Original Contribution

Loss of Mural Cells Leads to Wall Degeneration, Aneurysm Growth, and Eventual Rupture in a Rat Aneurysm Model

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Background and Purpose—The biological mechanisms predisposing intracranial saccular aneurysms to growth and rupture are not yet fully understood. Mural cell loss is a histological hallmark of ruptured cerebral aneurysms. It remains unclear whether mural cell loss predisposes to aneurysm growth and eventual rupture.

Methods—Sodium dodecyl sulfate decellularized and nondecellularized saccular aneurysm from syngeneic thoracic aortas were transplanted to the abdominal aorta of Wistar rats. Aneurysm patency and growth was followed up for 1 month with contrast-enhanced serial magnetic resonance angiographies. Endoscopy and histology of the aneurysms were used to assess the role of periadventitial environment, aneurysm wall, and thrombus remodeling.

Results—Nondecellularized aneurysms (n=12) showed a linear course of thrombosis and remained stable. Decellularized aneurysms (n=12) exhibited a heterogeneous pattern of thrombosis, thrombus recanalization, and growth. Three of the growing aneurysms (n=5) ruptured during the observation period. Growing and ruptured aneurysms demonstrated marked adventitial fibrosis and inflammation, complete wall disruption, and increased neutrophil accumulation in unorganized intraluminal thrombus.

Conclusions—In the presented experimental setting, complete loss of mural cells acts as a driving force for aneurysm growth and rupture. The findings suggest that aneurysms missing mural cells are incapable to organize a luminal thrombus, leading to recanalization, increased inflammatory reaction, severe wall degeneration, and eventual rupture. (Stroke. 2014;45:00-00.)

Key Words: aneurysm rupture ▪ degeneration ▪ inflammation ▪ intracranial aneurysm ▪ smooth muscle cells ▪ thrombosis

Subarachnoid hemorrhage attributable to saccular intracranial aneurysm (sIA) rupture is a devastating disease leading to stroke, permanent neurological damage, and death. The disease often affects relatively young patients, leaving half of the survivors with permanent disability, and results in lifetime costs more than double that of an ischemic stroke.1 The risk of sIA rupture increases with increasing aneurysm size and aneurysm wall irregularities.2,3 The biological mechanisms predisposing sIA to grow and rupture are not yet fully understood.

Human histopathologic studies have shown that loss of mural cells is a hallmark of ruptured aneurysms.5,6 Vascular smooth muscle cells of the aneurysm wall are able to undergo phenotypic modulation, proliferate, synthesize collagen matrix, and organize luminal thrombus, all which can strengthen the vessel wall.4,6,7 There is a growing body of evidence suggesting that loss of mural cells can tilt the balance between “repair and maintenance” and “degradation and destruction” of the sIA wall.6,8,11

We investigated in a rat model the hypothesis that loss of mural cells leads to destructive remodeling, aneurysm growth, and eventual rupture.

Materials and Methods

Study Design

After microsurgical in vivo pilot series (n=12) and ex vivo histological decellularization experiments (n=5), male Wistar rats (n=24; Harlan, Horst, The Netherlands) were randomly allocated to either the decellularized or nondecellularized aneurysm group. Serial magnetic resonance angiography (MRA) was used to follow-up the animals until study end point at day 28. All animals that died before this end point underwent autopsy. Animals were excluded from final histological analysis if the cause of death was suspected from aortic dissection, unknown, or when autopsy could not be performed within a time period of <12 hours. Study design, dropouts, and follow-up scheme are given in Figure 1.

Animals were housed in an animal room at 22°C to 24°C and 12-hour light/dark cycle with free access to pellet diet and regular tap water and received humane care in conformity with institutional guidelines. The experiments were reviewed and approved by...
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the Committee for Animal Welfare at the University of Helsinki, Finland. The rats were anesthetized by subcutaneous injection of medetomidine hydrochloride (0.5 mg/kg, Domitor; Orion, Espoo, Finland) and intraperitoneal injection of ketamine hydrochloride (50 mg/kg, Ketalar; Parke-Davis, Detroit, MI). Subcutaneous injection of buprenorphine (0.3 mg/kg, Temgesic; Schering-Plow Oy, Espoo, Finland) was used for analgesia.

Aneurysm Model and Graft Decellularization

Under an operating microscope, sidewall aneurysms were created by end-to-side anastomosis of an arterial saccular graft from the descending thoracic aorta of a donor rat to the infrarenal abdominal aorta of the recipient animal, as described previously.6,12 Alternately, the left and right inguinal region was dissected and the femoral vein isolated using venous catheters (Becton Dickinson and Company, Sparks, MD) for contrast agent (gadolinium-DOTA [Gd-DOTA]) injection and fluid resuscitation (glucose, 5%).

Although it has been shown that spontaneous thrombosis in sidewall aneurysms can be significantly reduced using an oblique cut of the aneurysm pouch13 and a minimized volume-to-orifice area,14 we decided to use a standardized perpendicular long-axis aneurysm creation in relation to the parent artery and standardized aneurysm dimensions to avoid group differences in aneurysm hemodynamics and hence rate of thrombosis.

A digital video camera (Sony Exwave HAD SSC-DC58AP, Tokyo, Japan) attached to the stereomicroscope (Leica M651, Wetzlar, Germany) was used to document preoperative aneurysm dimensions (width and length), microsurgical anastomosis procedures (total operating time, aortic clamping time, time for anastomosis creation, time to hemostasis, number of extra sutures, graft ischemia time, and complications), patency and pulsation of the graft, patency of distal abdominal aorta, and aneurysm harvest procedure including endoscopy at magnifications between ×6 and ×40 (see Methods and Table I in the online-only Data Supplement).

Untreated donor arterial grafts were immediately reimplanted in control rats. The saccular aneurysm to be decellularized was prepared as described by Allaire et al15 with slight modifications. Donor grafts were harvested and frozen in PBS at −4°C. The next day the grafts were thawed, rinsed with Milli-Q (Merck Millipore, Billerica, MA) water at room temperature, and incubated for 10 hours at 37°C in 0.1% sodium dodecyl sulfate (SDS) in Milli-Q water. The SDS-treated grafts were finally washed 3 times with gentle agitation, refrozen in PBS, and kept at −4°C until use. To assess the adapted decellularization process, thoracic aorta segments of 4 rats were harvested and assigned to various SDS incubation times. Three randomly selected axial cross sections of each aortic segment per incubation time point served for counting numbers of hematoxylin-stained nuclei in each vessel wall layer (adventitia, medial layer, and endothelium) separately. Digitalized microphotographs of representative fields were taken at ×40 magnification, blinded, and analyzed (K.B.) using the ImageJ1.47e (National Institutes of Health, Bethesda, MD) software.16 Near-complete graft decellularization was documented after 10 hours of SDS treatment (Figure I in the online-only Data Supplement).

MRI and Angiographic Evaluation

MRA studies were performed with a 4.7-T scanner (PharmaScan, Bruker BioSpin, Ettlingen, Germany) using a 90-mm shielded gradient capable

Figure 1. Flowchart of the study design and time scale of the serial magnetic resonance angiography (MRA) follow-up. SDS indicates sodium dodecyl sulfate. *Three rats died before surgery (anesthesia-related complication); †died before final follow-up; and ‡including 2 cases of aneurysm rupture.
of producing maximum gradient Germany amplitude of 300 mT/m with 80-ms rise time. A linear birdcage radiofrequency coil with inner diameter of 60 mm was used. Existing protocols for high-resolution time of flight-MRA were combined with contrast-enhanced MRA (see Methods in the online-only Data Supplement). All animals underwent high-resolution serial (day 0, 7, 14, 28) imaging to evaluate flow characteristics, parent vessel integrity, perianeurysmal environment, changes in aneurysm volume, and extension of spontaneous thrombosis. We reduced the imaging time points to 4 cycles to avoid excessive anesthesia.

Contrast-enhanced MRAs were analyzed and scored according to a scheme previously used to evaluate spontaneous thrombosis of experimental sidewall aneurysms in dogs. Aneurysm patency was categorized based on contrast filling in the aneurysms axial dimension as patent (>50%), partially thrombosed (<50%), or completely thrombosed (no aneurysm filling; Figure II in the online-only Data Supplement). Growing aneurysms were further analyzed using 3-dimensional active contour segmentation software itk-SNAP (V2.4, Pennsylvania, PA).

Tissue Preparation and Combined Macro- and Endoscopic Inspection

After final follow-up MRA, the animals underwent laparotomy and dissection of the aneurysm. The tissues were perfusion-fixed with 4% formalin and measured in all dimensions under the stereomicroscope. The posterior wall of the aorta was opened and evaluated by macro- and endoscopic intraluminal aneurysm surface inspection (Karl Storz Endoscopy, Tuttlingen, Germany). After overnight incubation in 4% formalin, the samples were transferred to PBS, dehydrated through a graded ethanol series, and embedded in paraffin.

Histology and Histological Analysis

Paraffin-embedded aneurysms were cut in the middle along the longitudinal axis and cut into consecutive 4-µm sections for hematoxylin–eosin, elastica van Gieson, and Masson-Goldner trichrome staining. All histological slides underwent qualitative analysis by 2 observers (J.F. and S.M.). Histological scoring was performed blinded to the treatment allocation. Slides were visualized under light microscopy (Carl Zeiss) and postprocessed using Adobe Photoshop CS 6 (V13.0, Adobe Systems). A 4-scale grading system was used to characterize the histopathologic findings (see Methods in the online-only Data Supplement).

Data Analysis and Statistics

Two-tailed Fisher exact test was used for comparison of dichotomized histological grades, aneurysm growth, and rate of thrombosis between decellularized and nondecellularized groups and growing and stable aneurysms, respectively. Two-tailed Student \( t \) test was performed to assess differences in surgical characteristics. Data were analyzed and visualized using GraphPad Prism statistical software V6.01 for Windows (GraphPad Software, San Diego, CA). Values are expressed as mean±SD and 95% confidence interval. A \( P \) value of <0.05 was considered statistically significant.

Results

MRI Follow-up

There were no significant differences between the aneurysm patency rates at any follow-up time point between the 2 groups (Table II in the online-only Data Supplement). Aneurysms in the nondecellularized group showed a linear course of thrombosis over time. Decellularized aneurysms exhibited a heterogeneous pattern of thrombosis and recanalization. Aneurysm growth occurred in 5 decellularized aneurysms (5/12; 42%). Four of the growing aneurysms increased in size during the first week and continued to grow thereafter (4/5; 80%). One aneurysm started to grow during the second week after creation (1/5; 20%). All nondecellularized aneurysm remained stable. Follow-up time points of all cases with schematic rate of growth and spontaneous thrombosis are visualized in Figure 2. Three-dimensional contrast-enhanced MRA visualization of growing aneurysms are given in Figure III in the online-only Data Supplement.

Aneurysm Growth and Rupture

Macroscopic measurement of width and length of nondecellularized aneurysms at creation (2.5±0.3 and 4.2±0.4 mm) and final follow-up (2.6±0.3 and 4.2±0.6 mm) confirmed that these aneurysms remained stable over time. In the decellularized aneurysm group, 4 aneurysms remained stable (4/12; 33%) and 4 grew to giant aneurysms (4/12; 33%) that were as large as 43×38×24 mm (Figure 3). Three of the growing aneurysms in the decellularized group ruptured during the observation period (3/4; 75%). Two of these ruptured aneurysms showed massive intraluminal thrombosis (2/3; 66%). One suspected case of growth and rupture had to be excluded from final histological analysis because of delayed autopsy. Case by case analysis including serial MRA and macroscopic and endoscopic findings of all animals of both groups are summarized in Figures IV and V in the online-only Data Supplement.

Histological Findings

There was a complete decellularization of all SDS-treated aneurysm grafts. The cells of the nondecellularized aneurysm wall control group remained viable (no damage attributable to the transplantation ischemia time). There were no signs of graft rejection, but walls of healthy control group aneurysms showed some areas of focal hypocellularity. The endothelial cells were lost in all aneurysms in both groups. Intraluminal thrombus was present in all but 1 aneurysm in both groups.

Decellularized aneurysms demonstrate higher grades of periadventitial fibrosis, significant enhanced aneurysm wall inflammation \( (P=0.03) \), and a trend toward increased neutrophil accumulation in the thrombus \( (P=0.08) \) when compared with nondecellularized aneurysms. Failure of thrombus organization and neointima formation was seen only in decellularized aneurysms (Figure 4). However, there was no significant difference of the dichotomized neointima score between the 2 groups (Figure VI in the online-only Data Supplement).

Analysis of histological characters between stable (decellularized group, \( n=4 \)) and nondecellularized group, \( n=8 \) and growing aneurysms (decellularized group, \( n=4 \)) revealed the following: growing aneurysms had marked adventitial fibrosis and inflammation \( (P=0.002 \) and \( P=0.03) \), wall disruption \( (P=0.008) \) with inflammation \( (P=0.003) \) and intramural hematomas \( (P=0.05) \), and increased neutrophil accumulation \( (P=0.001) \) in unorganized intraluminal thrombus formation \( (P=0.05) \) when compared with healthy nondecellularized control aneurysms. All of the aneurysms that remained stable during the observation period showed neointima formation (Figure VII in the online-only Data Supplement).

Wall dissection and mural hematomas were exclusively seen in decellularized aneurysms. Decellularized aneurysms also had a stronger neutrophil accumulation in the luminal thrombus and aneurysm wall (Figure VIII in the online-only Data Supplement).
Supplement). Aneurysms with moderate to severe neutrophil accumulation in the thrombus and increased wall inflammation demonstrated significant more mural dissections ($P=0.007$ and $P=0.02$). Aneurysm with increased neutrophil accumulation in the thrombus and increased wall inflammation showed a trend for mural hematomas ($P=0.05$ and $P=0.08$).

**Discussion**

We describe an experimental model for saccular aneurysms, in which aneurysms may start to grow and even spontaneously rupture. The results of this study demonstrate that aneurysms missing mural cells (smooth muscle cells and endothelial cells) are incapable to organize a luminal thrombus. Decellularization of the aneurysm wall leads to increased neutrophil accumulation, wall inflammation, and wall fragility. Neutrophil accumulation in the thrombus, wall inflammation, wall dissections, and intramural hematomas are in turn associated with aneurysm growth. For a more detailed discussion of potential causes involved in initial loss of endothelial cells and smooth muscle cells, please see our prior review.8

**Luminal Thrombus Formation**

Repeated follow-up MRA revealed that aneurysms with a healthy wall developed stepwise thrombosis, whereas decellularized aneurysms showed continually repeating cycles of clot formation, dissolution, and aneurysm recanalization. Histologically confirmed unorganized thrombus and failure of neointima formation was only noticed in decellularized aneurysms, which further strengthens the notion of impaired thrombus organization in aneurysms missing mural cells. Together, the radiological and histological findings indicate that aneurysms with loss of mural cells are less likely to form a stable thrombus. Results of previous studies already demonstrated the paramount importance of aneurysm wall smooth muscle cells in thrombus organization and neointima formation.6,19 Therefore, it can be hypothesized that loss of mural cells is causative for the failure of luminal thrombus transformation into stable fibrotic tissue.

A most interesting finding was that decellularized aneurysms did not only induce inflammation and damage (wall dissections and mural hematomas were exclusively seen in decellularized aneurysms) to the aneurysm wall, but also increased neutrophil accumulation in the luminal thrombus. Fibrin deposition and platelet-derived neutrophil-attracting chemokine released from the thrombus per se attract neutrophils.20 The additional increased neutrophil content in the luminal thrombus of decellularized aneurysm could be explained by the fact that ongoing degradation of red blood cells and degranulation of thrombocytes, platelets, and neutrophils...
trapped in the fibrin scaffold of an unorganized thrombus initi-
ates additional chemotropic responses and attracts even more
neutrophils.20,21 In abdominal artery aneurysms, intraluminal
thrombus is associated with wall instability, which seems to
contribute to growth and rupture.22

Aneurysm Growth and Rupture

Our data show that neutrophil accumulation in the thrombus
and wall inflammation are associated with aneurysm wall dis-
sections and mural hematomas. Aneurysm wall fragility is in
turn associated with aneurysm growth and eventual rupture.
Lack of viable mural smooth muscle cells, matrix degenera-
tion, intramural hematomas, aneurysm wall inflammation, and
intraluminal thrombus formation are known characteristics of
ruptured human sIA.4,5,11

A main source for matrix-degrading proteases are neutro-
phils trapped in unorganized thrombus.23 In addition, intralu-
minal thrombosis itself is not only a site of protease release
and activation, but also releases cytotoxic compounds and
induces inflammation throughout the wall, promoting further
matrix degradation.24 Moreover, the increased accumulation
of neutrophils in aneurysm walls missing mural cells may be
linked to the lack of cell barrier, meaning that macromolecular
plasma components such as lipids, complement, and immuno-
globulins diffuse freely to the decellularized wall matrix and
induce inflammation.

Loss of mural cells means also loss of aneurysm wall repair
(defense) mechanisms such as resynthesis of degraded colla-
gen,25 induction of antioxidant enzymes,26 or proteases inhibi-
tors.27 Together, these detrimental effects may tilt the balance
from aneurysm wall cicatrization to wall destruction which
promotes growth and eventual rupture.

Potential Clinical Relevance

Evaluation of the integrity of the mural cell population might
give some help for evaluation of rupture risk and assessment
The results of our study suggest that in case of complete loss
of mural cells the healing and cicatrization process is severely
interrupted. Although clinical experience and prior literature
clearly demonstrate that endovascular embolization of aneu-
rysms with healthy walls leads to neointima formation and
long-term occlusion of the aneurysm fundus, the long-term
results of embolization therapy in aneurysms with a decel-
larized, sick wall are likely to be much poorer because of
the defective cicatrization. This may explain why some
aneurysms regrow and rupture after initially successful endovascular treatment.

Sidewall Arterial Out-Pouch Model for the Study of Aneurysm Growth and Rupture

The present study describes a novel model that allows investigation of aneurysm wall inflammation, thrombosis, growth, and eventual rupture. The loss of mural cells that is characteristic for ruptured human intracranial aneurysms was modeled in an arterial pouch graft by destroying all mural cells (endothelial and smooth muscle) with SDS treatment. There are no microscopic changes in the extracellular matrix after SDS treatment. In prior studies, SDS treatment decreased vessel compliance via loss of smooth muscle cells, but did not induce collagen denaturation.

At the present time, the best models for molecular study of aneurysm formation and growth are the induced hypertension models developed by Hashimoto et al. Induction of intracranial aneurysms by hypertension is the only physiological intracranial aneurysm model available and recapitulates key characteristics of human intracranial aneurysms. Our decision to use the presented model was ultimately dictated by the need to compare arterial grafts with different wall characteristics (healthy and decellularized sick walls).

The presented model can be used to study basic biology concepts of aneurysm formation, although one needs to be aware of differences in hemodynamic characteristics and vascular biology between the aorta and cerebral arteries. With the exception of the Hashimoto et al.'s models and the model using cisternal injection of elastase to create cerebral artery aneurysms, this limitation needs to be considered in all currently used aneurysm models. Using the sidewall arterial out-pouch model, future experiments may allow testing the efficacy and interaction of endovascular devices within different wall conditions including growing aneurysms. Within these limitations, the model has great potential in elucidating the molecular disease process underlying aneurysm wall remodeling, growth, and rupture.

Conclusions

The findings suggest that aneurysms missing mural cells are incapable to organize a luminal thrombus, leading to aneurysm recanalization and increased inflammatory reactions, which in turn result in severe wall degeneration, aneurysm growth, and eventual rupture. The results provide further support that mural cells are of paramount importance for thrombus organization and aneurysm wall homeostasis.

Acknowledgments

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Disclosures

None.

References


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Supplemental Methods

Surgical Characteristics, Morbidity, and Mortality
A total of 41 animals were used. Despite randomization there were significant differences in
body weight and total operation time between the non decellularized and decellularized
aneurysm group. The overall prolonged operation time in animals receiving non
decellularized aneurysms is explained by the delay caused during graft harvesting. All other
assessed surgical characteristics did not differ between the two groups (Supplementary Table
I). There were no significant differences in aneurysm dimension (0.1 mm in baseline
characteristics for length and width) at the time of creation between the non decellularized
decellularized group.

Four animals of the non decellularized control group died prior to final follow-up. Two
animals died within the first week after creation and showed unspecific autopsy with an
unremarkable aneurysm site. One proximal and one distal dissection of the aorta with pseudo
aneurysm formation close to the anastomosis side were the suspected cause of death in the
remaining two animals. A total of six animals died in the decellularized aneurysm group.
Three rats died due to massive intra-abdominal bleeding of a ruptured enlarged aneurysm.
Two of these three rats underwent autopsy within 12 hours. One rat underwent delayed
autopsy and was therefore excluded for final histological assessment. One animal died within
the first week due to dissection of the aorta with otherwise unremarkable aneurysm site (no
change in aneurysm angioarchitecture). Two rats died due to anaesthesia related
complications during baseline and week one follow-up MRA, respectively.

Magnetic Resonance Angiography
After shimming and scout images, the three dimensional fast low-angle shot sequence (3D-
FLASH) was acquired (TR/TE = 15/2.5 ms, flip angle = 20°, field-of-view = 40 × 40 × 40
mm3, matrix size = 256 × 256 × 16 zero filling to 256 × 256 × 256 and acquisition time = 3
min 45 s). Afterwards the 3D FLASH (TR/TE = 15/2.5 ms, flip angle = 20°, field-of-view =
40 × 40 × 20 mm3, matrix size = 256 × 128 × 16 zero filling to 256 × 128 × 256 and
acquisition time = 1 min 32 s) with short imaging time was performed. At that time the
animals received a bolus injection of Gd-DOTA (1 ml/kg body weight, intravenously,
injection time < 3 s) and the 3D FLASH with short imaging time (CE-MRA) repeated twice
without delay between the scans (late CE-MRA). Altogether, MR imaging took
approximately 30 minutes.

With exception of four final follow-up scans there were no technical problems and high-
quality imaging of the perianeurysmal environment, aneurysm, and parent artery were
obtained in all cases. Abdominal aortic dissection with pseudo aneurysm formation occurred
in four animals in the non decellularized group and in one animal in the decellularized group.
In all other cases the parent arteries remained without adverse findings (no dilatation or
constriction). Bowel adhesion and perianeurysmal contrast enhancement after early and late
CE-MRA occurred irregularly in both groups.

Four Scale Histological Grading System
The following characteristics were assessed and scored as follows: Periadventitial
inflammation (0 = none, 1 = mild, 2 = moderate, 3 = severe), periadventitial fibrosis (0 =
none, 1 = mild, 2 = moderate, 3 = severe), aneurysm wall inflammation (0 = none, 1 = few (1-
3) spots, 2 = many (>4) spots, 3 = ubiquitous), aneurysm wall hematoma (0 = none, 1 = few
(1-3) spots, 2 = many (>4) spots, 3 = ubiquitous), aneurysm wall cellularity (0 = none, 1 =
few (1-3) spots, 2 = many (>4) spots, 3 = ubiquitous), aneurysm wall dissection (0 = none, 1 =
few (1-3) spots, 2 = many (>4) spots, 3 = ubiquitous), endothelial cellularity (0 = none, 1 = few (1-3) spots, 2 = many (>4) spots, 3 = ubiquitous), luminal thrombus (0 = absent, 1 = present), neutrophils in the thrombus (0 = none, 1 = mild, 2 = moderate, 3 = severe), and neointima formation (0 = none, 1 = organizing thrombus, 2 = organizing thrombus and neointima formation, 3 = mature neointima). Scores were dichotomized as (1) none/mild and moderate/severe, (2) no/few cells and focal hypo-cellularity/normal cell count, and (3) no neointima/organizing thrombus and organizing neointima/mature neointima.

Supplemental Figure I - SDS induced rat thoracic aorta segment decellularization over time.

Supplemental Table I - Surgical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>± SD</th>
<th>95% CI upper - lower</th>
<th>B</th>
<th>± SD</th>
<th>95% CI upper - lower</th>
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<tbody>
<tr>
<td>Mean body weight (grams)</td>
<td>423***</td>
<td>18</td>
<td>408 - 438</td>
<td>358</td>
<td>13</td>
<td>347 - 368</td>
</tr>
<tr>
<td>Mean operation time (minutes)</td>
<td>46**</td>
<td>2</td>
<td>44 - 48</td>
<td>9</td>
<td></td>
<td>29 - 43</td>
</tr>
<tr>
<td>Mean clamping time (minutes)</td>
<td>22</td>
<td>4</td>
<td>18 - 25</td>
<td>18</td>
<td>7</td>
<td>12 - 24</td>
</tr>
<tr>
<td>Mean anastomosis time (minutes)</td>
<td>15</td>
<td>3</td>
<td>12 - 18</td>
<td>13</td>
<td>5</td>
<td>9 - 17</td>
</tr>
<tr>
<td>Mean time of hemostasis (minutes)</td>
<td>2</td>
<td>2</td>
<td>1 - 3</td>
<td>2</td>
<td>1</td>
<td>1 - 3</td>
</tr>
<tr>
<td>Median (range) of additional sutures</td>
<td>0 (0-2)</td>
<td>-</td>
<td>-</td>
<td>0 (0-2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean graft ischemia time (minutes)</td>
<td>31</td>
<td>4</td>
<td>27 - 34</td>
<td>n/a</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Mean aneurysm width (millimetre)</td>
<td>2.5</td>
<td>0.3</td>
<td>2.3 - 2.8</td>
<td>2.6</td>
<td>0.2</td>
<td>2.4 - 2.8</td>
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<tr>
<td>Mean aneurysm length (millimetre)</td>
<td>4.2</td>
<td>0.4</td>
<td>3.8 - 4.6</td>
<td>4.1</td>
<td>0.6</td>
<td>3.6 - 4.5</td>
</tr>
</tbody>
</table>

A - Non decellularized aneurysms (control group); B - Decellularized aneurysms (SDS group); CI - confidence interval; n/a - not applicable; ** P ≤ 0.01; *** P ≤ 0.001.
Supplemental Figure II - CE-MRA based grading of aneurysm patency.

Red dashed-dotted lines indicate contrast-enhanced aneurysmal and parent artery filling. Yellow dashed-dotted lines represent borders of aneurysm thrombosis.

Supplemental Table II - MRA aneurysm patency rate.

<table>
<thead>
<tr>
<th>Patency</th>
<th>Group</th>
<th>Time point (weeks)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>No. of patent aneurysm (%)</td>
<td>A</td>
<td>58% (7/12)</td>
<td>60% (6/10)</td>
<td>40% (4/10)</td>
<td>38% (3/8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>75% (9/12)</td>
<td>44% (4/9)</td>
<td>43% (3/7)</td>
<td>50% (3/6)</td>
<td></td>
</tr>
<tr>
<td>No. of partially thrombosed aneurysm (%)</td>
<td>A</td>
<td>25% (3/12)</td>
<td>10% (1/10)</td>
<td>30% (3/10)</td>
<td>50% (4/8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>8% (1/12)</td>
<td>2/9 (22)</td>
<td>57% (4/7)</td>
<td>17% (1/6)</td>
<td></td>
</tr>
<tr>
<td>No. of completely thrombosed aneurysm (%)</td>
<td>A</td>
<td>17% (2/12)</td>
<td>30% (3/10)</td>
<td>30% (3/10)</td>
<td>13% (1/8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>17% (2/12)</td>
<td>33% (3/9)</td>
<td>0% (0/7)</td>
<td>33% (2/6)</td>
<td></td>
</tr>
</tbody>
</table>

A - Non decellularized aneurysms (control group); B - Decellularized aneurysms (sodium dodecyl sulphate treated group).
Supplemental Figure III - Growing decellularized aneurysm visualized by 3D-CE-MRA.
Supplemental Figure IV - Control group (non decellularized aneurysms); Case by case CE-MRA, endoscopic, and macroscopic follow-up.

- **a** - Non contrast enhanced scan; **b** - Dissection proximal to aneurysm; **c** - Artefact; **d** - Dissection distal to aneurysm; ***** - Aneurysm; ---- - Border of dissection.
Supplemental Figure V - Decellularized aneurysm group; Case by case CE-MRA, endoscopic, and macroscopic follow-up.

* - MRI not available; a - Dissection distal to aneurysm; b - Ruptured giant aneurysm.
Supplemental Figure VI - Histological findings of non decellularized and decellularized aneurysms.

Non decellularized aneurysms (control group, n = 8) versus decellularized aneurysms (sodium dodecyl sulfate treated group, n = 8); *$P \leq 0.05$; ***$P \leq 0.001$. 
Supplemental Figure VII - Histological findings of stable and growing aneurysms.

Stable aneurysms (n = 12, eight non decellularized and four decellularized aneurysms) versus growing aneurysms (n = 4, four decellularized aneurysms); * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. 
Supplemental Figure VIII - Histological characteristics of decellularized aneurysms.

(A) Periadventitial fibrosis; 20x magnification; Scale bar = 100 µm. (B) Aneurysm wall hematoma; 20x magnification Scale bar = 100 µm. (C) Neutrophils trapped in thrombus; 40x magnification, oil; Scale bar = 50 µm. (D) Complete disruption of the aneurysm wall; 20x magnification; Scale bar = 100 µm. All specimens are stained with HE.