

Effects of Flow Patterns and Hemodynamic Force on Vascular Endothelium in the Temporary Arteriovenous Shunt Loop in Rabbits

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Abstract

The purpose of this study was to investigate whether there is a risk of thrombosis in the temporary arteriovenous shunt loop (TAVSL). The authors established a TAVSL model in the rabbit. Experimental groups were divided into non-heparin treated and heparin treated. The maximum blood flow volume, blood viscosity, and radius of curvature were measured, and the Reynolds number and the shear stress were calculated. Computational fluid dynamics (CFD) was used to predict the flow pattern in the TAVSL, and these predicted data were compared with histological results. Early occlusion was noted in 70% (7/10) of the non-heparin-treated group and 22% (2/9) of the heparin-treated group. CFD analysis predicted a high shear stress at the arterial anastomosis region and the outer luminal surface of the curved section. The intimal structure at the luminal surface of the curved section was extensively lost histologically. In the patent group, severe stenosis of the lumen was noted at the apex of the loop due to an organized thrombus. Thus, thrombosis is likely to occur in the TAVSL due to endothelium injury caused by high shear stress, and this results in the formation of white thrombi at an early stage and an organized thrombus at a late stage.

Keywords

- ▶ arteriovenous shunt loop
- ▶ endothelial injury
- ▶ hemodynamic force

Autologous vein grafts have been widely used to bridge arterial and venous defects in reconstructive microsurgery. Preliminary arteriovenous fistula was reported as a convenient method for vein grafting.¹ Since then, many authors have reported favorably on the installation of the vein graft as a looped arteriovenous shunt fistula. However, some authors pointed out a risk of thrombus formation in the loop due to high-flow injury and secondary flow.^{2,3} We established an experimental model of preliminary arteriovenous fistula (termed temporary arteriovenous shunt loop [TAVSL] in this report) in the rabbit and investigated its thrombogenic risk by examining the flow pattern and histological findings.

Methods

All experimental procedures conformed to the guidelines for animal experimentation administered by the University of Kagawa, Kagawa, Japan.

1. Experimental Model and Groups

Japanese white male rabbits weighing 2.8 to 3.0 kg were used in all experiments. All surgical procedures were performed under general anesthesia in sterile conditions by the same surgeon using a surgical loupe and microscope. A vein graft to prepare the TAVSL was harvested from the right hind limb as described previously.⁴ The vein graft was subjected to

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hydrodistension with heparinized saline, the length was adjusted to 10 cm, and the distal and proximal ends were anastomosed end-to-end with the left femoral artery and vein, respectively, using a 10-0 monofilament nylon suture (►Fig. 1). Animals were divided into two groups with ($n = 9$) or without ($n = 10$) the intravenous administration of 250 IU heparin immediately before reperfusion of blood flow.

2. Measurements Items

1) Blood Flow (mL/min) of the Femoral Artery Before and After TAVSL Preparation

An ultrasonic transit time flow meter (T402-PB, ADInstruments, Inc., Colorado Springs, Colorado) and a 2-mm diameter probe were used. Blood flow of the femoral artery before TAVSL preparation was measured 10 minutes after the probe attachment and 2% Xylocaine (AstraZeneca, London, UK) application when blood flow had stabilized. Blood flow after TAVSL preparation was measured 30 minutes after reperfusion.

2) Maximum Blood Flow of the TAVSL (mL/min)

The maximum blood flow during the 30-minute measurement period after reperfusion of blood flow was regarded as the maximum blood flow.

3) Blood Viscosity (Pa · s)

Before TAVSL preparation, 1 mL of arterial blood was collected from the femoral artery, and its viscosity at 50 ppm was measured using a TVE-22L viscometer (Cole-Parmer, Vernon Hills, Illinois).

4) Radius of Curvature (cm)

The TAVSL after reperfusion of blood flow was photographed using a digital camera. The equivalent circle diameter was measured using image analysis software, Win ROOF (Ver.6.1, Mitani Corporation, Fukui, Japan), and the value recorded was regarded as the radius of curvature (►Fig. 1).

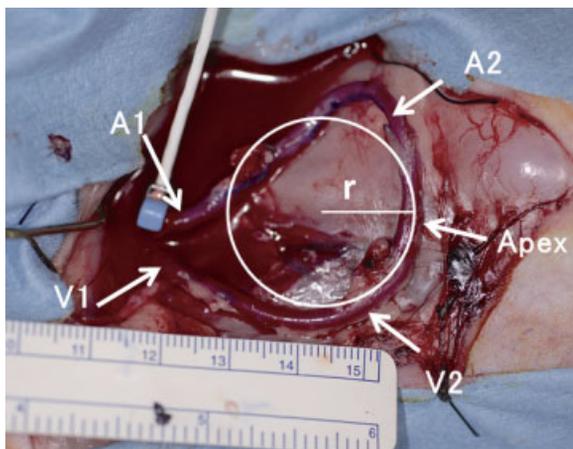


Fig. 1 Preparation of temporary arteriovenous shunt loop: femoral artery, vein graft, femoral vein. Histological specimens were harvested from A1, A2, Apex, V1, and V2. Equivalent circle diameter was measured using image analysis software, Win ROOF (Ver.6.1).

3. Histological Study

Animals in which blood flow decreased to 0 (mL/min) within 30 minutes after reperfusion were designated as an early occlusion group. In the early occlusion group, the loop vessel was immediately harvested for histological study. Animals in which blood flow remained patent for more than 30 minutes were designated as a patent group. One of the patent loop vessels in the non-heparin-treated group (rabbit no. 10) was harvested for histological study in the early phase. In the remaining patent group, the surgical wound was closed, and 250 IU/day of heparin was administered intramuscularly for 2 weeks in the heparin-treated group. The loop was harvested after 2 to 5 weeks, colored with a green tissue marker on its convex border. All histological specimens were fixed in fixed in 10% formalin and cross-sections of the loop were prepared from five regions: the anastomosis region between the femoral artery and vein graft (A1), the apical region of the loop (Apex), the middle region between A1 and Apex (A2), the anastomosis region between the femoral vein and vein graft (V1), and the middle region between V1 and Apex (V2) (►Fig. 1). Histological sections were stained with hematoxylin and eosin (HE), phosphotungstic acid-hematoxylin (PTAH), and Elastica van Gieson (EVG). The area of thrombus formation in the lumen was histologically measured in the regions (A1, A2, Apex, V2, and V1) as follows using image analysis software, Win ROOF (Ver.6.1) (►Fig. 2A):

Percentage area of thrombosis in the early occlusion group

$$= \frac{\text{white thrombi area} + \text{blood cell aggregates area}}{\text{whole vascular luminal area}} \times 100 (\%)$$

Percentage area of organized thrombus in the patent group

$$= \frac{\text{organized thrombus area}}{\text{whole vascular luminal area}} \times 100 (\%)$$

4. Flow Pattern Analysis

Flow pattern analysis in the TAVSL was performed based on the measured values employing the Reynolds-Averaged Navier-Stokes Equation (RANS) as a governing equation. Numerical analysis was performed on the condition that no turbulent flow, such as Reynolds stress, was generated because the flow was laminar. Adding to this, the blood flow was one of typical non-Newtonian fluid flows. Therefore, the power law model was adopted for blood viscosity. In this model, blood viscosity was expressed as a function of local velocity gradient.

5. Statistical Analysis

Measurement results are presented as the means \pm standard error of the mean (SEM). An unpaired t -test was performed, and $p < 0.05$ was regarded as significant.

Results

The TAVSL was occluded within 30 minutes in 7 of 10 (70%) and 2 of 9 (22.2%) in the non-heparin-treated and heparin-treated groups, respectively (►Table 1). In the group in which

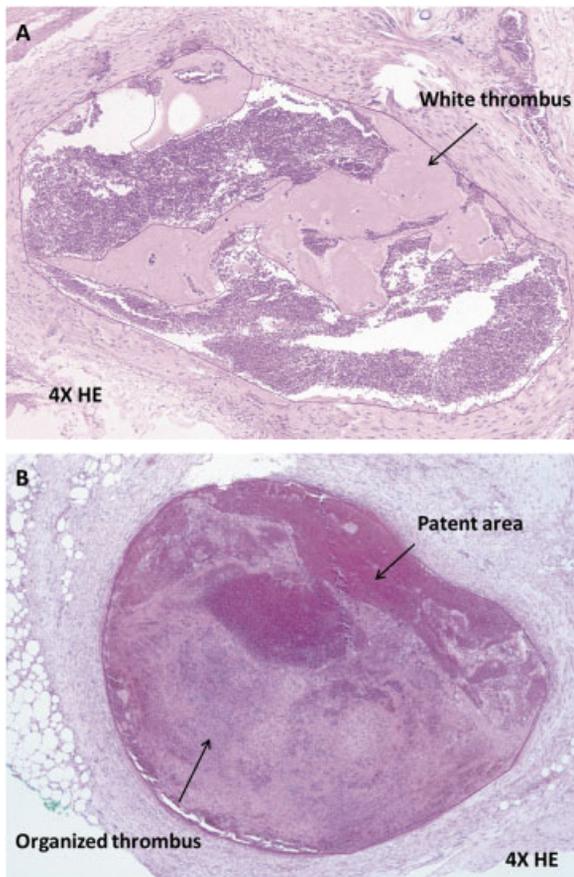


Fig. 2 Measurement of thrombosis area. (A) In early occlusion group, area of white thrombi and blood cell aggregates were measured. (B) In patent group, area of organized thrombus was measured.

the TAVSL was patent for 30 minutes, all were patent until harvesting.

1. Results of Measurement Items (►Table 1)

1) Blood Flow (mL/min) of the Femoral Artery Before TAVSL Preparation

The average blood flows in the femoral artery were 7.40 ± 0.40 mL/min ($n = 10$) and 7.22 ± 0.40 mL/min ($n = 9$) in the non-heparin-treated and heparin-treated groups, respectively.

2) Blood Flow (mL/min) of the Femoral Artery After TAVSL Preparation

Blood flow of the femoral artery reached the maximum immediately after reperfusion and then slowly decreased to 10 to 20 mL/min. In the early occlusion group, the flow further decreased and occluded within 30 minutes, whereas it gradually increased and retained patency in the patent group.

3) Maximum Blood Flow in the TAVSL (mL/min)

The average maximum blood flows were 76.6 ± 11.34 mL/min ($n = 9$) and 91.4 ± 9.89 mL/min ($n = 7$) in the non-heparin-treated and heparin-treated groups, respectively ($p = 0.365$),

and 67.3 ± 10.6 mL/min ($n = 7$) and 95.3 ± 9.45 mL/min ($n = 9$) in the early occlusion and patent groups, respectively ($p = 0.0686$). Maximum blood flow in the TAVSL was greater than that of the femoral artery before TAVSL preparation by ~ 10 times on average and reached a maximum of ~ 20 times.

4) Blood Viscosity (Pa · s)

The average blood viscosity was 2.98 ± 0.32 ($n = 10$) and 3.54 ± 0.18 ($n = 9$) in the non-heparin-treated and heparin-treated groups, respectively ($p = 0.157$) and 3.01 ± 0.336 ($n = 9$) and 3.45 ± 0.21 ($n = 10$) in the early occlusion and patent groups, respectively ($p = 0.261$).

5) Radius of Curvature (cm)

The average equivalent circle diameter was 1.94 ± 0.199 cm ($n = 6$) and 1.98 ± 0.158 cm ($n = 5$) in the non-heparin-treated and heparin-treated groups, respectively ($p = 0.867$), and 1.96 ± 0.185 cm ($n = 4$) and 1.96 ± 0.175 cm ($n = 7$) in the early occlusion and patent groups, respectively ($p = 0.988$).

6) Reynolds Number

The Reynolds number (Re) was calculated using the measured maximum blood flow (mL/min), radius of the vessel ($r = 1$ mm), and viscosity (Pa · s): $Re = 313 \pm 68.8$ ($n = 9$) and $Re = 303 \pm 32.1$ ($n = 7$) in the non-heparin-treated and heparin-treated groups, respectively ($p = 0.721$), and $Re = 301 \pm 182$ ($n = 7$) and $Re = 227 \pm 284$ ($n = 9$) in the early occlusion and patent groups, respectively ($p = 0.827$). The maximum Reynolds number in the experimental groups was 608 (no. 3) in the early occlusion group, in which viscosity was the lowest (1.39).

7) Shear Stress (dyn/cm²)

Shear stress (τ) generated on the luminal surface of the loop vessel in the straight section was calculated using the equation below:

$$\tau = \frac{4\mu Q}{\pi R^3} \quad (Q: \text{blood flow, } \mu: \text{viscosity, } R: 1 \text{ mm})$$

The average shear stress values were 48.4 ± 10.4 dyn/cm² ($n = 9$) and 67.3 ± 8.90 dyn/cm² ($n = 7$) in the non-heparin-treated and heparin-treated groups, respectively ($p = 0.206$), and 38.9 ± 8.26 dyn/cm² ($n = 7$) and 70.6 ± 8.90 dyn/cm² ($n = 9$) in the early occlusion and patent groups, respectively ($p = 0.0235$). The maximum shear stress was 100 dyn/cm² observed in no. 13 in the patent group.

4. Histological Study

The TAVSL was harvested from eight animals in the early occlusion group (six and two in the non-heparin-treated and heparin-treated groups, respectively) and seven animals in the patent group (two and five, respectively) (►Table 2).

1) Histological Findings in the Early Occlusion Group

A high percentage area of thrombosis was noted at A1 and V1 in seven of eight, suggesting that thrombus formation at A1 and/or V1 was the cause of early occlusion (►Fig. 3A). White

Table 1 Summary of Data

GROUP	No.	Blood flow of FA (ml/min)		Harvest	Patency	Max flow vol (ml/min)	Viscosity (Pa • s)	ECD (cm)	Reynolds number	Shear stress (dyn/cm ²)							
		Pre-TAVSL	30 minutes post-TAVSL														
Non-heparin treated	1	7	0	Same day	Occlusion	Not measured	4.00	Not measured	—	—							
	2	6	0														
	3	9	0														
	4	8	0														
	5	7	0														
	6	7	0	2 wks	Patent	39	1.77	1.58	233	15							
	7	9	56														
	8	6	25														
	9	6	0														
	10	9	26														
Heparin-treated		avg 7.4		Same day	Patent	avg 76.6	avg 2.98	avg 1.94	avg 313	avg 48.4							
	11	7	60								5 wks	Patent	Not measured	3.61	Not measured	—	—
	12	6	40														
	13	6	61	2 wks	Patent	122	3.86	—	164	46							
	14	7	0														
	15	7	84	3 wks	Patent	120	3.67	2.24	330	93							
	16	8	0														
	17	9	96	3 wks	Patent	105	3.60	1.45	309	80							
	18	9	55														
19	6	38	2 wks	Patent	78	3.64	1.89	227	60								
	avg 7.22									2 wks	Patent	avg 91.4	avg 3.53	avg 1.98	avg 303	avg 67.3	

Abbreviations: ECD, equivalent circle diameter; TAVSL, temporary arteriovenous shunt loop.

Table 2 Percentage Area of Thrombosis at A1, A2, Apex, V2

GROUP	Heparin	No.	A1	A2	Apex	V2	V1	Histological diagnosis
Early occlusion group	Non-treated	1	67.2	20	2.8	0	3.1	White thrombosis at A1
		2	89.2	15.4	12.8	8.4	71.3	White thrombosis at A1, V1
		3	45.9	1.5	12.4	1.33	6.78	White thrombosis at A1
		4	17.8	12.8	57.6	5.39	1.26	White thrombosis at Apex
		5	91.4	35.6	21.2	2.76	20.4	White thrombosis at A1
		6	11	5.4	23.2	9	91	White thrombosis at V1
	Treated	14	2.2	0	2.25	0	45	White thrombosis at V1
		16	78.9	3.3	9.45	13.5	47.6	White thrombosis at A1, V1
Patent group	Non-treated	10	0	0	0	0	0	No thrombus
		11	0	0	90 ^a	76.9 ^a	76.9	Severe stenosis at Apex, V2, V1
	Treated	12	0	0	0	0	0	No thrombus
		13	0	0	0	0	0	No thrombus
		15	0	49.7 ^a	85.6 ^a	80 ^a	52.4	Severe stenosis at Apex, V2
		17	0	0	0	0	0	No thrombus
		19	0	90 ^a	74.4 ^a	76.4 ^a	0	Severe stenosis at A2, Apex, V2

^aLines of Zahn was noted.

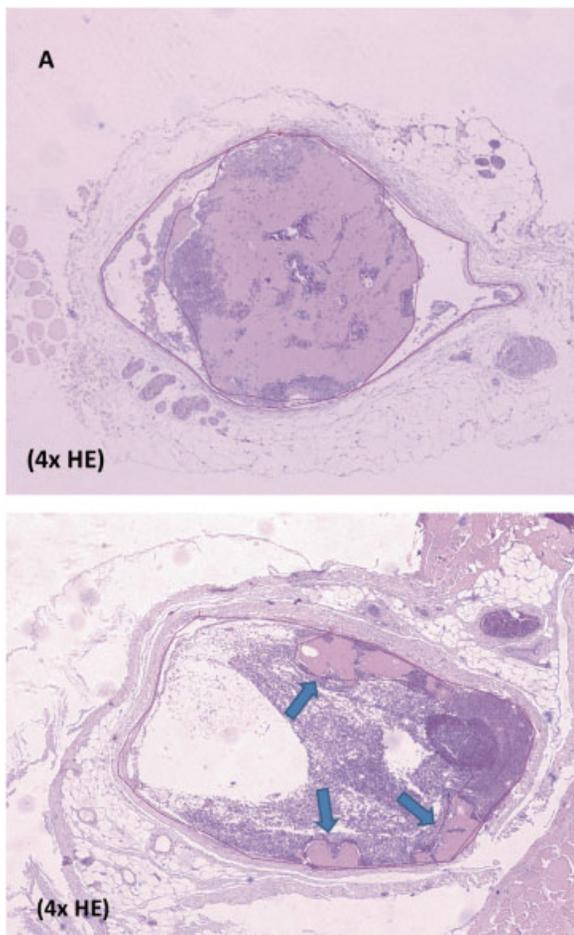


Fig. 3 Histology in early occlusion group. (A) White thrombosis at arterial anastomosis (A1). (B) White thrombi adhered to outer luminal wall in the curved section (➡).

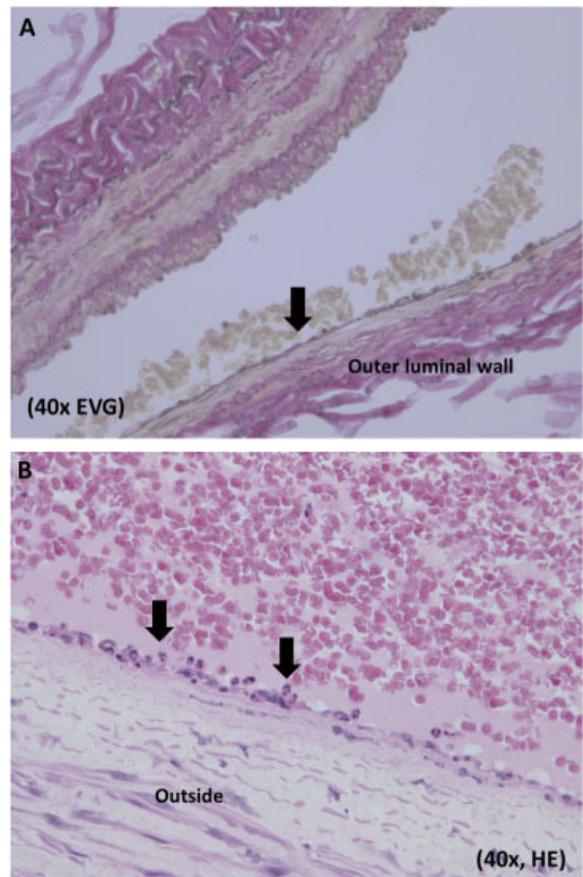


Fig. 4 Histology in the patent group. (A) The intimal structure was extensively lost in the outer luminal wall in the early phase (➡, Elastica van Gieson stain). (B) White blood cell adherence to the intimal surface on the outside of the curved section at 3 weeks (➡, hematoxylin and eosin stain).

thrombi adhered to the outer luminal surface in the curved section of the loop in all specimens of the early occlusion group (►Fig. 3B), and these white thrombi may have been the cause of early occlusion in one animal (no. 4) (►Table 2).

2) Histological Findings in the Patent Group

(1) In the Early Phase (1 Hour after Reperfusion)

The intimal structure of the loop vessel was extensively lost in the outer luminal wall of the curved section. Extension, rupture, and loss of the internal elastic lamina were also noted (►Fig. 4A).

(2) After 2 to 5 Weeks

The percentage area of thrombosis was 0% in all A2, A1, Apex, V2, and V1 regions in three of six (►Table 2). Characteristics of the histology were intimal thickening in the vessel wall on the inside of the curved section and adherence of white blood cells to the outer luminal surface of the curved section (►Fig. 4B). In the other three of six, a high percentage area of organized thrombus was noted in the curved section of the loop (A2, Apex, and V2) (►Table 2). These organized thrombi were formed in layers—that is, “lines of Zahn”—that are alternating pale pink bands of platelets with fibrin and red bands of red blood cells (RBCs) forming a true thrombus, and only a narrow patent area remained on the inside of the lumen (►Fig. 5A). Organized thrombus was adherent to the outer luminal surface of the loop vessel, and fibrosis was also noted (►Fig. 5B).

3. Flow Pattern Analysis

In the TAVSL, blood from the femoral artery flowed into the afferent straight oblique section of the vein graft after removal of the vascular clamp, passed through the curved section, and entered the femoral vein through the efferent straight oblique section of the vein graft, forming an arteriovenous shunt flow. The mean and maximum Reynolds numbers were approximately 300 and 608, respectively, and the flow in this experimental model was regarded as laminar. The flow pattern in the TAVSL was analyzed as a non-Newtonian fluid (steady flow) based on the measured values, and the following findings were obtained:

1. Fast flow was formed in the region near the outer luminal wall of the curved section, and this velocity distribution remained until the downstream, straight, oblique section of the vein graft (►Fig. 6A).
2. Velocity decreased in the region near the inner luminal wall of the entrance of the curved section, forming a field with a flow velocity of nearly 0, where there could be blood stagnation (►Fig. 6B).
3. At the apex of the curved section, a pair of whirlpools were formed by secondary flows directing from the inside toward the outside through the center of the lumen and vice versa near the vessel wall, and another pair of whirlpools from the outside toward the inside (►Fig. 6C).
4. The shear stress value on the outer luminal wall was higher in the curved section than in the straight one (the mean value was ~ 1.36 times greater) (►Fig. 7).

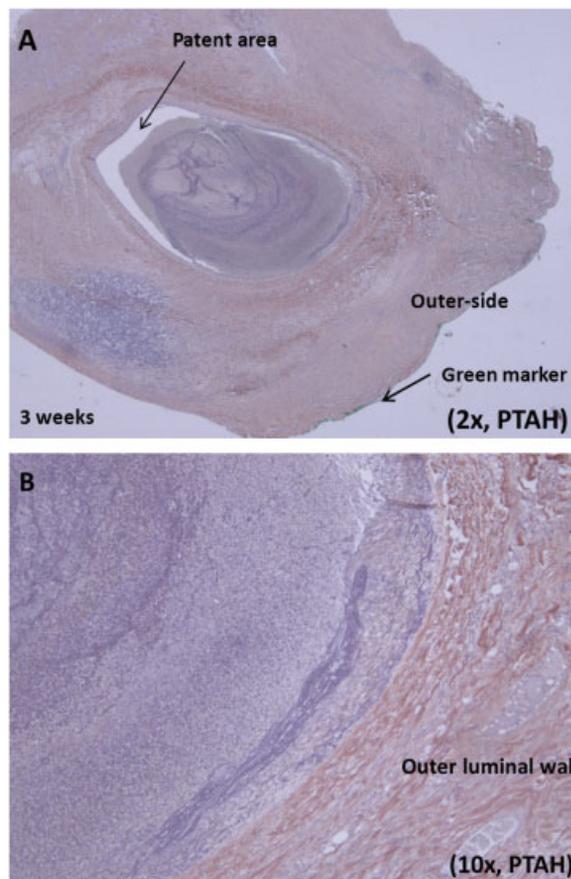


Fig. 5 Histological finding of organized thrombus (lines of Zahn) (phosphotungstic acid-hematoxylin stain). (A) In heparin-treated patent group at 3 weeks (no. 14), organized thrombus was formed in layers and only a narrow patent area remained on the inside of the lumen. (B) Organized thrombus was adherent to the outer luminal wall, and fibrosis was noted.

5. The shear stress value in the arterial anastomosis region was equivalent to that in the region near the outer luminal wall of the curved section. This was calculated on the condition of uniform flow as an inlet boundary condition in consideration that blood flow enters the loop when a vascular clamp is removed.

Discussion

Papers describing TAVSL-free flaps published between 1982 and 2009 were reviewed,^{3,5-24} and thrombosis was reported in 15% (33 of 222) of cases when the procedure was performed in one stage. In two-staged procedures, thrombosis was reported in 27.8% (37 of 133), including cases in which the loop was already occluded at the time of microvascular tissue transfer (►Table 3). Combining one- and two-staged procedures, the incidence of thrombosis was 19.7% (70 of 355), higher than that with conventional vein grafting. Regarding the higher incidence of thrombosis and the low salvage rate of the TAVSL, Acland² warned of “a high-flow thrombus” and stated that it was sometimes formed at the arterial end of the graft. In the present experimental model,

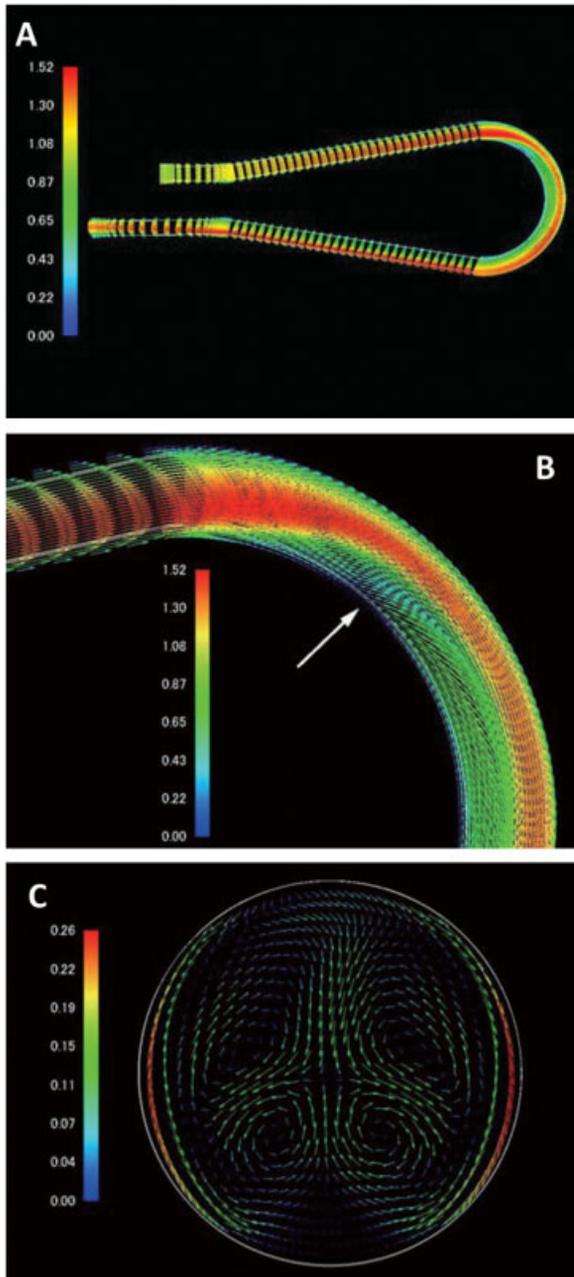


Fig. 6 Flow pattern in the temporary arteriovenous shunt loop (non-Newtonian fluid). (A) Velocity distribution. (B) Stasis in the region near the inner luminal wall of the entrance of the curved section (→:flow velocity of nearly 0). (C) Secondary flow at the top of the curved section.

although vascular anastomosis was performed by a senior microsurgeon, early thrombotic occlusion occurred at a high rate. The cause of early occlusion was white thrombi at the arterial anastomosis in seven of eight, and the apex of the curved section in one of eight histologically evaluated animals. White thrombi were also noted throughout the loop specimen in all eight animals with early occlusion, which is consistent with publication findings, “thrombosis of the loop”²³ and “extensive thrombosis throughout the vein graft.”⁵ On the other hand, no thrombus was noted in half (three of six) of the patent group, but organized thrombus

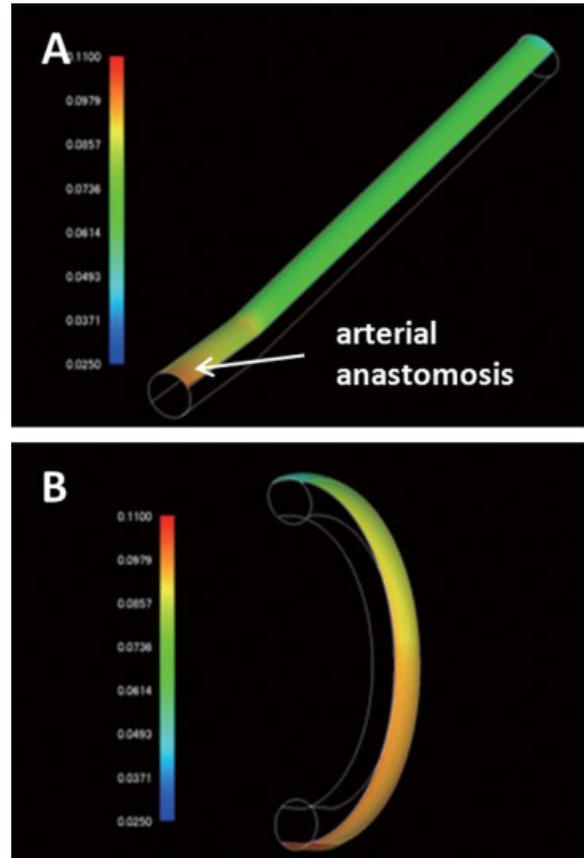


Fig. 7 Shear stress distribution in the temporary arteriovenous shunt loop. Shear stress on the outer luminal wall in the straight section (A) and in the curved section (B).

was formed at the curved section of the loop in the other half, showing severe stenosis of the lumen. Thus, it was clarified that white thrombus formation readily occurred at the arterial anastomosis site and in the loop vessel under the condition of the TAVSL in the early stage, and severe stenosis or occlusion was likely caused by organized thrombus in the late stage, which is consistent with clinical reports (→Table 3). On histological examination of the patent group in the early phase, the endothelium was extensively lost in the outer luminal wall of the curved section, showing severe endothelial injury. It has been reported that most endothelial cells of the vein graft were lost, and platelets adhered to the vascular inner surface when a vein graft was placed between arteries in peripheral revascularization.²⁵ Ishikawa et al²⁶ reported that more than 80% of the endothelium of the vein graft detached immediately after vein grafting between arteries in dogs. Because approximately 20 dyn/cm² of shear stress acts on the arterial endothelium under physiological conditions,²⁷ it was thought that the endothelium of the vein graft may be mostly detached by approximately 20 dyn/cm² of shear stress. In our experimental model, the mean shear stress was 38.9 ± 8.26 dyn/cm² and 70.6 ± 8.90 dyn/cm² in the early occlusion and patent groups, respectively, and the maximum shear stress was 100 dyn/cm², showing that values were two to five times higher than 20 dyn/cm². In addition,

Table 3 Summary of Reports Describing TAVSL-free Flaps More than Two Cases (1982–2009)

Author	Reported year	No. of cases	No. loop complication	Thrombosis at		
				Arterial anastomotic site	Venous anastomotic site	Within Loop site
One-staged procedures						
Freedman ⁵	1989	25	3	–	–	3
Earle ⁶	1990	2	0	–	–	–
Vlastou ⁸	1992	26	2	Not specified		
Angel ⁹	1993	10	2	–	–	2
Mulholland ¹⁰	1993	5	1	1	(1*combined)	–
German ¹³	1996	20	4	–	–	4
Ritter ³	1996	9	1	Not specified		
Lineaweaver ¹⁴	1997	3	0	–	–	–
Karanas ¹⁵	1998	6	1	1	–	–
Lin ¹⁸	2004	28	8	8	(1* combined)	–
Bruner ¹⁹	2004	26	3	–	–	3
Oswald ²¹	2007	12	2	–	–	2
Vogt ²²	2007	10	2	–	–	2
Cavadas ²³	2007	37	2	2	–	–
Demiri ²⁴	2009	3	0	–	–	–
Subtotal		222	31 (14%)	12	(2*)	16
Two-staged procedures						
Sorenson ⁷	1990	9	2	–	–	2
Silveira ¹¹	1993	12	2	–	–	2
Devash ¹²	1995	11	0	–	–	–
German ¹³	1996	7	0	–	–	–
Ritter ³	1996	8	6	–	–	6
Atiyeh ¹⁶	2001	19	8	–	–	8
Antohi ¹⁷	2001	5	1	1	–	–
Lin ¹⁸	2004	6	3	2	–	1
Bruner ¹⁹	2004	5	5	–	–	5
Sunar ²⁰	2004	15	3	–	–	3
Vogt ²²	2007	17	3	–	–	3
Cavadas ²³	2008	19	4	–	–	4
Subtotal		133	37 (27.8%)	3	–	34
Total		355	68 (19.2%)	15	–	50

Abbreviation: TAVSL, temporary arteriovenous shunt loop.

flow pattern analysis showed that shear stress higher than these values acted on the outer luminal surface of the curved section and the luminal surface of the arterial anastomosis region (A1), supporting histological findings of extensive endothelial detachment at Apex and A1. Furthermore, suture material added to the endothelial injury at the arterial anastomotic site, resulting in the high incidence of thrombotic occlusion. Based on these findings, the mechanism of early thrombus formation in the TAVSL was thought to be as follows: (1) Endothelium of the vein graft was extensively

detached by high shear stress predisposing to platelet adherence. In particular, stronger shear stress acted on the arterial anastomosis region and outer luminal surface of the curved section, which severely injured the endothelium and widely exposed collagen in subendothelial tissue. (2) Platelet adherence then followed (primary aggregation), mediated by von Willebrand factor, and the intrinsic pathway of coagulation was simultaneously promoted. Adhering platelets were activated by shear stress, and platelet aggregation (platelet plug) increased vascular resistance and reduced blood flow volume

in the early phase. (3) A fibrin mesh formed by the intrinsic pathway of coagulation entrapped the plug and became stable (white thrombus). Blood flow may become slower and stagnate when white thrombus formation increases, and combined with red thrombi, early occlusion may occur. When fewer white thrombi are formed, fibrinolysis may act and retain patency. In the heparin-treated group, the TAVSL was patent in many animals, which may have been due to the inhibition of fibrin formation by heparin, reducing white thrombus formation.⁸

On flow pattern analysis, the blood flow velocity became 0 (mL/min) in the region near the inner luminal wall of the entrance of the curved section. Although flow pattern analysis in this experimental model was performed on the assumption of steady flow, there were many similarities between dye particle motions in pulsatile flow and steady flow,²⁸ suggesting that stagnation will occur and thrombosis will be promoted at this site combined with endothelial injury. In addition, in the one-staged procedure where a vascular clamp might be applied to the apex region of the curved section (the arterial end of the graft) during vascular anastomosis, where aggregation activity is enhanced and platelet plug and white thrombi have diffusely formed due to endothelial injury, there is extra risk for thrombosis at this site. Once thrombosis develops under these conditions, the loop is filled with thrombi in a vicious circle, termed a "thrombus loop,"²² which may make salvage difficult.¹⁸ On the other hand, in the patent group, organized thrombus was formed in layers (lines of Zahn) and adhered to the outer luminal wall of the curved region, leaving only a narrow patent portion on the inside of the lumen, which becomes a risk factor in the two-staged procedure. These lines of Zahn were formed by repeated white thrombus and red blood cell aggregation, suggesting that (1) high shear stress acted and severely injured the endothelium of the outer luminal wall in the curved section, on which initial white thrombus formation occurred; and (2) thrombus formation repeatedly occurred to grow into organized thrombus. Although patency of the loop was retained for 2 to 5 weeks, organized thrombus formation may have progressed and caused severe stenosis of the lumen. Thus, when a TAVSL is used as a two-staged procedure, the possibility that an organized thrombus is formed in the top curved section prior to the second stage microvascular anastomosis should be considered.

Shear stress is proportionate to blood flow, vascular diameter, and curvature of the TAVSL, and inversely proportionate to vascular length. This means that a sharper curve, high flow, and larger diameter of the TAVSL will be the most likely situation for endothelial injury and encourage thrombosis acutely. Clinically, since arterial inflow, diameter and length of the vein graft, and curvature of the TAVSL vary among patients, results differ with each case. Since there is a risk of shear stress-induced thrombus formation in a TAVSL in both one-staged and two-staged procedures, it should be carefully used in clinical practice with systemic anticoagulation. Conventional vein grafting or the blind loop method^{2,29,30} reported by Acland is recommended. The use of an arteriovenous vascular bundle may be another choice.³¹

Summary

We established an experimental model of a temporary arteriovenous shunt loop and performed flow pattern analysis and a histological study of the loop vessel. High shear stress acts on the outer luminal wall of the loop and readily injures the endothelium, which is likely to enhance the intrinsic coagulation system and thrombus formation in the early stage. It was also clarified that there is a risk of severe stenosis caused by organized thrombus in the late stage.

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References

- 1 Threlfall GN, Little JM, Cummine J. Free flap transfer—preliminary establishment of an arteriovenous fistula: a case report. *Aust N Z J Surg* 1982;52(2):182–184
- 2 Acland RD. Refinements in lower extremity free flap surgery. *Clin Plast Surg* 1990;17(4):733–744
- 3 Ritter EF, Anthony JP, Levin LS, et al. Microsurgical composite tissue transplantation at difficult recipient sites facilitated by preliminary installation of vein grafts as arteriovenous loops. *J Reconstr Microsurg* 1996;12(4):231–240
- 4 Tanaka Y, Crowe DM, Wagh M, Morrison WA. New source of vein graft for rabbit experimentation. *Microsurgery* 1999;19(4):167–170
- 5 Freedman AM, Meland NB. Arteriovenous shunts in free vascularized tissue transfer for extremity reconstruction. *Ann Plast Surg* 1989;23(2):123–128
- 6 Earle AS, Feng LJ, Jordan RB. Long saphenous vein grafts as an aid to microsurgical reconstruction of the trunk. *J Reconstr Microsurg* 1990;6(2):165–169
- 7 Sørensen JL, Muchardt O, Reumert T. Temporary arteriovenous shunt prior to free flap transfer. *Scand J Plast Reconstr Surg Hand Surg* 1990;24(1):43–46
- 8 Vlastou C, Earle AS, Jordan R. Vein grafts in reconstructive microsurgery of the lower extremity. *Microsurgery* 1992;13(5):234–235
- 9 Angel MF, Chang B, Clark N, Wong L, Ringelman P, Manson PN. Further clinical use of the interposition arteriovenous loop graft in free tissue transfers. *Microsurgery* 1993;14(8):479–481
- 10 Mulholland S, Boyd JB, McCabe S, et al. Recipient vessels in head and neck microsurgery: radiation effect and vessel access. *Plast Reconstr Surg* 1993;92(4):628–632
- 11 Silveira LF, Patricio JA. Arteriovenous fistula with a saphenous long loop. *Microsurgery* 1993;14(7):444–445

- 12 Devansh S. Prefabricated recipient vascular pedicle for free composite-tissue transfer in the chronic stage of severe leg trauma. *Plast Reconstr Surg* 1995;96(2):392-399
- 13 Germann G, Steinau HU. The clinical reliability of vein grafts in free-flap transfer. *J Reconstr Microsurg* 1996;12(1):11-17
- 14 Lineaweaver W, Hui KCW, Kaufmann D. Cross-facial vein grafting in complicated flap reconstructions of the face and mandible. *J Reconstr Microsurg* 1997;13(8):545-549
- 15 Karanas YL, Yim KK, Johannet P, Hui K, Lineaweaver WC. Use of 20 cm or longer interposition vein grafts in free flap reconstruction of the trunk. *Plast Reconstr Surg* 1998;101(5):1262-1267
- 16 Atiyeh BS, Khalil IM, Hussein MK, Al Amm CA, Musharafieh RS. Temporary arteriovenous fistula and microsurgical free tissue transfer for reconstruction of complex defects. *Plast Reconstr Surg* 2001;108(2):485-488
- 17 Antohi N, Stingu C, Stan V. The use of prefabricated recipient vascular pedicle for free tissue transfer in severe limb injury. *The Rom J Plast Surg* 2001;9:421-425
- 18 Lin CH, Mardini S, Lin YT, Yeh JT, Wei FC, Chen HC. Sixty-five clinical cases of free tissue transfer using long arteriovenous fistulas or vein grafts. *J Trauma* 2004;56(5):1107-1117
- 19 Br uner S, Bickert B, Sauerbier M, Germann G. Concept of arteriovenous loupes in high-risk free-tissue transfer: history and clinical experiences. *Microsurgery* 2004;24(2):104-113
- 20 Sunar H, Aygit CA, Afsar Y, Halici U, Duran E. Arterial and venous reconstruction for free tissue transfer in diabetic ischemic foot ulcers. *Eur J Vasc Endovasc Surg* 2004;27(2):210-215
- 21 Oswald TM, Stover SA, Gerzenstein J, et al. Immediate and delayed use of arteriovenous fistulae in microsurgical flap procedures: a clinical series and review of published cases. *Ann Plast Surg* 2007;58(1):61-63
- 22 Vogt PM, Steinau HU, Spies M, et al. Outcome of simultaneous and staged microvascular free tissue transfer connected to arteriovenous loops in areas lacking recipient vessels. *Plast Reconstr Surg* 2007;120(6):1568-1575
- 23 Cavadas PC. Arteriovenous vascular loops in free flap reconstruction of the extremities. *Plast Reconstr Surg* 2008;121(2):514-520
- 24 Demiri EC, Hatzokos H, Dionyssiou D, Megalopoulos A, Pitoulias G, Papadimitriou D. Single stage arteriovenous short saphenous loops in microsurgical reconstruction of the lower extremity. *Arch Orthop Trauma Surg* 2009;129(4):521-524
- 25 Davies MG. Intimal hyperplasia: basic response to arterial and vein graft injury and reconstruction. In: Rutherford RB ed. *Vascular Surgery*. 6th ed. Philadelphia: Saunders; 2005:149-172
- 26 Ishikawa M, Sasajima T, Kubo Y. Re-endothelialisation in autogenous vein grafts. *Eur J Vasc Endovasc Surg* 1996;11(1):105-111
- 27 Ando J. Perception and response to blood flow by vascular endothelial cells. *Jap J Jikken Igaku* 1995;13:133-138
- 28 Back MR, Cho YI, Crawford DW, Back LH. Fluid particle motion and Lagrangian velocities for pulsatile flow through a femoral artery branch model. *J Biomech Eng* 1987;109(1):94-101
- 29 Brown EE, Chang WT, Jones NF. Temporary arteriovenous loop between the saphenous vein and the first plantar metatarsal artery in toe-to-hand transfers. *J Hand Surg Am* 2006;31(9):1543-1545
- 30 Topalan M, Bilgin-Karabulut A, Ermis I. "Extracorporeal loop" (blind loop): an alternative microsurgical technique for prelaminated flap transfer. *J Reconstr Microsurg* 2002;18(3):155-158, discussion 159
- 31 Echo A, Bullocks JM. Use of the descending branch of the lateral femoral circumflex vessels as a composite interposition graft in lower extremity reconstruction. *Microsurgery* 2011;31(3):241-245