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The Effect of Placing Flow-Diverting Stents in Intracranial Collateral Arteries of Miniature Pig

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AEF 1 Jiwei Wang
A 2 Yanan Ding
C 3 Qiuxia Wang
C 4 Yanan Wang
A 5 Shiqing Mu
A 6 Lixin Bi
BDG 5 Youxiang Li

1 Beijing Neurosurgical Institute, Capital Medical University, Beijing, P.R. China
2 Department of Neurosurgery, Affiliated Hospital of Hebei University, Baoding, Hebei, P.R. China
3 Department of Respiratory Medicine, Baoding No. 1 Central Hospital, Baoding, Hebei, P.R. China
4 Department of Pathology, Affiliated Hospital of Hebei University, Baoding, Hebei, P.R. China
5 Department of Interventional Neuroradiology, Tiantan Affiliated Hospital of Capital Medical University, Beijing Neurosurgical Institute, Beijing Engineering Research Center for Interventional Neuroradiology, Beijing, P.R. China
6 Department of Tuberculosis, Affiliated Hospital of Hebei University, Baoding, Hebei, P.R. China

Corresponding Authors: Shiqing Mu, e-mail: shiqingmu623@sina.com, Youxiang Li, e-mail: youxiangli623@sina.com
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Background: Flow-diverting stent (FDS) has been suggested as an effective intracranial aneurysm treatment. However, the effects of FDS on collateral branches of an aneurysm parent artery still remain unknown. Thus, the present study aimed to comprehensively evaluate the effects of placing a FDS in the intracranial collateral artery, using a miniature pig animal model.

Material/Methods: Ten healthy miniature pigs were included in the study: one pig was reserved as a control and the remaining nine pigs were placed in three experimental groups: FDS (i.e., Pipeline), LVIS, and Solitaier-AB stent groups. Pigs in the experimental groups were examined by cerebral angiography immediately after stent placement, followed by hemodynamic analyses. In addition, magnetic resonance imaging (MRI) of the brain of pigs in the experimental groups was performed to inspect the brain for obstruction and blood flow. Stents were examined for the growth of neointimas.

Results: The results showed that neointimas, consisting of smooth muscle cells, collagenous fibers, and macrophages, were 0.67 mm thick on average and partially covered the stent wires. The thickness of neointimas in the FDS group was significantly higher than in the two conventional intracranial stent groups. There was no obvious obstruction identified in collateral arteries where the FDS was placed.

Conclusions: These results indicated that neointimas in collateral arteries of a miniature pig would be slightly thickened after one month of FDS placement; and FDS was shown to be safe for collateral arteries.

MeSH Keywords: Arteries • Neointima • Neurosurgery

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Background

Intracranial aneurysm is a very common neurological disease, which is suffered by 5–10% of the general population [1,2]. It is a major health care concern since the rupture of an aneurysm can lead to subarachnoid hemorrhage, placing the patient in great danger [2,3]. It has been suggested that the occurrence, development, and even rupture of aneurysms are mainly caused by hemodynamic factors [4]. In recent years, the flow-diverting stent (FDS), which can change the hemodynamic environment of an aneurysm, has been widely used in clinical practice as a treatment for intracranial aneurysm [5]. Particularly, FDS has the advantage of a high metal coverage rate as well as a low mesh rate, thus promoting the recovery from intracranial aneurysm disease by reducing blood exchange between the aneurysm and its parent artery, which could lead to thrombosis and growth of neointimas if not managed.

Although stents have been widely used in treating arterial aneurysm, it is still unknown whether stents affect blood flow in other vessels besides the aneurysm parent artery. To address this concern, several studies have already been conducted to evaluate the effects of different stents on arteries in different part of the body. For example, Whitbread et al. [6] implanted self-expanding stents in abdominal aortas of large white pigs with stent wires covering the opening of renal arteries. No obstructions were observed in renal arteries when checked six weeks after surgery by angiography and histological examination. However, using a scanning electron microscope, neointimas were found partially covering the stent wires across the opening of the renal arteries. In addition, Lee et al. [7] implanted 18 nitinol mesh stents into abdominal aortas, iliac arteries, and renal arteries of six dogs. All artery branches remained unobstructed when checked by angiography at one day, three weeks, four weeks, and 14 weeks after surgery. Moreover, Masuo et al. [8,9] put stainless steel stents into abdominal aortas of healthy rabbits, as well as artery atherosclerotic rabbits, with stent wires covering the lumbar arteries' branches. Lumbar arteries of all healthy rabbits expect for one were unobstructed. In these rabbits, new blood vessel endothelial cells partially covered the stent wires located at the opening of the lumbar arteries, narrowing the arteries. Fortunately, the narrowing was not serious enough to affect the hemodynamics of the arteries. However, the situation was worse for the artery atherosclerotic rabbits, where very thick intimal hyperplasia was observed. Wakhloo et al. [10] implanted five self-expanding stents into the vertebral arteries of dogs, with stents covering intramuscular vessels (of about 0.5 mm diameter), which were derived from a cervical segment of a vertebral artery. Covered vessels were kept unobstructed with no blockage or narrowing of the opening observed on follow-up angiography nine months later. Levy et al. [11] compared the application of self-expanding stents and balloon-mounted stents in dog brain arterial thrombotic treatments and found that thin stent wires in self-expanding stents led to safer release and smaller impairments to vessel walls. Lanzino et al. [12] also reported that thinner stent wires and larger mesh could avoid or alleviate blocks in collateral arteries. These studies used different animal models to investigate the effects of different stents on arteries in different parts of the body, and the results consistently showed that the self-expanding stents led to relatively safe release, and small impairments to vessels.

Although FDS has been popularly used in clinical practice, it still remains unclear whether the placement of a FDS device in an aneurysm parent artery would induce any effects on perforating arteries and collateral branches. Indeed, such an understanding is important for the application of FDS in the treatment of intracranial aneurysm. In clinical practice, collateral arteries are unavoidably covered when using FDS in intracranial aneurysm treatment. For example, the ophthalmic artery, the anterior choroidal artery, as well as the posterior communicating artery will be covered in the treatment of internal carotid aneurysm; or the posterior cerebral artery, as well as the anterior cerebellar artery, will be covered when a stent is implanted in the basilar artery. Thus, the present study aimed to investigate the effects of FDS on collateral branches with the adoption of an animal model using miniature pigs. The study compared the effects of three types of stents: the FDS and two other conventional self-expanding stents (i.e., LVIS stent and Solitaire-AB stent) on collateral branches.

Material and Methods

Animals

Ten healthy miniature pigs (either gender, 15–20 kg) were purchased from the Animal Centre of Capital Medical University. This study was approved by the Tiantan Affiliated Hospital of Capital Medical University.

Experimental procedures

One pig was reserved as a control group, and nine pigs were equally divided into three self-expanding stent subgroups (n=3 for each group): one FDS (i.e., Pipeline) group, one LVIS stent group, and one Solitaire-AB group. The stents were placed in intracranial arteries of pigs in each subgroup, respectively. Pigs were treated with 10 mg/kg aspirin and clopidogrel by gavage every day beginning three days before surgery and continuing until 30 days after surgery. Anesthesia induction was performed by intramuscular injection of 0.2 mL/kg Su Mian Xin (i.e., 846 complex, combinations of xylazole, EDTA, DHE, and haloperidol), followed by intravenous anesthesia maintenance (1% pentobarbital sodium, 10 mg/kg for two doses).

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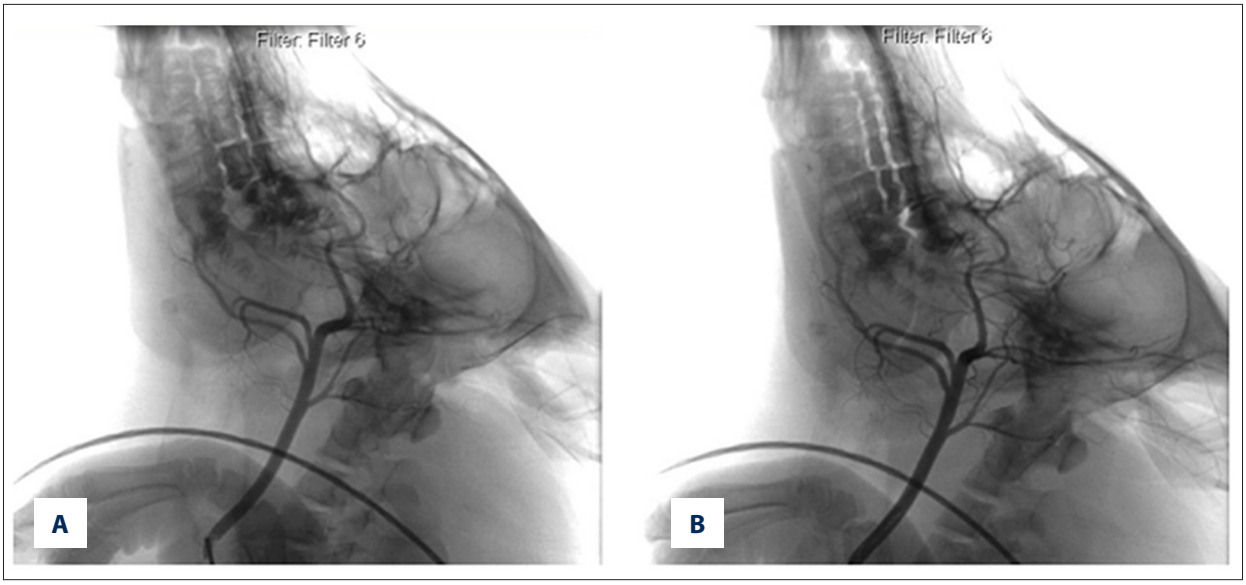

Figure 1. (**A, B**) Results of angiography. No stenosis in arteries and no obstruction in collateral branches were observed after stent implantation.

The right femoral artery was exposed in a sterile environment, and a 5F-vascular sheath was inserted by arteriotomy of the femoral artery. Intravenous heparin (100 U/kg) injection was then conducted. A 5F-guiding catheter was inserted into the internal carotid artery, and contrast agent was injected. Micro guide wire was positioned into the internal carotid artery and was pulled out after guiding the matching micro catheter into the internal carotid artery. A stent was inserted into one internal carotid artery segment containing at least one collateral branch with the guidance of the micro guide wire. Angiography was once again performed after pulling out the micro guide wire of the inserted stent. The guiding catheter as well as the vascular sheath were evacuated, and the femoral artery was ligated. After recovery from anesthesia, each pig was injected with penicillin sodium solution (200,000 U) as anti-inflammatory prophylactic. Treated pigs were fed an experimental animal center diet and received brain MRI scans one month later. Then the pigs were sacrificed by an overdose of thiopental sodium.

Pathologic examination

For each pig, the artery with the stent was extracted, cleaned by normal saline, and fixed in 4% formalin solution for at least 24 hours. Artery tissues were excided longitudinally. The coverage of stents by neointimas, and any obstruction of branches were observed by microscopy. After observation, stent wires were picked out carefully from artery tissues with the help of a dissecting microscope. The tissues were dehydrated conventionally and embedded in paraffin. Then the tissues were cut into 5 mm slices and treated by hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining. IHC staining was conducted mainly for labeling α-actin (first antibody, mouse anti-human monoclonal antibody, Shanghai Jiehao Biotechnology Co., Ltd.), CD68 (first antibody, mouse anti-human monoclonal antibody, Shanghai Jiehao Biotechnology Co., Ltd.), and endothelin-VIII (first antibody, mouse anti-rabbit monoclonal antibody, Beijing Zhongshanjinqiao Company) by SP method.

Patency of the artery and its collateral branches after the stent had been implanted was assessed by a neurologist physician with more than 20 years of experience analyzing the results of angiograms. One month after the stent implantation, a brain MRI scan of brain infarctions was evaluated by a radiologist with more than 20 years of experience analyzing the results of brain MRI scans. Stenosis of the vessels and patency of collateral branches covered by net wires were also observed visually by cutting open specimens longitudinally. Coverage of neointimas was observed under a microscope (Leica MZ12). A pathologist with more than 10 years of experience was invited to help measure the thickness of neointimas and observe their compositions.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 and results were represented by mean ±SD. Comparisons among multiple groups were conducted by single factor variance of analysis (ANOVA). Pairwise comparisons in the same group were performed by Fisher Least Significant Difference (LSD) paired-t test. Significance level was set as $p<0.05$.

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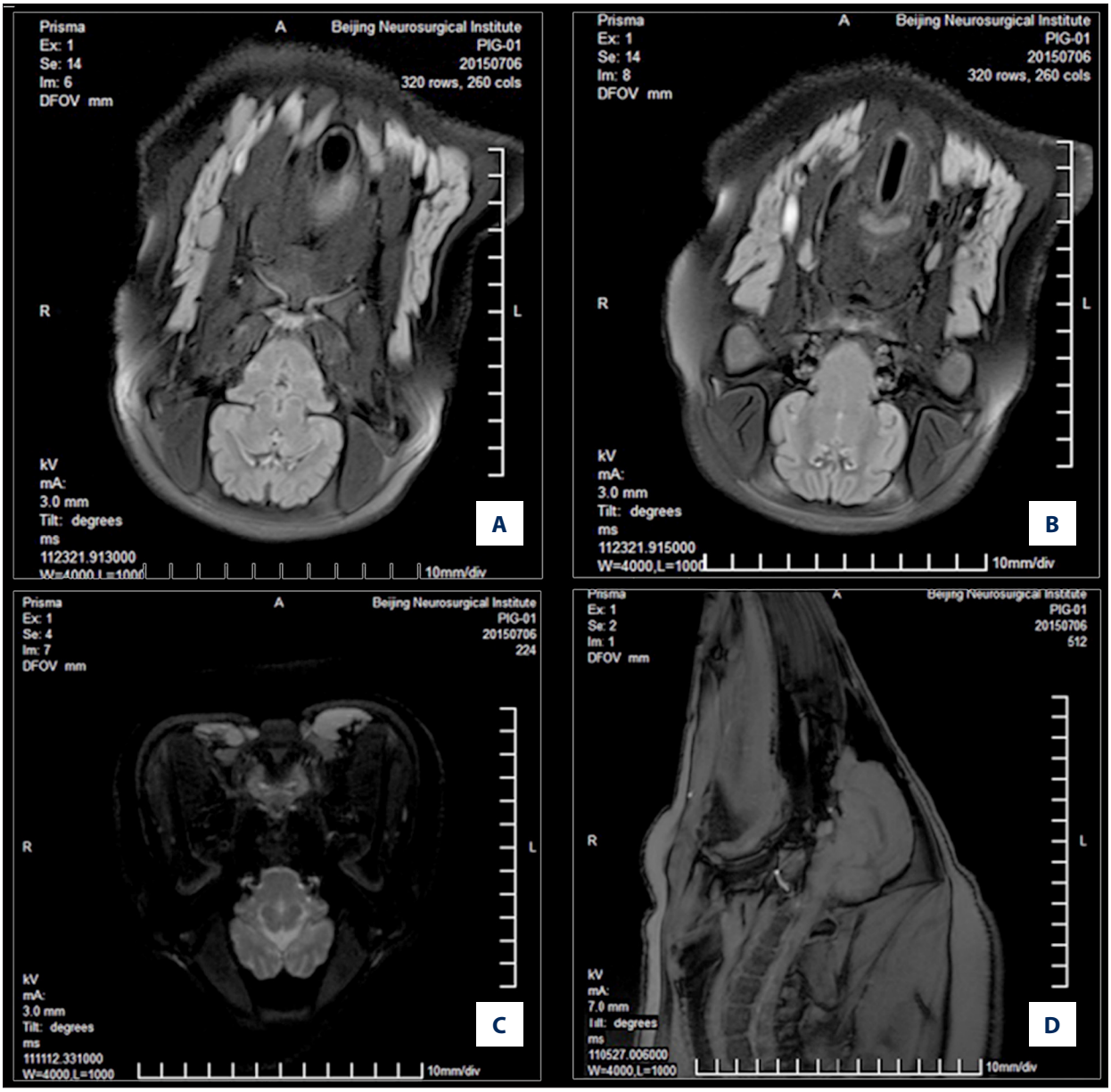

Figure 2. MRI brain scan one month after stent implantation. No brain infarction was observed in MRI images.

Results

Angiography and MRI characteristics

Angiograms of the experimental groups showed that for all animals, there was no stenosis in the arteries and no obstruction in the collateral branches after stents had been implanted (Figure 1). As revealed by the MRI brain scans, no brain infarction occurred in any of the experimental animals at one month after the stent implantations (Figure 2).

Morphology characteristics

After the vessels was cut open longitudinally, it was observed that the vascular walls were attached closely by stent wires. The stent wires were covered by a layer of semitransparent oyster white neointimas, which were smooth and even. Neointimas on stent wire surfaces were a little higher than those at stent wires holes, but their transitions were mild. No thrombus was observed in any of the stents. Each stent was covered by tiny collateral vessels. Stent wires crossed the opening of perforator vessels and were covered by a thin layer of transparent neointimas. The opening of collateral vessels was not covered by neointimas, which was confirmed by the unobstructed outflow

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Table 1. Thickness of neointimas in each experimental group (mm).

Group	n	Thickness of neointimas
Lvis	3	0.58±0.02*
Solitaier-AB	3	0.61±0.02*
Pipeline	3	0.82±0.03
F		90.529
P		<0.001

* Represents $p<0.05$ when compared with Pipeline group.

of injected normal saline. Neointimas in pigs with FDS were significant thicker than those in pigs with conventional intracranial self-expanding stents. Stent wires crossing collateral vessels were also covered by neointimas, and although the neointimas were thicker, the collateral vessels were still open.

Pathology characteristics

Neointimal hyperplasia was significant more serious ($p<0.05$) in pigs with a FDS than those with a conventional intracranial self-expanding stent as revealed by the average thickness of neointimas, i.e., 0.82±0.03 mm, 0.59±0.02 mm, and 0.62±0.02 mm for pigs with FDS, LVIS stents, and Solitaier-AB stents, respectively (Tables 1 and 2). Spindle cells formed neointimas on the surface of stent wires. Further revealed by H&E staining and IHC staining, α-actin positive vascular smooth muscle cells, collagen fibers, and macrophages were the major components of neointimas (Figures 3–6).

Discussion

FDS has been widely used in treating intracranial aneurysm in recent years but its effects on collateral arteries has seldom been reported. In the present study, effects of FDS on collateral branches of the aneurysm parent artery were studied based on a miniature pig model and FDS was compared with two other conventional self-expanding stents. It was found that collateral arteries were obstructed by none of the stents used in the experiments, though FDS led to thicker neointimas than conventional self-expanding stents.

Previous studies have revealed three major effects on collateral arteries with implanted stents: stent jail, neointimal hyperplasia, and snowplowing effect. Stent jail is when stent wires directly cover the opening of collateral arteries obstructing blood flow. This effect usually occurs immediately after stent implantation. Neointimal hyperplasia can occur after the stent is implanted into the artery, as the stent can promote depositions of clots and lead to an inflammatory response, which may stimulate proliferation of smooth muscle cells. This process can cause serious neointimal hyperplasia and vessel narrowing [13–15], and is usually observed later in stent-caused obstructions of collateral arteries. Snowplowing effect occurs when atheromatous plaques realign longitudinally after stent implantation and obstruct collateral arteries. This effect usually occurs in coronary disease treatment with stents [16,17], and is also observed in patients accepting intracranial aneurysm treatment with stents.

In this study, the effects of stent jail and neointimal hyperplasia, induced by the placement of FDS, were studied and FDS effects were compared with the effects of two other conventional self-expanding stents. Miniature pigs were used in this study because their internal carotid arteries have similar construction

Table 2. Characteristics of pathology, angiography, and brain MRI scanning for each exerimental group.

Group	Animal	Pathology *neointimas thickness*	Angiography *vessel narrowing*	Brain MRI *cerebral infarction*
Lvis	1	0.58 mm	0	0
	2	0.59 mm	0	0
	3	0.58 mm	0	0
Solitaier-AB	4	0.61 mm	0	0
	5	0.61 mm	0	0
	6	0.62 mm	0	0
Pipeline	7	0.82 mm	0	0
	8	0.80 mm	0	0
	9	0.81 mm	0	0

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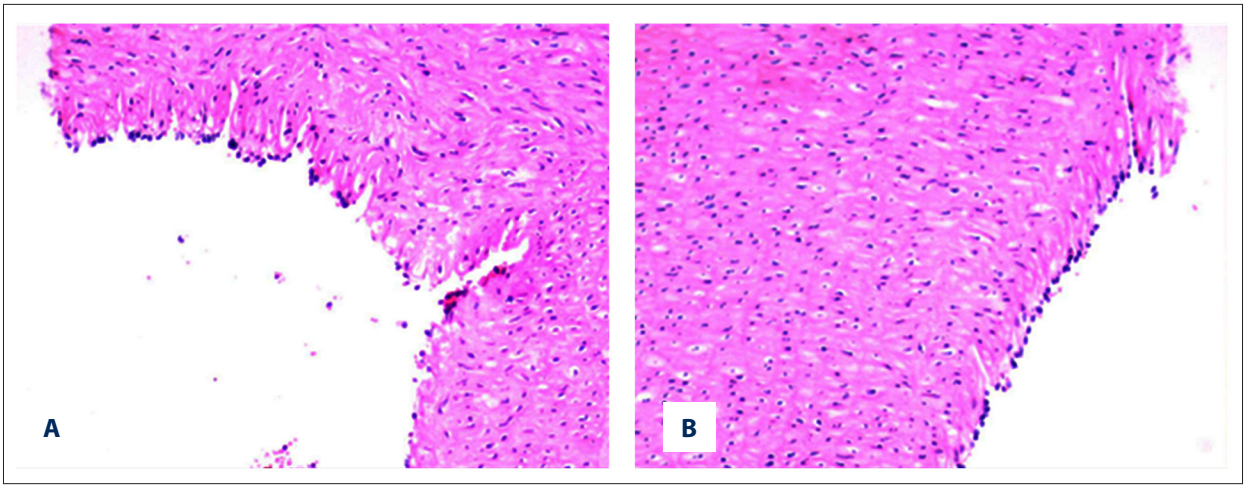

Figure 3. (**A, B**) Pathology of components of new vessels after stents removed (H&E staining). New vessels consisted of new endothelia cells as well as myofibroblasts under the endothelia cells. The thickness of new vessels was about 1 mm.

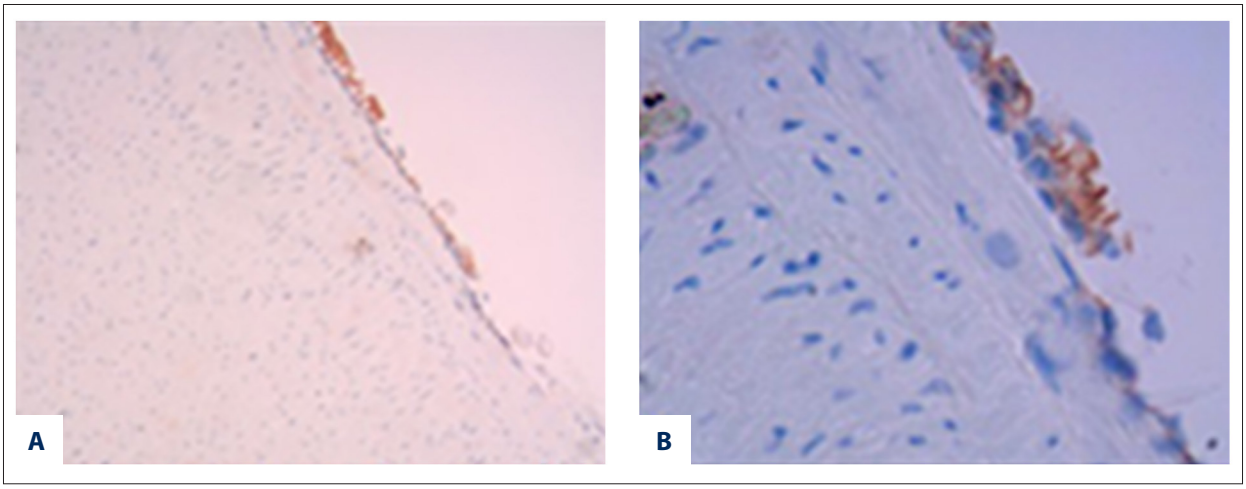

Figure 4. The expression of factor VIII protein of endothelial cell cytoplasm in the neointimal IHC staining (endothelial factor III). Brown granular, lumpy expression indicates moderate intensity. (**A**) Low magnification; (**B**) High magnification.

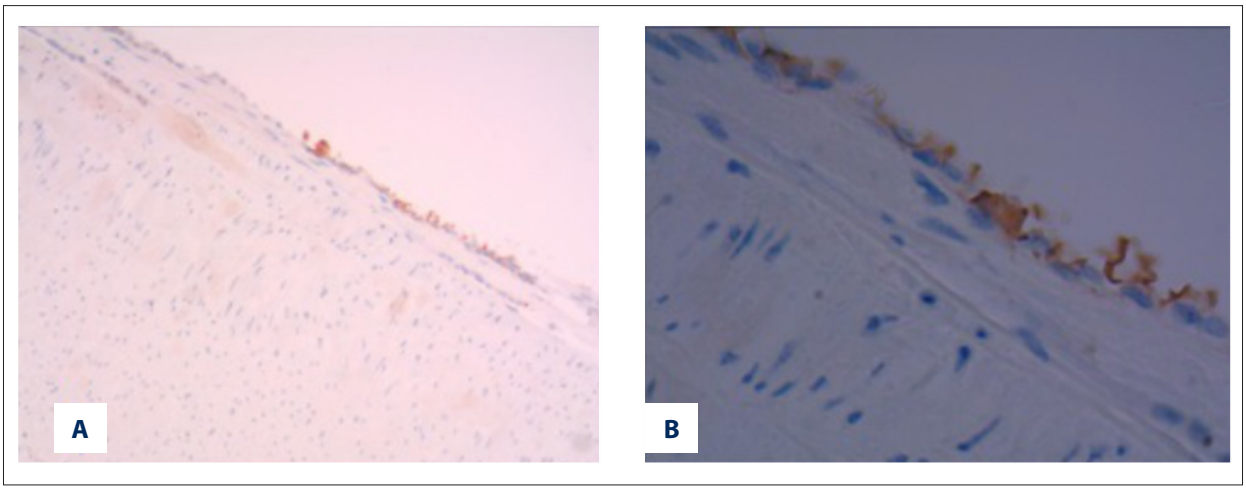

Figure 5. The expression of CD68 (in brown particles) of endothelial cell cytoplasm in the neointimal IHC staining. (**A**) Low magnification; (**B**) High magnification.

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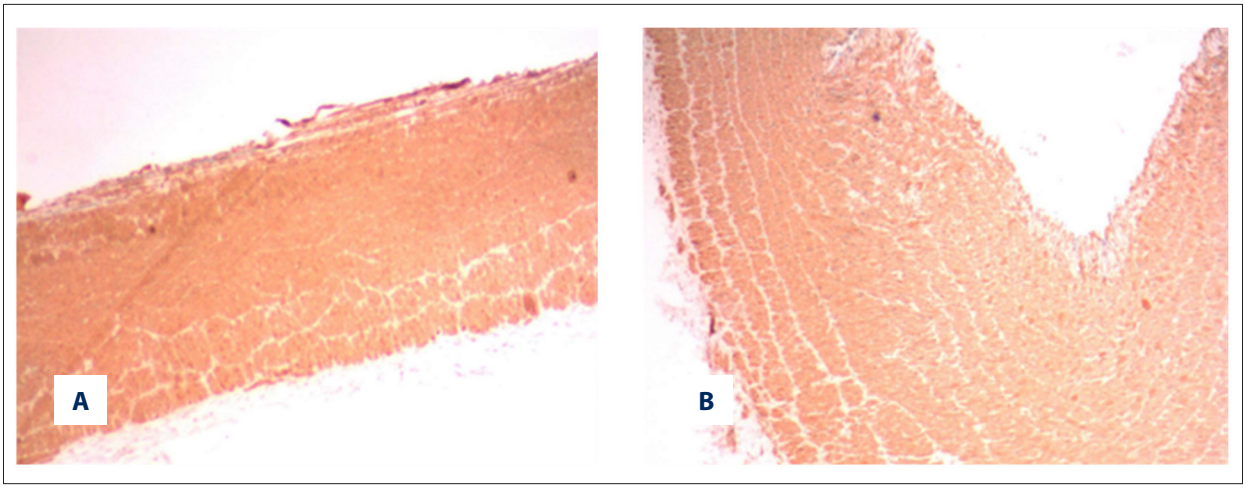

Figure 6. (**A, B**) The expression of cytoplasm muscle fibers of endothelial cells in the neointimal IHC staining (α-actin α-Actin), as indicated by the brownish red lumps, and diffusion indicates high positivity.

to human intracranial arteries. Angiography immediately after surgery revealed that there was no obstruction in the collateral arteries for all three types of stents, suggesting that the stent jail effect was very small for FDS as well as the other two types of stents. In our study, neointimas were observed for all three types of stents, and neointimas in pigs with FDS were significantly thicker ($p<0.05$) than others due to the higher coverage rate of the FDS. However, no cerebral infarction was revealed by brain MRI scans and the blood flow in the collateral arteries was unobstructed, suggesting that neointimal hyperplasia was also not serious for FDS or the other two types of stents. A similar phenomenon was observed in other related studies [18,19]. As revealed by IHC staining, the stent surface was covered by mature endothelial cells, which are the components of normal vessel walls and can stop platelet aggregation and thrombosis. Hence, FDS did not affect blood flow in the collateral arteries and was as safe as conventional stents when implanted in the internal carotid artery.

In clinical practice, coverage of collateral arteries is unavoidable when using FDS (i.e., Pipeline) in intracranial aneurysm treatment. For example, the ophthalmic artery, the anterior choroidal artery, and the posterior communicating artery will be covered in the treatment of internal carotid aneurysm; or the posterior cerebral artery and the anterior cerebellar artery will be covered when a stent is implanted in the basilar artery. In most cases, collateral arteries will be unobstructed, e.g., three-fourths of cases where the ophthalmic artery is covered, will be asymptomatic. The results in our study further confirmed clinical findings and provided preliminary explorations into FDS related pathology. In this study, stents were placed in arteries with a diameter larger than 1 mm, which is the situation in most basilar arteries, e.g., posterior cerebral arteries, superior cerebellar arteries, and anterior cerebellar arteries. For arteries with diameters smaller than 1 mm, the situation is more complex because the vessels are tiny and are hard to reveal by angiography. The effects of FDS on these arteries will be assessed in the future studies.

Concusions

Indeed, this study had several limitations. (1) The sample size in each group was very small and should be expanded in future studies. (2) The observation time was very short after stent implantation. (3) The clinical environment could not be comprehensively compared with healthy miniature pigs. (4) The results were only for arteries visible by angiography. Further studies are still needed to examine whether FDS is safe for all vessels.

Conflict of interests

The authors declare no conflict of interest.

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