinner layer of smooth muscle cell in the aorta patch angioplasty (Fig. 2A). At day 30, after aorta patch angioplasty, the smooth muscle cell layer became thicker and most cells were spindle shaped; however, after IVC patch angioplasty, there were several layers of smooth muscle cells near the surface of the neointima, but only some cells were spindle-shaped, suggesting a more synthetic phenotype in the venous cells (Fig. 2A). Above the smooth muscle cells was a layer of vWF-positive cells (Fig. 2A). Some vWF-positive cells colocalized with VCAM-1 (Fig. 2A,B). There were also more vWF and p-eNOS dual-positive cells in the aorta patch angioplasty neointima compared to the IVC patch angioplasty both at day 7 and day 30 (Fig. 2A,B). There were some SM-MHC-positive SMC in the neointima, both at day 7 and day 30, that colocalized with alpha-actin-positive SMC (Fig. 2A). There were also some vimentin-positive SMC in the neointima both at day 7 and day 30 (Fig. 2A,B). In addition, there were also CD68-positive cells in the neointima, patch body and the adventitia; there were more CD68-positive cells in the neointima of IVC patch angioplasty compared to the aorta patch angioplasty at day 7 and day 30 (Fig. 2A,B). There were more CD3 positive cells in the neointima after IVC patch angioplasty compared to aorta patch angioplasty (Fig. 2A,B).

To determine if increased proliferation or less apoptosis contributed to the thicker neointima in the venous patch compared to the arterial patch, we examined cell turnover in the aorta and IVC patch angioplasty neointima. There were more PCNA-positive cells in the IVC patch angioplasty neointima compared to the aorta patch angioplasty neointima at day 7 (Fig. 3A,B). There was a significantly lower number of PCNA-positive cells at day 30 compared to day 7 (Fig. 3A,B). While there was a significantly larger number of cleaved caspase-3 positive cells in the IVC angioplasty compared to the aorta angioplasty at day 7, there was a similar fraction of cleaved caspase-3 positive cells in the aorta and IVC angioplasty at day 30 (Fig. 3A,C). In the neointima, some cells were immunoreactive with both anti-PCNA and anti- α -actin antibodies, suggesting SMC proliferation in the neointima (Fig. 3A). There were more PCNA-positive smooth muscle cells in the IVC patch angioplasty neointima compared to the aorta patch angioplasty neointima at day 7 (Fig. 3A,D).

Neointimal cell identity

EPC play important roles in neovascularization and vascular adaptation, including re-endothelialization after injury.^{23,32,33} There was a layer of CD34 and VEGFR2 dual-positive cells on the luminal side of the neointima in both the aorta and IVC angioplasty (Fig. 4A). There was no difference in the number of CD34 and VEGFR2 dual-positive cells in the neointima, both at day 7 and day 30 (Fig. 4B). There were similar numbers of PCNA-positive, CD34-positive, and VEGFR2-positive cells in the neointima, both at day 7 and day 30 (Supporting Information Fig. 1A,B).

We examined neointimal endothelial cell identity using immunofluorescence. In the embryo, Eph-B4 is a determinant of venous identity, whereas Ephrin-B2 is a determinant of arterial identity; venous cells express Eph-B4 both during development as well as in adult veins.²¹ Immunofluorescence showed that in the aorta polyester patch angioplasty neointima, vWF-positive cells colocalized with the arterial identity markers Ephrin-B2 and notch-4 at day 30, but with rare expression of the venous identity markers Eph-B4 and COUP-TFII (Fig. 4C–G), while in the IVC polyester patch angioplasty neointima, vWF-positive cells colocalized with Eph-B4 and COUP-TFII at day 30, but with rare expression of Ephrin-B2 and notch-4 (Fig. 4C–G). There was a significantly higher number of Ephrin-B2- and notch-4-positive endothelial cells in aorta angioplasty neointima compared to the IVC polyester patch angioplasty (Fig. 4D,E). There was also a significantly higher number of Eph-B4- and COUP-TFII-positive endothelial cells in IVC angioplasty neointima compared to those in aorta polyester patch angioplasty neointima (Fig. 4F,G). There was a similar expression of Ephrin-B2 and notch-4 in the PCNA and vWF dual-positive cells in the aorta angioplasty neointima at day 30; and likewise, there was similar expression of Eph-B4 and COUP-TFII in the PCNA and vWF dual-positive cells in the IVC angioplasty neointima at day 30 (Supporting Information Fig. 1C,D). In toto, these data are consistent with endothelial cells acquiring the identity of their arterial or venous microenvironment after prosthetic patch angioplasty.

Higher blood flow decreases venous neointimal thickness

A distal AVF increases prosthetic graft patency in human patients requiring vascular surgery.^{34,35} We previously showed that in a pericardial patch angioplasty model there was a thinner neointima on the IVC patch angioplasty in the presence of a distal AVF.³¹ Therefore, we examined whether there is a decreased neointimal thickness in the rat IVC polyester patch angioplasty in the presence of a distal AVF. After AVF creation, the color of blood flow in the IVC changed to red immediately (Supporting Information Fig. 2A). The patency of the fistula was 100% percent at days 7 and 14, and the IVC lumen area distal to the fistula increased in the presence of AVF at days 7 and 14 (Supporting Information Fig. 2A–C). Both at day 7 and day 14, there was a significantly thinner neointima and larger lumen area in the IVC angioplasty in the presence of a distal AVF compared to the neointima and lumen in the IVC angioplasty without an AVF (Fig. 5A,B); however, there was no difference in cell infiltration into the patch or in the adventitia thickness whether there was an AVF or not (Fig. 5A,B). There was also no difference in CD34 and VEGFR2 dual-positive cells on the neointimal surface, both at day 7 and day 14, without or with an AVF (Fig. 5C,D). Interestingly, there were also more vWF and p-eNOS dual-positive cells in the IVC patch angioplasty neointima, both at day 7 and day 14, in the presence of an AVF (Fig. 5D and Supporting Information Fig. 2D). There were fewer CD68-positive and PCNA-positive cells in the IVC patch angioplasty in the presence of an AVF compared to the IVC patch angioplasty alone, both at day 7 and day 14 (Fig. 5C,D and Supporting Information Fig. 2D). There were a similar number of cleaved caspase-3 positive cells in the neointima of both the IVC patch angioplasty with or without an AVF, at days 7 and 14 (Fig. 5C,D and Supporting Information Fig. 2D); these results are consistent with the AVF reducing SMC proliferation and neointimal hyperplasia that forms on the patch. Interestingly, there were more Ephrin-B2- and notch-4-positive endothelial cells in the presence of an AVF compared to IVC patch angioplasty alone (Fig. 5E,F); there were also more Eph-B4- and COUP-TFII-positive endothelial cells in the presence of an AVF compared to IVC patch angioplasty alone (Fig. 5E,F). These results suggest that the presence of the fistula environment alters neointimal thickness as well as the identity of neointimal endothelial cells.

DISCUSSION

Although some aspects of the healing of polyester vascular grafts have been described,^{36,37} the identity of the neointima that forms on polyester grafts, and its differential expression in different environments, has not been previously reported. We show that polyester patches develop thicker neointima in the venous environment compared to the arterial environment (Figs. 1–3). In addition, polyester patches heal with accumulation of EPC that differentially acquire arterial or venous identity depending on the vessel environment into which they are implanted (Fig. 4). Interestingly, addition of an AVF not only diminishes neointimal thickness that forms on polyester patches implanted into the venous environment, but the venous neointimal cells acquire dual arterial-venous identity (Fig. 5). These results suggest that prosthetic patches heal *in vivo* and support differential accumulation of vascular progenitor cells in different environments.

Polyester is widely used for surgical repair of large arteries, such as its use as aortobifemoral bypass grafts and for aortic aneurysm repair^{38,39}; however, polyester is used much less frequently in the venous system. Graft thrombogenicity with low venous blood flow and the tendency of vein walls to collapse are thought to be responsible for the high occlusion and failure rates of prosthetic grafts placed in the venous system⁴⁰; venous polyester grafts had only 25% patency at 3 months in a dog model.⁴¹ Consistent with these observations, we show that in the rat polyester patch model, the luminal neointima was significantly thicker in veins compared to that in arteries (Fig. 1). Low magnitudes of shear stress, such as found in the venous system, are associated with a thick neointima and increased SMC proliferation compared to higher magnitudes of shear stress such as found in the arterial system^{42,43}; in addition, other factors such as increased growth factor synthesis and secretion as well as lower oxygen tension promote proliferation in the venous cells compared to arterial cells.^{42,44–47} Thus, increased neointimal hyperplasia in the venous system provides another potential reason why prosthetics are infrequently used for venous reconstruction.^{48–51} Similarly, in a pericardial patch model, that is, a collagen-based scaffold, we have previously shown that patch venoplasty is associated with greater neointimal thickness and EPC infiltration compared to patch arterioplasty²⁴; however, our data show similar amounts of EPC infiltration into venous and arterial polyester patches (Fig. 4A), suggesting potentially different mechanisms of healing in polyester and pericardial patches. Polyester is noncompliant compared to other commonly implanted vascular biomaterials, and accordingly may support less neoendothelialization.^{52–55}

Smooth muscle cells play an important role in pathogenesis of neointimal hyperplasia.⁵⁶ In the neointima that forms on both venous and arterial patches, alpha-actin/MHC dual-positive cells were present with greater number of these cells in arterial compared to venous patches (Fig. 2A). Although the role of the environment on SMC differentiation and phenotype change is recognized, the particular factors that regulate these processes remains unclear.^{57,58}

Vascular surgeons create distal AVF in human patients to increase blood flow and patency, both in arterial reconstruction^{34,35} as well as in venous reconstruction.^{59–61} Similar results were shown in animal models, with increased flow inhibiting SMC migration and reducing neointimal hyperplasia.^{48,50,62,63} Pericardial patch venoplasty is also associated with reduced neointimal thickness in the presence of an AVF,²⁴ similar to our results with polyester patch venoplasty (Fig. 5). These results provide rationale for the clinical use of AVF with polyester venous reconstruction, despite the ongoing clinical controversy regarding the effects of distal AVF on long-term lower extremity prosthetic arterial graft patency.^{64–70}

Although vascular cell identity is genetically predetermined and present in the embryo, the arterial or venous microenvironment can also play a role in determining endothelial cell identity^{21,71}; however it is not established whether vessel identity is plastic in adult cells or whether plasticity is restricted to embryonic, stem cell, and neoplastic environments.^{72–75} We show that the neointima that forms in adult rats acquires arterial identity in the aorta and venous identity in the IVC (Fig. 4); these results show that vessel identity may be plastic in adults. Interestingly, in the presence of an AVF, venous neointima acquires both arterial and

venous identity (Fig. 5E,F). It is not clear whether shear stress is the principle stimulus for this change in identity,^{71,72,76,77} or whether other environmental features such as pressure or oxygen tension are important stimuli. However, acquisition of dual arterial-venous identity in the fistula environment is distinctly different than our previous results showing loss of venous identity, without gain of arterial environment, in the vein graft.⁷⁸ It is possible that gain of dual identity in the fistula environment reflects neointimal formation on an acellular pericardial or polyester patch, whereas vein graft adaptation reflects venous endothelial injury; however, the mechanisms by which a fistula may induce dual arterial-venous identity are not known.

In summary, we showed that conventionally used polyester patches attract EPC after implantation, with acquisition of specific identity dependent on the microenvironment into which the patch is implanted. Addition of an AVF reduces venous neointimal hyperplasia and induces gain of dual arterial-venous identity. These results provide rationale for the clinical observation that addition of a distal AVF increases the patency of vascular bypass grafts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Increased neointimal thickness after polyester patch angioplasty in rat aorta and inferior vena cava (IVC). A: H&E and EVG staining of the preimplantation polyester patch; scale bar, 100 μm . B: Polyester patch angioplasty of the rat aorta and IVC at day 7; A, aorta; IVC, inferior vena cava; P, patch; ruler markings are 1 mm. C: H&E staining of the polyester patch on postoperative days 7 and 30. Upper row, low power (scale bar, 1 mm); middle and lower rows, high power shows the neointimas and patches at day 7 or 30 after angioplasty; P, patch; L, aorta or IVC lumen; N, neointima; scale bar, 100 μm . D: Bar graph showing increased neointimal thickness; *day 7, $p = 0.0093$; *day 30, $p = 0.0293$; $n = 4-7$. E: Bar

graph showing increased cell infiltration into the patch body (mean number of cells counted in four high power fields); $n = 4-7$. F: Bar graph showing adventitial thickness; $n = 4-7$.

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FIGURE 2.

Cell composition of the neointima after polyester patch angioplasty. A:

Immunohistochemistry and immunofluorescence analysis of the neointima at day 7 or 30.

First row, merge of vWF (green), α -actin (red), and DAPI (blue); second row, merge of vWF (green), VCAM-1 (red), and DAPI (blue); third row, merge of vWF (green), p-eNOS (red), and DAPI (blue); fourth row, merge of MHC (green), α -actin (red), and DAPI (blue); fifth row, merge of vimentin (green), α -actin (red), and DAPI (blue); sixth row, CD3 (red) and DAPI (blue); seventh row, immunohistochemistry analysis of the CD68. L, lumen; scale bar, 100 μ m; $n = 4-7$. Arrows show the positive cells. B: Bar graphs showing endothelial confluence, vWF and VCAM-1 dual-positive cells (*day 7, $p = 0.0128$; *day 30, $p = 0.0102$), vWF and p-eNOS dual-positive cells (*day 7, $p = 0.0002$; *day 30, $p < 0.0001$), MHC and α -actin dual-positive cells (*day 7, $p = 0.0223$; *day 30, $p = 0.0003$), vimentin and α -actin dual-positive cells (*day 30, $p = 0.0001$), CD3 (*day 7, $p = 0.0249$), and CD68 (*day 7, $p = 0.0026$; *day 30, $p = 0.0010$).

FIGURE 3.

Cell turnover after polyester patch angioplasty. A: Immunohistochemistry and immunofluorescence analysis of the neointima at day 7 or 30. Upper row, immunohistochemistry stained with PCNA; middle row, immunohistochemistry stained with cleaved caspase-3; lower row, merge of α -actin (green), PCNA (red), and DAPI (blue); scale bar, 100 μ m. L, lumen; N, neointima; arrows show the positive cells or dual-positive cells; $n = 4-7$. B: Bar graph showing the number of neointimal PCNA-positive cells, *day 7, $p = 0.0005$; *day 30, $p = 0.0025$; $n = 4-7$. C: Bar graph showing the percentage of neointimal cleaved caspase-3 positive cells; * $p = 0.0636$; $n = 4-7$. D: Bar graph showing the number of neointimal PCNA-positive smooth muscle cells; * $p = 0.0076$; $n = 4-7$.

FIGURE 4.

Neointimal endothelial cell identity after polyester patch angioplasty. A:

Immunofluorescence analysis for CD34 (green), VEGFR2 (red), and DAPI (blue) of the neointima at day 7 (upper row) and day 30 (lower row); yellow arrow shows CD34 and VEGFR2 dual-positive cells in the neointima; L, lumen; N, neointima; scale bar, 100 μ m; $n = 4-7$. B: Bar graph showing the CD34 and VEGFR2 dual-positive cells number in the neointima; $n = 4-7$. C: Immunofluorescence analysis of the neointima at day 30; analysis for: first column, merge of vWF (green), Ephrin-B2 (red), and DAPI (blue); second column, merge of vWF (green), notch-4 (red), and DAPI (blue); third column, merge of vWF (green), Eph-B4 (red), and DAPI (blue); fourth column, merge of vWF (green), COUP-TFII (red), and DAPI (blue); L, lumen; N, neointima; scale bar, 100 μ m; yellow arrow shows the dual-positive cells. $n = 4-7$. D-G: Bar graphs showing the percentage of Ephrin-B2 ($*p = 0.0056$), notch-4 ($*p = 0.0090$), Eph-B4 ($*p = 0.0027$), and COUP-TFII ($*p = 0.0050$) positive endothelial cells.

FIGURE 5.

Fistula flow decreases venous neointimal thickness. A: Representative H&E stained photomicrographs showing patches harvested at day 7 and day 14 with or without a distal aortocaval fistula; upper row, low power, scale bar, 1 mm; lower row, high power photomicrographs showing the neointima; scale bar, 100 μm; P, patch; N, neointima; L, IVC lumen; $n = 3$. B: Bar graphs showing the lumen area (*day 7, $p = 0.0082$; *day 30, $p = 0.0041$), neointimal thickness (*day 7, $p = 0.0334$; *day 30, $p = 0.0032$), adventitial thickness, number of cells infiltrated into the patch; $n = 3$. C: Upper row, merge of CD34 (green), VEGFR2 (red), and DAPI; middle row, merge of α -actin (green), PCNA (red), and DAPI; lower row, merge of CD68 (green), Ki67 (red), and DAPI (blue); scale bar, 100 μm; P, patch; N, neointima; L, IVC lumen; $n = 3$; yellow arrow shows the positive cells. D: Bar graphs showing CD34 and VEGFR2 dual-positive cells number, vWF and p-eNOS dual-positive cells number (*day 7, $p = 0.0004$; *day 30, $p = 0.0002$), CD68-positive cells number (*day 7, $p = 0.0478$; *day 30, $p = 0.0220$), PCNA-positive cell percentage (*day 7, $p = 0.0060$; *day 30, $p = 0.0223$), cleaved caspase-3 positive cell percentage, PCNA-positive smooth muscle cells number (*day 7, $p = 0.0078$; *day 30, $p = 0.0029$), and Ki67-positive macrophages number (*day 7, $p = 0.0241$; *day 30, $p = 0.0132$) in the neointima; $n = 3$. E: Comparison of the neointima with or without a distal aortocaval fistula at day 14; analysis for: first column, merge of vWF (green), Ephrin-B2 (red), and DAPI (blue); second column, merge of vWF (green), notch-4 (red), and DAPI (blue); third column, merge of vWF (green), Eph-B4 (red), and DAPI (blue); fourth column, merge of vWF (green), COUP-TFII (red), and DAPI (blue); N, neointima; scale bar, 100 μm; yellow arrow shows the dual-positive

cells. $n = 3$. F: Bar graphs showing percentage of Ephrin-B2 ($*p = 0.0026$), notch-4 ($*p = 0.0005$), Eph-B4 ($*p = 0.0228$), and COUP-TFII ($*p = 0.0237$) positive endothelial cells; $n = 3$.

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