The size mismatch in an end-to-end vascular anastomosis between the host vessel and the graft may cause flow disturbance and possibly result in thrombosis. To test the hypothesis that intentionally induced swirling flow in an end-to-end anastomosis could suppress flow disturbance, impeding thrombus formation by affecting platelet adhesion, a comparative study was designed to investigate the effect of swirling flow on the adhesion and activation of platelets in a glass sudden tubular expansion tube coated with calf skin type I collagen. The results revealed that the swirling flow generated in the expansion could reduce the length of the flow recirculation zone distal to the expansion and significantly reduce the total number of adherent platelets in the test tube when compared with that for the normal flow. No significant difference was observed in the activation of platelets between the swirling flow group and the normal flow group. This study therefore suggests that intentionally introduced swirling flow in an end-to-end anastomosis has no adverse effect on platelet activation and may indeed be a solution to improve the patency of end-to-end microvascular anastomoses by suppressing thrombus formation. ASAIO Journal 2010; 56:172–179.

Although the actual causes and mechanism of thrombosis are complex and not completely understood, it is now well recognized that local hemodynamic factors play important roles in the adhesion of platelets and the onset and localization of thrombosis. Generally speaking, platelet thrombus in vivo are frequently found in disturbed flow regions, such as in blood vessel bifurcations, T joints, and downstream of stenoses.1–3 In these areas, blood flow is disturbed and flow streamlines separate from the vessel wall, forming vortices. With components of flow directed toward the wall, these recirculation zones are typically characterized by low shear conditions and long “residence time” for any materials contained within the regions.4,5 Platelets may recirculate in these regions long enough to become activated by the wall or by coagulation factors.6 Some of the recirculating platelets may also adhere to the vessel wall or form small aggregates, making such regions susceptible to thrombus formation.

Sudden expansions, which often occur at arterial stenoses, in the common carotid artery after endarterectomy and at end-to-end anastomoses, are the most common geometries that may cause flow recirculation. Anastomosis is the main component of vascular surgery. A common issue in vascular surgery is the anastomotic failure often caused by the size discrepancy (or size mismatch) between the host vessel and the graft.7 The sudden change in vessel diameter can lead to blood flow separation with the formation of flow recirculation, hence likely inducing platelet adhesion and thrombus formation.2 This size mismatch problem is more prominent in microvascular anastomoses. To reduce the risk of anastomotic thrombosis due to the size mismatch, a number of techniques have been used, including mechanical expansion, oblique cut, sleeve procedure, and mechanical devices.8 Unfortunately, these techniques are still far from ideal to manage the size mismatch problem.

Hemodynamic studies revealed that blood flow in the aortic arch took a form with a corkscrew-like pattern.9–11 Besides the ascending aorta, there are many places in the circulation where spiral or swirling blood flow was observed. Stonebridge and Brophy12 evidenced the existence of spiral blood flows in human infraringuinal blood vessels and found that out of the 75 arteries examined, 51 had spiral folds on their endoluminal surfaces. Combining with the observations by others,9,13,14 they argued that spiral blood flow was a normal physiological flow phenomenon, at least in parts of the circulation. Frazin et al.15 even believed that spiral or helical flow may account for a significant amount of normal organ perfusion from branch vessels because of the centripetal spin induced in blood. Stonebridge and Brophy13 hypothesized that the rifled endoluminal surfaces of the arteries might reflect inherent structural features in the elastic wall of the vessels and spiral flow could exert a beneficial effect on the mechanisms of endothelial damage repair. The study by Caro et al.16 had supported this hypothesis and demonstrated that spiral flow may lead to relative uniformity of wall shear, and inhibition of flow stagnation, separation, and instability. Spiral flow can also reduce the size of disturbed flow zones in endovascular stents.17

We believe that the spiral flow observed in the circulation may also be beneficial to prevent the adhesion of platelets to the vessel wall, which is the first stage of thrombogenesis. We therefore hypothesize that by intentionally inducing swirling or spiral flow in vascular end-to-end anastomosis that geometrically resembles a sudden tubular expansion because of its size mis-
match, flow disturbance at the anastomotic sites may be suppressed, hence reducing the adhesion of platelets and the risk of anastomotic thrombosis. To test this hypothesis, we designed a comparative study using a glass sudden tubular expansion as our model and investigated the effect of spiral flow on the adhesion and activation of platelets.

Methods

Experiment

Materials. Type I monomeric collagen from calf skin, bovine serum albumin (BSA) and Arg-Gly-Asp-Ser (RGDS) were purchased from Sigma Chemical Co (St Louis, MO). Fluorescein isothiocyanate-labeled antibodies against activated GPI-IbIIa (PAC-1), PE labeled antibodies against P-selectin (CD62P), and negative control PE-labeled Mouse IgG1 were from Becton Dickinson (Pharmingen, NJ).

Spiral Flow Guider Design and Experimental Setup. In this study, a spiral flow guider for the test expansion tube was fabricated to generate a stable helical (or swirling) flow in the test expansion tube. Figure 1 shows the geometry of the flow guider. The guider had an axial length of 6.5 mm. The internal diameters of its inlet and outlet were 2 and 3 mm, respectively, and the outer diameters of its outlet were 6 mm. The spiral flow guider model was created using the computer-aided design (CAD) software SolidWorks 2006. To meet the requirement of size and precision, the guider was fabricated of a photosensitive resin with laser rapid prototyping (LRP) technology.

The schematic drawing of the experimental perfusion system is also shown in Figure 1. The test section (the expansion tube) of the experiment was a thin-walled straight glass tube with a length of 50 mm and an inner diameter of 6 mm. The sudden tubular expansion in the present study was geometrically similar to the one in the study of Karino and Goldsmith. For comparison, both the swirling flow test section and normal flow test section were used in the experiment. For the normal flow model, the upstream section of the expansion tube was a 3-mm inner diameter, 6 mm outer diameter straight tube that was also fabricated of a photosensitive resin with LRP technology. For the swirling flow model, the glass tube was connected to the spiral flow guider that was used to generate swirling flow in the test tube. Both the spiral flow guider and the straight resin tube for the normal flow experiment were fitted tightly and concentrically with the glass tubes so that they formed a 3- to 6-mm axisymmetric sudden tubular expansion. Washed platelet suspension was perfused through the test tube by an infusion pump (Cole-Parmer Instrument Company, Vernon Hills, IL).

Platelet Preparation. Washed platelets were obtained using a modified protocol of Viisoreanu and Gear. Briefly, venous blood from healthy volunteers was collected into a 1:7 final volume of acid-citrate-dextrose (ACD). Anticoagulated blood was then centrifuged at 1300 r/min for 8 minutes at 22°C to collect platelet-rich plasma (PRP). The collected PRP was then centrifuged at 1500g for 2 minutes and the supernatant removed. The remaining platelets were washed twice at 1500g for 2 minutes in CGS (120 mM NaCl, 12.9 mM sodium citrate, 30 mM glucose and 1 mg/ml BSA, pH 6.5, 37°C) and was finally resuspended in modified Tyrode buffer (MTB, 2.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 150 mM NaCl, 2.5 mM KCl, 12 mM NaHCO₃, 5.5 mM glucose, 1 mM CaCl₂, 1 mM MgCl₂, 1 mg/ml BSA, pH 7.4). Considering a large volume of platelet suspension solution required for each experiment, platelet concentration of the perfusion solution was adjusted to 3 × 10⁵ platelets per microliter instead of 3 × 10⁶ platelets per microliter as usually used by others. The prepared
platelet suspension was allowed to rest for 1 hour at room temperature so that platelets could return to their resting state.

Perfusion Experiment. Following the method described by Karino and Goldsmith, the distribution of platelet adhesion to the wall of the expansion tube was studied and compared between the swirling flow and the normal flow groups. Briefly, before each adhesion experiment, the straight glass tube of the expansion was rinsed with distilled water. The inner wall of the tube was coated with 50 μg/ml calf skin type I collagen in 5 mM acetic acid overnight at 4°C. Only a section of the tube (from the origin, L = 0 to L = 25 mm) was coated. The spiral flow guider, the straight resin tube for the normal flow experiment and the bare section of the test tube, which was not coated with the collagen were then blocked with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 hour at 37°C to prevent the uncoated surfaces from activating the platelets. After the tubular expansion was assembled to the flow system by inserting the flow guider or the straight resin tube into the glass tubes, 18 ml of platelet suspension were perfused through the test section at a steady flow rate of 0.3 ml/s (Re = 120, based on the inner diameter of the upstream section of the expansion and the measured viscosity of the perfusion solution, 3 mm and 1.1 × 10\(^{-3}\) kg m\(^{-1}\) s\(^{-1}\), respectively) with an infusion pump at room temperature for 1 minute. Immediately after the completion of the perfusion, the test glass tube was removed, gently flushed with modified Tyrode buffer, and then fixed with 4% paraformaldehyde in PBS. The perfusion flow rate was the same for both the normal and the swirling flow experiments.

Once dried, the test tube was observed with an inverted microscope (Olympus Optical Co., Ltd., Japan) to study the distribution of platelet adhesion. The counting for platelets adhering to the inner wall of the glass expansion tube was facilitated by projecting the image onto a computer display using a video camera (Olympus Optical Co., Ltd.) that was connected to the computer. A counting process very similar to the process described by Karino and Goldsmith was followed throughout the experiment. The counting was started at the origin and ended in the axial distance 25 mm (L = 25 mm) with an interval of 1 mm. Therefore, the total counting locations were 25. The results, giving the mean platelet adhesion densities of three independent experiments, were normalized with the average downstream platelet adhesion density. The average downstream platelet adhesion density was the mean value of the platelet adhesion densities at the last three locations of the expansion, as our experiment showed that the platelet adhesion densities at the last three locations remained almost constant, and the values of which had no significant difference between the swirling flow and the normal flow groups. Then the normalized platelet density (NPD) was plotted as a function of distance L from the origin of the expansion.

Flow Cytometric Analysis of Platelet Activation. To study the influences of flow disturbance on platelet activation under swirling (spiral) flow and normal flow conditions, resting platelet sample (the control) and the platelet samples collected immediately on the completion of flow experiments were tested by fluorescence-activated cell sorter analysis. To detect platelet receptor expression, fluorescent monoclonal antibodies (PAC-1 FITC, CD62P PE) were added to the samples from the three different groups (the control, the normal and the swirling flow groups). Meanwhile, the isotype control (Mouse IgG1 PE, PAC-1 FITC, and RGDS) was used to detect any nonspecific association. Platelets were incubated with those antibodies in the dark at 22°C for 20 minutes and then were fixed with cold (2°C–8°C) 4% paraformaldehyde in the dark for at least 30 minutes. Samples were analyzed on a flow cytometer (Becton Dickinson, Rutherford, NJ) using BD CellQuest Pro Software. Because the activation of washed platelets cannot be totally avoided during preparation, unstimulated platelets were analyzed in parallel with all in vitro samples to measure specific effects of the shear stress or collagen stimulation. A small elliptical gate was drawn at the down left corner of the cell population in the FSC versus SSC dot plot to ensure that all the detected cells were single intact platelets.

Statistical Analysis

Results of the NPD were presented as means ± standard deviation. Statistical relevance was determined using analysis of t test with p values less than 0.05.

Numerical Simulation of the Flows in the two Experimental Test Tubes

To study the effect of swirling flow on platelet adhesion in the disturbed flow region, the flow generated in the expansion tube with the spiral flow guider was characterized numerically and compared with the flow in the normal flow model.

Geometric Model. The geometrical configurations of the two flow models for the flow simulation were the same as those of the two test tubes used in the perfusion experiment. As shown in Figure 2, the model used in the spiral flow study was a rigid tube combined with the spiral flow guider. As for the comparison, the model used in the normal flow study was also a rigid tube, but with a 3-mm ID, 6 mm OD, straight tube as its upstream section. The length and the inner diameter of both test tubes are 50 and 6 mm, respectively.

Assumptions. The experimental perfusion solution in this study was the MTB solution, which was assumed to be a homogeneous, incompressible, and Newtonian fluid. Its viscosity and density were measured as 1.1 × 10\(^{-3}\) kg m\(^{-1}\) s\(^{-1}\) and 1040 kg m\(^{-3}\), respectively. In the flow simulation, the Reynolds number was set at 120 based on the flow rate in the experiment. At such a rather low Re, though the spiral flow guider may somewhat disturb the flow, the flow in the tube with the flow guider should still remain laminar.

Boundary Conditions. 1) Because flow disturbance must have occurred at the inlet of the models because of the connection between the experiment test tube and the infusion pump, a uniform velocity profile was assumed at the inlet based on the flow rate of 0.3 ml/s used the experiment; 2) the outlet condition was set to be outflow, which applied a zero diffusion flux condition like a fully developed flow; and 3) the wall of the tubes was assumed to be rigid and nonslip.

Solution. The numerical simulation was based on the three-dimensional incompressible Navier-Stokes equations as follows:

\[
\rho (\bar{u} \cdot \nabla) \bar{u} + \nabla p - \mu \Delta \bar{u} = 0
\]

\[
\nabla \cdot \bar{u} = 0
\]
In this study, the Fluent Discrete Phase Model was used to predict particle residence time in the flow models to better understand the influence of flow patterns on platelet activation. Particles were released upstream of the expansion tube. The particle residence time is defined as the time that particles reside in the area near the wall.

Finite volume method was used in the simulation. The computational meshes of the models created using the CAD software Gambit 2.2 (ANSYS, Inc., Canonsburg, PA) were unstructured hexahedron grids combined with tetrahedron grids. The commercially available computational fluid dynamics code, FLUENT 6.2 (ANSYS, Inc.) was used in the numerical simulation. Discretization of the pressure and momentum at each control volume was in a second-order scheme. The iterative process of computation was terminated when the residual of mass and velocity were all less than the convergence criterion, $1.0 \times 10^{-5}$.

To verify grid independence, numerical simulations were performed on different grid sizes. We used the Size Function and Boundary Layers in Gambit to refine the meshes in the computational domain, especially in areas where the velocity gradient was steep. The average wall shear stress (WSS) and the length of flow recirculation zone were selected to check grid independence in this work. The result showed that grid independence was achieved at 905,847 cells for the spiral flow model and 701,902 cells for the normal flow model. Figure 3 shows the differences in WSS between coarser mesh and finer one when grid independence was achieved.

**Results**

**General Flow Patterns**

Figure 4 shows the flow streamlines obtained numerically in the spiral flow and normal flow models. The flow simulation shows that for both models, flow is disturbed with the formation of flow recirculation just distal to the expansion. However, there exists an essential difference in the disturbed flow for the two models. In the normal flow model, the disturbed flow distal to the expansion is in the form of an axisymmetrical close annular vortex, whereas the one in the spiral flow model is asymmetrical open recirculation flow. The flow simulation results show that under the flow rate used in the experimental perfusion study, the length of the annular vortex in the normal flow model is decreased in the swirling flow condition. Letter R indicates the location of the reattachment point.
flow model is approximately 15.0 mm. However, because of its asymmetrical nature, the length of the recirculation zone in the spiral flow model is not a constant value. It varies with the circumferential location of the model. The mean value of the recirculation flow length averaged in the spiral flow model is approximately 6.9 mm. Therefore, the numerical results demonstrated that spiral flow created in the expansion tube could significantly reduce the length of the flow recirculation zone distal to the expansion, hence suppressing flow disturbance there.

Wall Shear Stress Distribution

The distribution of the area-weighted average WSS along the expansion tube of the two models is shown in Figure 5. For the normal flow, the WSS first increases, reaching a peak value, and then decreases gradually to a minimum (zero at the reattachment point). After that, it increases again, approaching the value of a fully developed laminar flow in a circular tube. For the spiral flow model, however, the WSS decreases a little after reaching the peak value, and then becomes a constant value. Because of the rotation of the flow, at each corresponding location, WSS in the swirling flow model is always higher than that in the normal flow model.

Residence Times

Figure 6 shows the distribution of the area-weighted average residence time obtained from numerical simulation. As evident from the Figure, particles in the vicinity of the expansion and the reattachment point have the longest residence time because of the stagnant flow condition, suggesting an increased contact between the circulating platelets and the wall in these areas. Results also show the effect of swirling flow on reducing the length of the flow recirculation zone, as the area with high residence time is larger in the normal flow model.

Adhesion of Platelets

When suspensions of washed platelets flowed through the flow test tubes for 1 minute, platelets were found to adhere to the collagen-coated sections of the expansion tubes. The effect of sudden expansion geometry on platelet adhesion under swirling flow and normal flow conditions was evident in Figure 7. It was observed that under spiral flow condition, the distribution of adherent platelets in the flow recirculation zone was quite uneven in most samples. This was probably attributed to the asymmetrical nature of the open recirculation flow under spiral flow condition. However, it was found that when the adherent platelets were counted over the entire area of each subdivision of the corresponding location, there were certain reproducible regularities and tendencies in the NPD curve as shown in Figure 7. Under both flow conditions, the general trend of the NPD curve was the same. Beginning at the origin of the expansion, the NPD first increased, reaching the peak value, and then decreased gradually, and finally approached a constant value of $1.01 \pm 0.21$ about 1 mm downstream from the reattachment point. For both flow groups, platelet adhesion was found maximum in the flow recirculation zone and then dropped to the constant value of 1.01 immediately distal to the reattachment point. Nevertheless, the curve of the NPD for the spiral flow group was different than that for the normal flow group. Under the spiral flow condition, as shown in the Figure, a much sharper peak in the NPD curve was observed when compared with that for the normal flow group. For the spiral flow group, NPD reached the highest value of $1.46 \pm 0.26$ at $L = 4$ mm, and decreased to the constant value at $L = 8$ mm, approximately 1 mm downstream from the flow reattachment point. On the other hand, for the normal flow group, NPD reached the peak value of $1.54 \pm 0.27$ at the distance of 7 mm from the origin, and decreased to the constant value at a distance of 14 mm from the origin. The peak value of NPD between the two flow conditions showed a significant difference ($p = \ldots$)
From $L = 6.0$ mm to $L = 12.0$ mm along the expansion tube, NPD under spiral or swirling flow condition was significantly lower than that under normal flow condition ($p < 0.05$). At each corresponding location along the first 5 mm section of the expansion tube, the spiral flow group had slightly higher NPD; however, the total number of adherent platelets in the disturbed flow region for spiral flow group was much lower than that for normal flow group.

**Activation of Platelets.** The expression of platelet surface makers P selectin and activated GPIIbIIIa on the platelet surface were measured after perfusion experiments. Figure 8 is the example histograms of the results of flow cytometric analysis. The $t$ test showed that the percentage of activated GPIIbIIIa positive platelets were significantly higher for the two experimental groups compared with the resting platelets (the control group) (15.8%, 17.0%, versus 7.0%, $p < 0.05$), but there was no significant difference in the percentage of activated GPIIbIIIa positive platelets between the spiral and normal flow groups. The percentage of P selectin positive platelets among the spiral flow group, the normal flow group, and the control group (the resting platelets) had no significant difference ($p > 0.05$).

**Discussion**

The presence of spiral blood flow patterns at several sites of the cardiovascular system has been widely documented. The implication of swirling flow in the vascular system has been suggested. We believe that the swirling motion of blood flow in the human aortic arch is a typical example of “form follows function” in the vascular system. The swirling or spiral flow can eliminate stagnation flow regions and prevent the adhesion of platelets and the accumulation of atherogenic lipids on the wall of the ascending aorta. Therefore, thrombosis and atherosclerotic plaques can hardly form in the ascending aorta.

Studies have shown that due to the size mismatch of an end-to-end anastomosis, blood flow may be inevitably disturbed, especially, in a microvascular anastomosis. The size mismatch is a predisposing factor to anastomotic complications such as thrombotic occlusions. Therefore, eliminating or suppressing flow disturbance in an anastomosis might be a solution to the thrombus problem of end-to-end anastomoses.

In this study, we proposed to apply the mechanism of swirling flow in the arterial system to the management of size mismatch in microvascular anastomosis. We hypothesized that intentionally created swirling flow in the anastomosis could suppress flow disturbance, impeding the occurrence of thrombus formation by affecting platelets adhesion, hence, could increase the anastomotic patency. To test this hypothesis, we studied the adhesion of platelets along the wall of a glass sudden tubular expansion tube under swirling flow and normal flow conditions. The swirling flow in the test tube was created by a well-designed spiral flow guider.

A typical feature of the experimental results under both flow conditions was the obvious variation in platelet adhesion along the test expansion tube, which is very similar to the experimental result obtained by Karino and Goldsmith.2 For both flow conditions, platelet adhesion was greatly elevated in the flow recirculation zone distal to the expansion and the highest platelet adhesion occurred at a position corresponding to the vortex center, where the wall shear rate was the highest theoretically. Then beyond the reattachment point, the adherent platelet density quickly reached a constant value that was markedly lower than the mean value of platelet density in the disturbed flow region.

The flow simulation in the test expansion tube showed that by intentionally introducing a swirling flow in the expansion, flow disturbance could be effectively restrained, reducing the size of the disturbed flow zone from 15.0 to 6.9 mm. As a result, the swirling flow in the test expansion tube significantly reduced the total number of adherent platelets in the disturbed flow region when compared with that for the normal flow.

According to Karino and Goldsmith, the observed increase in the NPD in the flow recirculation zone was mainly due to the curvature of the flow streamlines near the tube wall. In other words, they believed that in the flow recirculation zone, the flow streamlines carried platelets toward the wall near the
reattachment point and away from the wall near the origin of the expansion, and this in turn led to the elevation of NPD there. This explanation may also apply well to our experiment. Nevertheless, there exists a slight difference in the NPD curve between our experiment and theirs. In their study, they observed another minor peak in the NPD curve just downstream of the flow reattachment point, which did not occur in our experiment. Another puzzling result observed in our study was that, for both the spiral flow and the normal flow groups, the adherent platelet density dropped to a constant value immediately beyond the reattachment point. There was no significant variation \((p > 0.05)\) between the two flow conditions, although at each corresponding location the WSS in the swirling flow model was always higher than that in the normal flow model. The discrepancy observed between our study and theirs was possibly attributed to the sizes of the expansion tubes used. Our test expansion tube was much larger than the model used by Karino and Goldsmith.\(^2\) Therefore, our expansion tube might not be sensitive enough to detect the minor peak in the NPD curve as observed by them.\(^2\) In addition, although the WSS in the swirling flow model was higher than that in the normal flow model, the difference was only 0.1 dyn/cm\(^2\), which might not be high enough to result in significant difference in NPD in those regions.

To test the hypothesis that intentionally inducing swirling blood flow in a small-caliber arterial graft can suppress acute thrombus formation, we previously studied the effect of swirling flow on platelet adhesion and activation in a straight glass tube with matched diameter.\(^24\) The work was aimed at investigating whether the advantages of swirling flow such as enhancing near wall velocity and WSS might be beneficial to reduce the adhesion of platelets to the wall, and the results showed that platelet adhesion density under spiral flow condition was significantly lower than that under normal flow condition until about 12 mm from the origin. This means that swirling flow may have implication in the design of small caliber arterial grafts. As size mismatch may exist at the anastomosis after vascular grafts were implanted, in the present article, similar methods were used to study the effect of spiral flow on platelet adhesion and activation in a sudden expansion tube. We hope to verify whether swirling flow can suppress flow disturbance and hence reduce platelet adhesion, so as to demonstrate that swirling flow might be beneficial to increase patency not only in a matched diameter graft but also in the end-to-end anastomosis after the graft was implanted. Different from the results of the previous work that platelet adhesion increased simply along the tube, the platelet adhesion in this study first increased, reaching the peak value, and then decreased gradually, and finally approached a constant value, which was mainly due to the flow disturbance caused by the sudden expansion.

Besides thrombotic occlusion, long-term occlusion due to intimal hyperplasia caused by flow disturbance and low shear stress is also a common issue that may lead to anastomotic failure.\(^25\) Endothelial cells are known to play an important role in the pathogenesis of intimal hyperplasia, especially when they are disturbed,\(^26\) as WSS changes may alter the endothelial cells in shape and function.\(^27\) In addition, monocytes are also significant components in the initiation and progression of intimal thickening, and studies indicated that flow disturbance with low WSS could promote monocyte adhesion.\(^28\) Our numerical simulation showed that under swirling flow condition, flow disturbance could be suppressed by reducing the size of the disturbed flow zone from 15.0 mm to 6.9 mm, and WSS was increased compared with normal flow condition. These effects of spiral or swirling flow might be beneficial to reduce the endothelial dysfunction as well as monocyte adhesion in the region distal to end-to-end anastomosis, and hence increase the long-term patency of anastomosis.

The pathologic dilation of an artery (usually named aneurysms) is another long-term complication. Blood flow dynamics has been shown to play an important role in the progression of this disease. Previous investigations showed that high WSS seems to be a predisposing factor for the initiation of aneurysm.\(^29\)–\(^31\) According to Fukuda et al.,\(^31\) an excessive increase in WSS may cause the induction of inducible nitric oxide synthase that can synthesize nitric oxide (NO). The synthesized NO may lead to aneurysm formation. Although this study indicated that the swirling flow could suppress flow disturbance distal to the expansion and increase WSS there, the level of the WSS still remained in normal physiological range and was far below the level that could lead to aneurysm formation. Therefore, we may believe that the current swirling flow model might not induce aneurysmal dilatation. Nevertheless, attention should be focused on keeping WSS at normal level to prevent aneurysm formation when designing swirling flow device.

Our present experiment showed that under both flow conditions platelet was activated when compared with the resting group, and the numerical result also showed that particle residence time was higher in the disturbed flow regions. The activation of platelets over resting controls may be due to the prolonged contact of platelets with the collagen-coated surface caused by flow stagnation and recirculation in the disturbed flow regions. There was no significant difference in the activation of platelets between the swirling flow and the normal flow groups, which indicates that the swirling flow itself did not activate platelets more when compared with the normal flow. Therefore, introducing swirling flow intentionally in an end-to-end anastomosis may not have adverse effect on platelets that may lead to thrombus formation.

It has long been known that platelet adhesion may be affected by local hemodynamic factors including WSS and local convective-diffusive transport of platelets.\(^4\)–\(^34\) Previous studies demonstrated that disturbed flow with flow recirculation was predisposed to platelet adhesion.\(^2\)–\(^3\) From this study, we therefore have reason to believe that creating swirling flow in an end-to-end microvascular anastomosis is an effective way to manage the flow disturbance due to the size mismatch between the host vessel and the graft, hence solving the problem of anastomotic failure by suppressing thrombus formation.

Here, it should be mentioned that in this study, only a platelet suspension instead of whole blood was used in the platelet adhesion experiment. Although our perfusion assay system was able to analyze the effect of flow pattern (swirling or normal flow) on the adhesion of platelets in a sudden expansion tube, it is imperative to elucidate their exact roles under more physiological conditions such as using whole blood. Another limitation of this study is that as each of the perfusion experiments requires a large volume of platelet suspension (36 ml), we diluted the platelet concentration of the perfusion solution \((3 \times 10^4\) platelets per \(\mu l)\) that was appar-
ently lower than the normal platelet concentration in blood. Although this low platelet concentration is unphysiological, the results still can shed some lights on the effect of spiral flow on platelet adhesion in disturbed flow regions.

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References


BENEFICIAL EFFECT OF SWIRLING FLOW ON PLATELET ADHESION