The Asymmetric Vascular Stent
Efficacy in a Rabbit Aneurysm Model

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Background and Purpose—Development of hemodynamic modifying devices to treat intracranial aneurysms is an active area of research. The asymmetrical vascular stent (AVS), a stent containing a low-porosity patch, is such device. We evaluate AVS efficacy in an in vivo intracranial aneurysm model.

Methods—We created 24 elastase rabbit model aneurysms: 13 treated with the AVS, 5 treated with standard coronary stents, and 6 untreated controls. Four weeks after treatment, aneurysms underwent follow-up angiography, cone-beam micro-CT, histological evaluation, and selective electron microscopy scanning.

Results—Four rabbits died early in the study: 3 during AVS treatment and 1 control (secondary to intraprocedural vessel injury and an unrelated tumor, respectively). AVS-treated aneurysms exhibited very weak or no aneurysm flow immediately after treatment and no flow in all aneurysms at follow-up. Standard stent-treated aneurysms showed flow both after treatment (5/5) and at follow-up (3/5). All control aneurysms remained patent during the study. Micro-CT scans showed: 9 of 9 scanned AVS aneurysms were occluded, 6 of 9 AVS were ideally placed, and 3 of 9 low-porosity region partially covered the aneurysm neck; standard stent-treated aneurysms were 1 of 5 occluded, 2 of 5 patent, and 2 of 5 partially patent. Histology results demonstrated: for AVS-treated aneurysms, advanced thrombus organization in (9/9); for standard stent-treated aneurysms, (1/4) no thrombus, (2/4) partially thrombosed, and (1/4) fully thrombosed; for control aneurysms (4/4), no thrombus.

Conclusion—The use of AVS shows promise as a viable new therapeutic in intracranial aneurysm treatment. These data encourage further investigation and provide substantial support to the AVS concept. (Stroke. 2009;40:959-965.)

Key Words: aneurysm □ asymmetrical □ elastase □ hemodynamics □ modification □ stent model □ vascular

Intracranial aneurysm (IA) treatment using flow alteration induced by stents is a subject studied intensely by a number of endovascular research groups.1–18 The primary hypothesis is that flow diversion can induce IA thrombus formation, thereby resulting in aneurysm exclusion from the circulation.19 The goal is to achieve aneurysm exclusion while maintaining a low probability of perforator occlusion,19 in-stent restenosis, thromboembolic events, or vessel injury. Potential benefits of such a treatment would include reduced procedure time, lower dome perforation risk, shorter recovery time, reduced aneurysm recanalization, and reduced costs.

Various groups have reported both in vitro and in vivo results of aneurysm treatment with commercially available stents.1,7–10,13 However, stent-only treatment did not attain the desired reproducibility of IA occlusion.2,7,8 Aneurysm dome hemodynamics are very different from one case to another as indicated by computational fluid dynamics analysis of patient-specific aneurysm geometries.1 As a result, current commercially available stents do not provide sufficient flow diversion to consistently alter significantly intra-aneurysmal flow.

We developed a novel stent, called an asymmetrical vascular stent (AVS), that is extremely low-porosity in a relatively small, specific portion of the stent.2,5,11,14 The device was built by adding a fine low-porosity stainless steel mesh onto an existing standard high-porosity stent structure. Ideally the device is deployed so the dense patch covers the aneurysm neck. IA flow alterations with the new device have been studied extensively with phantoms4,14 or using computational fluid dynamics.3,11 Phantom studies4 using particle image velocimetry indicated radical flow changes with AVS-treated aneurysms compared to traditional stent designs. We
reported 2 orders of magnitude decrease in the velocity and vorticity, and reduced aneurysmal wall shear stress. Addi-
tionally, in vitro angiographic studies using the AVS demonstrated drastic reduction of iodine contrast in the aneurysm
dome and prolonged contrast presence after injection, signi-
fying decreased flow within the aneurysm.

Preliminary AVS in vivo verification was performed in a
small pilot study using canine aneurysms model. The study
indicated that the treatment method is possible, the aneurysms
were adjacent to and spanned the entirety of the aneurysm neck,
and prolonged contrast presence in the aneurysm for a very long time,
indicating a strong indication of slow flow within the aneurysm.

Materials and Methods
Aneurysm Creation
All procedures were approved by the Institutional Animal Care and
Use Committee of the State University of New York at Buffalo and
were conducted according to guidelines established by the animal
welfare act. Twenty-four New Zealand White rabbits (7.5 ± 0.75 kg;
6–8 kg; mean ± SD, range) underwent right carotid aneurysm creation
using previously described techniques. After 3 weeks of
aneurysm maturation, the subjects underwent angiographic evaluation
and treatment. The subjects were divided into 3 cohorts: those
marked the patch quadrants to allow for rotational placement accuracy of
alignment are shown on the bottom left. The side view shows
the projection image corresponding to the radiographic view
while the transverse view shows a cut perpendicular to the project-
ion plane passing through the dotted line. In the radigraphic images taken before and after the deployment, the white
arrows indicate the positions of the AVS markers.

Angiographic data were acquired before the procedure, immediately
after the procedure (after stent deployment), and at 4 weeks of
follow-up. Contrast inflow was graded on a 3-tiered scale: fast (F),
slow (S), and occluded (O). Fast flow is characterized by a rapid
aneurysm opacification with likewise relatively quick contrast clear-
ance ≤5 seconds. The contrast is observed to enter the aneurysms as
a small focused jet. Slow flow enters the aneurysm in a more
cloud-like dispersion and remains opacified for >5 seconds. Oc-
ccluded flow is characterized by zero contrast entering the aneurysm.

Contrast pooling, another monitored aspect, occurs when gravita-
tional forces acting on the contrast are stronger than the hemody-
namic ones. This unbalance causes the contrast to settle in
the aneurysm for a very long time, >1 minute in some cases. Previous
work indicates contrast pooling as a very strong indication of slow
flow in certain areas of the aneurysms.

Cone-Beam Micro-CT Analysis
After 4 weeks of follow-up, aneurysms were fixed by pressure
perfusion at time of euthanization using 0.9% neutral buffered
formalin, then explanted and stored in the same solution at 4°C for
48 hours for further analysis. Nineteen of 20 samples were scanned
using cone-beam micro-CT. The cone-beam micro-CT scanner
consists of a microfocal spot x-ray tube, a rotary stage, and a
microangiographic detector with a 45-μm pixel size. Aneurysm
samples were connected to an air-flow circuit for 1 to 3 minutes to
remove as much of the neutral-buffered formalin solution as possi-
ble. In this way, air was used as contrast agent to visualize the vessel lumen and the potentially untreated portions of the aneurysms.

**Histological Analysis**

For histological evaluation, aneurysm domes were excised from the parent vessel, processed, embedded in paraffin, and cut in 2-mm-thick slices taken from the midpoint of the aneurysm in the coronal orientation (same direction as the parent artery) using a rotary microtome (HM355S; Microm International). Slides were stained with hematoxylin and eosin for overall structural analysis and Masson Tri-Chrome for determination of collagen deposition. Unlike the angiographic and micro-CT evaluations wherein stent visualization prevented appropriate blinding, microscopic analysis was performed by 2 observers in a blinded fashion. A grading scale was used to evaluate the occurrence of aneurysm dome and neck organized thrombus: zero for <2% thrombus; 1 for 2% to 10% thrombus; 2 for 10% to 50%, 3 for 50% to 90%, and 4 if >90% of the area was filled with organized thrombus.

**Scanning Electron Microscopy**

Scanning electron microscopy (SEM) was performed on 1 specific AVS-stented artery specimen. This specimen was fixed using isotonic 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate (Polysciences). The arterial trunk was longitudinally cut, opposite the aneurysm neck, and the vessel walls were opened, and SEM of the parent vessel performed for an “in-vessel” view of the surface covering the stent and the stent patch. The samples were washed in distilled water, dehydrated in ethanol (70% to 100%), dried with hexamethyldisilazane (Polysciences), and stored overnight in a desiccator. SEM was performed using a field emission scanning electron microscope (Hitachi #9000) in high-vacuum mode.

**Statistical Analysis**

Data are presented as mean±SD and range. Statistical analysis was performed using GraphPad InStat (GraphPad Software Inc). Student t tests assuming equal variances were used to determine statistical significance of aneurysm geometry and aneurysm histology. Fisher exact test was used for angiographic categorical results. Significance was defined as P<0.05.

**Results**

**Aneurysm Creation**

Aneurysmal geometries were: vessel size (4.47±0.29 mm; 4.07–5.12 mm); aneurysm neck (3.32±0.75 mm; 2.11–4.69 mm); aneurysmal dome size (5.79±2.83 mm; 2.63–12 mm); and

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**Table. Summary of the Angiographic, CT, and Histology Results**

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<th>Group</th>
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<th>Before Procedure</th>
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*For histology some samples were excluded because of severe artifacts or defects that occurred during the processing of the aneurysms.

Flow classification: pooling (P), fast (F), slow (S), and occluded (O). The histology grading scale: 0 for <2% thrombus; 1 for 2% to 10% thrombus; 2 for 10% to 50%, 3 for 50% to 90%, and 4 if >90% of the area was filled with organized thrombus. NA indicate not applicable.
dome-to-neck ratio (1.80±0.84; 0.88–2.78). There were no statistically significant differences among the 3 experimental cohorts.

**Procedural Results**

Twenty-four rabbits were divided in 3 groups: AVS-treated (13/24), stent-treated (5/24), and (6/24) control. Relevant procedure was successful in (10/13) AVS, (5/5) stent, and (6/6) control. Vessel injury before stent deployment occurred in 3 AVS animals that were euthanized as required by Institutional Animal Care and Use Committee protocol. Each of these euthanizations occurred early in the study (3 of the first 4 animals) and was the result of vessel perforation during AVS positioning (1 superficial cervical artery and 2 axillary artery injuries). Once appropriate techniques were learned, zero of the final 9 animals had perforation.

Follow-up was performed on the successfully treated subject groups (10/10) AVS, (5/5) stent, and (5/6) control. One out of 6 controls had a mammary gland tumor and was excluded. Cone-beam micro-CT (µ-CT) was performed in (9/10) AVS, (5/5) stent, and (5/5) control. One of 10 of the AVS sample was used (SEM), which excluded the micro-CT analysis. All 20 samples explanted at follow-up underwent preparation for dome and neck histology analysis. During the embedding and cutting, some samples were severely damaged or affected by artifacts and were not graded. The neck was graded in 8 of 10 in AVS group, 3 of 5 in stent group, and 4 of 5 in control group. The dome was graded in 9 of 10 in AVS group, 3 of 5 in stent group, and 4 of 5 of controls.

For the AVS cohort, aneurysm neck coverage was complete in 7 of 10 and only partial in 3 of 10. Neck partial coverage was 50% for patient 16, 75% for patient 17, and >90% for patient 18.

**Angiographic Analysis Results**

Angiographic results are summarized in the Table. Eighteen of 20 surviving animals’ aneurysms (90%) showed strong initial aneurysm contrast flow, whereas 2 of 10 showed slow contrast followed by pooling: patient 6 (stent cohort) and patient 11 (AVS cohort). Immediately after traditional stent treatment, 2 aneurysms (40%) showed fast contrast diffusion, 2 (40%) showed slow perfusion (patient 8, patient 10), and 1 (20%) had full occlusion (patient 9). Immediately after AVS treatment, 9 aneurysms (90%) were completely obliterated, whereas 1 (10%) showed slow perfusion (patient 17).

At 28 days of follow-up, AVS-treated aneurysms resulted in more consistent aneurysm occlusion compared to treatment with traditional stents (P<0.01) or no treatment (P=0.01). All 5 control aneurysms were patent. Two of the stent-treated aneurysms (40%) had fast flow (patients 8 and 10), 2 had slow perfusion (patients 6 and 7), and 1 had occlusion (patient 9). All AVS-treated aneurysms were occluded.

**Cone-Beam Micro-CT Results**

The cone-beam micro-CT scanner was used to evaluate the stent placement with respect to the aneurysm neck and thrombus formation. Central CT slices taken across the vessel and the aneurysm domes are shown in Figure 2 for 1 case each: untreated, treated with a stent, and treated with an AVS.

CT scans of the 5 untreated animals showed patent aneurysm domes with no sign of thrombosis. Two stent-treated aneurysms (patients 8 and 10) were patent with no sign of thrombus formation, 2 others had remnant necks (patients 6 and 7), and 1 had full thrombosis (patient 9). All AVS-treated aneurysms were occluded.

**Histology Results**

Histology results are summarized in Table 1 and Figure 3 (top section). Four aneurysms from the control cohort successfully underwent histological analysis. None of the control aneurysm necks contained thrombus; however, 2 of 4 had some thrombus in the dome; 1 had ~4% organized thrombus, and 1 contained 2% to 4% unorganized clot.

Four of 5 stent-treated aneurysms underwent histology analysis; 1 demonstrated severe cutting artifacts and could not be graded. All 4 remaining aneurysm domes had thrombus; 2 contained 10% to 50% organized thrombus, 1 had >50% organized thrombus, and 1 filled with only organized thrombus.

Nine of 9 AVS-treated aneurysms that underwent histological analysis contained organized thrombus in both the neck and in the dome; of these, 8 had >50% organized thrombus in the dome with organized tissue spanning the neck.

At 28 days of follow-up, AVS-treated aneurysms neck histology showed more organized thrombus compared to treatment with traditional stents or no treatment. The t test analysis indicate better neck occlusion for the AVS, 3.88±0.35, than for the regular stents, 1.3±0.2, with a large statistical significance P=0.004. Results for dome filling with organized thrombus for stent vs AVS samples were 2.5±1.29 and 3.11±0.78 (P>0.05). All 5 control aneurysm necks were patent.
SEM Results

After initial processing for SEM, low-magnification light microscopic inspection (5×) of the stented aneurysm and neck region revealed new tissue growth within the patched aneurysm neck and on some large stent struts (Figure 3). The entire fine mesh patch was covered with a shiny layer consistent with endothelialization. Details obtained with the electron microscope are shown in Figure 3. Although this SEM analysis demonstrated new intima growth over the stent struts, stent mesh, and aneurysm neck, we cannot extrapolate that this occurred in all other cases given there was only 1 specimen analyzed with SEM.

Discussion

We demonstrate in vivo efficacy of a new AVS designed for the sidewall IA treatment. Ten aneurysms were successfully treated. AVS-treated aneurysms resulted in more consistent aneurysm occlusion compared to treatment with traditional stents or no treatment.

All aneurysms occluded after treatment remained occluded at follow-up. Immediately after deployment, occlusion was observed in 90% of the AVS-treated aneurysms and in only 1 (20%) of the stent group. Interestingly, whereas the 1 AVS that demonstrated slow flow immediately after deployment went on to total occlusion, both of the 2 stent-treated animals that showed changes from fast to slow flow immediately after treatment demonstrated a return of fast flow at week 4 of follow-up. Because the use of stent-assisted coiling has become increasingly used over the past 5 years, it has become evident that current, traditional stents do not consistently lead to IA thrombosis, and recanalization remains a significant problem.25–31 This promising initial data suggest that the use of an AVS-type device may substantially increase IA occlusion rates while decreasing recanalization rates.

All surviving AVS-treated animals had complete aneurysm occlusion at the 28-day follow-up regardless of the percent aneurysms neck coverage measured after micro-CT scanning. These results compare favorably to recent endoluminal flow disruption device literature.6 It should also be noted that at least 50% of aneurysm neck coverage was achieved in all cases. We find it encouraging that such a high rate of successful occlusion can be achieved with only a portion of the stent being low-porosity and anticipate continued success with improving AVS designs. However, caution must be expressed as the current AVS iteration has substantial mechanical limitations.

First, whereas AVS placement was ideal in 66% of the 9 micro-CT scanned AVS-treated aneurysms, balloon rotation during inflation caused slight misplacement of 3 stents. It is likely that such technical difficulties will be alleviated once a self-expanding stent is used as the base structure rather than a balloon-mounted stent, thereby removing the substantial rotation component-associated with balloon-mounted stent deployment. It should be noted, though, that despite partial neck coverage the hemodynamics were modified sufficiently to cause full IA thrombosis in all aneurysms at week 4 of follow-up.

Second, the AVS prototype described herein cannot likely be used in its current configuration for the more tortuous
vascular approach encountered in human neurovascular anatomy. The fine stainless mesh patch adds stiffness to the already fairly rigid balloon expandable device. Our 3-point flexure rigidity measurements show a 15% to 20% increase in the stiffness of the AVS compared to the bare stent. In any case, these data provide important proof of concept in a small animal model and are an important step in the translation of this technology toward clinical application. Current commercially available self-expanding stents that are specifically designed for intracranial use have greatly improved navigability compared to the previously used balloon-mounted coronary stents and as a result have greatly increased the number of lesions treatable by endovascular means.25–31

Three deaths occurred in the AVS cohort. All 3 were within the first 4 AVS treatment experiments and were secondary to vessel injury during AVS alignment. Because accurate localization of the aneurysm neck is essential for AVS treatment, we would advance the AVS distal to the aneurysm neck during roadmap acquisition, thereby avoiding the roadmap misregistration. These vessel injuries occurred while advancing the AVS past the aneurysms location. One injury was secondary to AVS advancement into the superficial cervical artery and 2 were secondary to AVS advancement into the axillary artery. These complications were eliminated once we were aware of the potential injury and careful attention was paid to the distal vasculature, and by using AVS no longer than 15 mm. The clear value of this learning curve is evidenced by the fact that none of the remaining 9 animals had this complication.

A potential complication of endoluminal flow disruption is perforator occlusion. Whereas we experienced no occurrence of nearby vessel thrombosis, the current model does not allow adequate assessment of this potential complication attributable to the lack of small vessel origins in the vicinity of the aneurysm. Data exist documenting excellent perforator patency maintenance with stents of ~70% porosity,6,32 well <80% porosity of the majority of the AVS. We are currently working to assess the risk of perforator occlusion resulting from low-porosity mesh coverage of vessel ostia.

The current study shows treatment of sidewall aneurysms only; however, we are currently endeavoring to develop self-expanding AVS alternatives with substantially improved flexibility and navigability, and we anticipate that such a retooling will also lead to improved applicability of this technology. To increase flexibility, in recent preliminary studies33,34 we replaced the stainless steel patch with a polyurethane membrane (ChronoFlex AR; Cardiotech International Inc). We simulated treatment in patient-specific sidewall13 and basilar-tip34 aneurysm phantoms using the AVS as a flow diverter. The optical flow study revealed significant flow reduction in the aneurysms, with partial aneurysm orifice blockage. Extension of the current latest AVS fabrication to self-expanding stents is expected in the future.

Conclusion
We demonstrate a high degree of efficacy using a new endoluminal flow diverter, the AVS, to treat aneurysms in a rabbit elastase aneurysm model. After AVS treatment, 100% of aneurysms were occluded at week 4 of follow-up. Complications were encountered early in the series but, once identified, were completely avoidable in the latter portion of the study. These data support the development and likely eventual potential benefit of AVS technology in sidewall aneurysm treatment. However, substantial improvements in stent flexibility and deployment methods are likely necessary before translation to humans can be achieved.

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