Aortic valve replacement in sheep with a novel trileaflet mechanical heart valve prosthesis without anticoagulation

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ABSTRACT

Background: Even after decades of intensive research, an ideal heart valve prosthesis remains elusive. Shortcomings of conventional devices include reduced durability of bioprostheses and the thrombogenicity of mechanical substitutes, necessitating anticoagulation and resulting in imperfect hemodynamics. Here we present in vivo results of a novel mechanical heart valve prosthesis aiming for freedom from anticoagulation.

Methods: Four female sheep had their aortic valves replaced using the novel mechanical heart valve (size 21 mm), with no postoperative anticoagulation treatment. This trileaflet heart valve was designed with the pivots in the systolic central flow. Hemodynamics, biochemistry, hematology, and macroscopy and microscopy were studied at 90 days in 2 sheep and at 1 year in the other 2 sheep.

Results: Mean (<6 mm Hg) and peak (<10 mm Hg) aortic transvalvular gradients remained low during the study period. Aortic regurgitation was trivial, and central traces were only rarely observed. The rate of thrombotic events was very low, with none macroscopically and microscopically visible thrombotic material on the device. Biochemistry and hemotology were unchanged without hemolysis. In 3 sheep, the fibrous pannus and mitral leaflet were partially folded over the edge of the annular body. Apart from organic/inorganic deposits on the leaflets after 1 year, the ultrastructurally evaluated leaflets were similar to those of nonimplanted controls.

Conclusions: The preliminary in vivo results of this novel anticoagulation-free aortic mechanical heart valve are promising with excellent hemodynamics and a very low risk of thrombotic events. (JTCVS Open 2021;7:76-88)
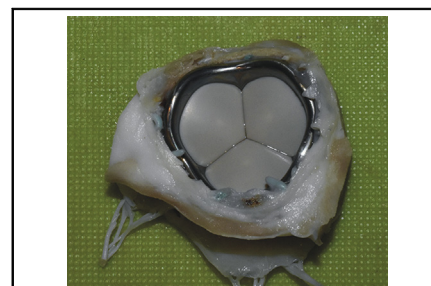
Novel trileaflet mechanical heart valve explanted after 375 days in a sheep model.

CENTRAL MESSAGE

In sheep, a mechanical aortic valve replacement with central hinge pivot position showed excellent hemodynamics, a low rate of thrombotic events, and no hemolysis without anticoagulation therapy.

PERSPECTIVE

An ideal aortic valve substitute including lifelong durability, no need for anticoagulation and excellent hemodynamics could have a disruptive impact on global health burden. The crucial design feature of the novel mechanical heart valve is the central position of the pivots to provide systolic washout. The encouraging preliminary in vivo results move forward to an ideal aortic valve substitute.

▶ Video clip is available online.

Despite intensive research, an ideal heart valve replacement prosthesis is not available.[1,2] The materials and designs of commonly used mechanical valves have not experienced a disruptive evolution.[1] Almost all clinically used mechanical valves are produced from pyrolytic carbon and consist of a circular ring that bears the pivots of 2 leaflets. Most bioprostheses are made from xenogenous pericardium

mounted in an artificial frame. Known shortcomings are the lifelong need for anticoagulation, the increased risk of stroke and bleeding, as well as the restricted quality of life with mechanical valves, the limited durability of bio-prostheses, and the reduced hemodynamic performance of both.[2] Amelioration of these shortcomings is of paramount importance to relieve individual and global health burdens.

To approach this goal, we developed a novel trileaflet mechanical valve to be used without anticoagulation, with a low pressure gradient and no regurgitation in an iterative process over the last 30 years. One major point is the central position of the pivots in a systolic high-flow area compared with hinges in the housing, where the flow is considerably lower. A flow dynamic study and an auspicious in vitro study have already been performed, and thus the next step in the development of this valve was an in vivo study. Here we present the in vivo results for this novel prosthesis in a sheep model.

METHODS
Novel 3-Leaflet Mechanical Prosthesis
The novel mechanical prosthesis consists of 3 leaflets and is described in detail elsewhere.[3,4] In brief, the housing is made of medical-grade titanium-aluminium-vanadium alloy (TiAl6V4), a standard material for medical devices because of the good biological and hematologic properties. The leaflets are manufactured from poly-ether-ether-ketone, which has also good biological properties and allows for manufacturing of varying shapes and sizes. These leaflets are fixed on struts with hemispherical hinges (Figure 1). The hinges are located in the central flow for maximal systolic washout.

For this study, a valve size of 21 mm was used. This is the most appropriate size in the sheep model and is frequently used in humans for the aortic position.

Study Animals
Four animals were used in the study, of age 24 months, age 26 months, age 68 months, and unknown age, to accommodate target weight (78-79.5 kg) at the time of implantation, with the goal of maintaining nearly the same aortic annulus size within the natural deviation. The International Organization for Standardization (ISO) 10993 (Biological Evolution of Medical Devices) part 2 (animal welfare requirements) was applied, and the study was also reviewed and approved by the Institut Mutualiste Montsouris Recherche (IMMR; Paris, France) Institutional Study Animal Care and Use Committee before study initiation (Ethics Approval UKSH GLP 18-59). All animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*.

Study Design
Novel prostheses (21 mm) were implanted in the aortic position in 4 sheep at IMMR in compliance with ISO 10993 (parts 2, 6, and 11), the Food and Drug Administration Good Laboratory Practice regulations 21 CFR Ch. I Part 58, IMMR's standard operating Procedures and Quality Assurance and the ETHI-SOP-30 Ethics Management, registered at the Comité national de réflexion éthique sur l'expérimentation animale (France) under Ethics Committee no. 37. Dr Sievers and the animal experimental specialized stuff of IMMR performed the surgeries.

Anasthesia was administered following the IMMR's standard operating procedures using morphine (0.2 mg/kg) and midazolam (0.25 mg/kg) for premedication and intravenously applied sodium thiopental (5-10 mg/kg), maintained by orotracheal intubation, oxygen (100%), and isoflurane (1%-2%).

Via a left intercostal thoracotomy at the level of the third intercostal space, the ascending aorta was exposed, followed by cannulation of the left femoral and carotid arteries and left jugular vein. This is the method most commonly used by the IMMR's surgeons and follows the IMMR's standard protocol. Normothermic blood cardioplegia was used prior to transsecting the aorta at the sinotubular junction. After excision of the native aortic leaflets the novel mechanical prosthesis was implanted with 12 polyfilament sutures with Teflon pledgets (Seracore 2/0, 2xHRT-17, with 6 × 3 × 0.5 mm pledgets; Serag-Wiessner, Naila, Germany). The aortotomy was closed, interposing a Dacron tube to relief tension on the

FIGURE 1. Novel mechanical heart valve, aortic view (*left*) and ventricular view (*right*) with the joints in the central flow.

anastomoses. The average cardiopulmonary bypass time was 75 minutes, and the average cross-clamp time was 60 minutes.

Each study animal was extubated on the table and observed continuously in an isolation pen at the IMMR Paris site during the immediate postoperative period until the ovine was breathing comfortably and demonstrating stable vital signs, as determined by the staff veterinarian or study animal health technician. Once stable, each study animal was observed every several hours for the next 24 hours. Any atypical observations were recorded on the animal's case report form. For analgesia, finadyne (2 mg/kg intramuscularly [i.m.]) and morphine sulfate (0.3 mg/kg i.m. and 0.2 mg/kg i.m. 2 × 24 hours) were administered at anesthesia weaning and as needed for the first 48 hours. Antibiotic treatment consisted of cefazolin 20 mg/kg/i.m./2 × 24 hours for 8 days.

Postoperatively, all 4 sheep were treated with enoxaparin (Lovenox) 0.1 mL/10 kg/2 × 24 hours within the first month (days 0 to 30). Lovenox was given because of concerns about hypercoagulability in the short term as well as tissue integration of the prostheses directly after operation. Afterward, the animals were treated with aspirin 250 mg/i.m./1 × 24 hours until humane killing. The original protocol aimed to kill all 4 sheep after 90 days; however, the excellent results after the first 2 explantations prompted us to extend the survival time to 1 year for the remaining 2 animals. To identify possible issues in the hardest conditions, 1 of the latter animals received no antithrombocyte and anticoagulation medication in the previous month.

During follow-up echocardiographic evaluation, biochemistry (eg, urea, creatine, glucose, plasma proteins, phosphorus, total bilirubin) and hematology (eg, red blood cells, hemoglobin, hematocrit, white blood cells, platelets, free hemoglobin) were performed on a regular basis (days 0 [only hematology], 30, 60, 90, 120, 180, 240, and 300 and before explantation). For the echocardiographic evaluation, the sheep were anesthetized. Echocardiography was done both transthoracically and transesophageally, depending on the feasibility of obtaining the best images. The medical specialist evaluated leaflet motion subjectively. Histologic evaluation, including ultrastructural analysis, was conducted in compliance with ISO 10993-Part 6 and IMMR POT-SOP-160 and was performed by experienced pathologists at the IMMR. Evaluation of embolization of all relevant organs (lung, liver, spleen, kidney, lymph node draining the side of implantation, and brain) was performed macroscopically during autopsy and photographically documented and microscopically afterward (Figure 2).

Data Analysis

We decided not to perform statistical analyses because of the limited number of sheep, and present only original data.

RESULTS

During follow-up, no clinical complications were observed, including no stroke or bleeding events. All sheep were clinically unremarkable.

Weight Development

The mean weight of the 4 sheep was 78.6 ± 0.75 kg before implantation, remained stable through week 13 (77.4 ± 4.5 kg), and then increased through week 52 (94 ± 8.13 kg).

Biochemistry and Hematology

Biochemistry and hematology parameters were normal in all 4 sheep. The hemolysis was normal over the entire study period. Full individual biochemistry and hematology panels are presented as Tables E1-E8.

Cardiac Output

Cardiac output was approximately within the range of 2 to 7 L/minute, except for an output of 10.2 L/minute in 1 animal at day 300.

Transvalvular Gradient

Mean aortic transvalvular pressure gradients remained low during the study period (<6 mm Hg), except for an elevated gradient (12.7 mm Hg) at day 60 in 1 sheep (Figure 3). Peak aortic transvalvular gradients were low (<10 mm Hg) throughout the study period except for an elevated level (19.7 mm Hg) in 1 sheep at day 60 (Table 1).

Preliminary Results of Aortic Valve Replacement in Sheep with a Novel Trileaflet Mechanical Heart Valve Prosthesis without Anticoagulation

New mechanical heart valve in sheep without anticoagulation.

Implantation

Explantation

2x 90 days

2x 1 year

Results

- first 30 days Lenovox
- day 30 to sacrifice only aspirin
- one sheep of the 1 day group got no medication for the last month
- full time hemodynamic follow-up

Overall
- very low thromboembolism
- low pressure gradient
- no / trace regurgitation

These preliminary results are a glimpse for a durable mechanical heart valve substitute without anticoagulation.

FIGURE 2. Animal study of a novel trileaflet mechanical heart valve prosthesis without anticoagulation.

FIGURE 3. Evolution of the mean aortic transvalvular pressure gradient in each sheep during follow-up. The gradients remained low during the study except for 13 mm Hg in one sheep (#71204) at day 60, which decreased to 5 mm Hg at 90 days.

Effective Orifice Area (EOA)

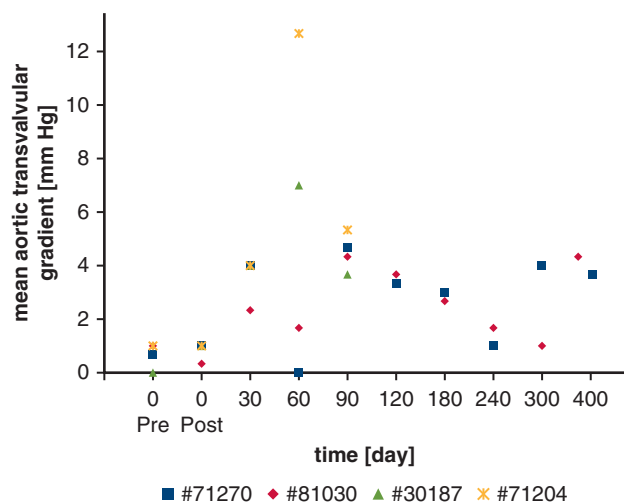Mean EOA, calculated from either maximal velocity (V_{max}) or velocity time index (VTI), decreased from the time of implantation (V_{max}, 2.53 cm^2/VTI, 2.65 cm^2) through day 90 (1.12/1.16 cm^2), and then increased through day 240 (2.82/2.88 cm^2) and again decreased at day 300 (2.11/2.15 cm^2) (Table 1).

Aortic and Mitral Regurgitation

Aortic regurgitations were trivial, central traces were rarely observed, and only a trace paravalvular leak was observed at day 30 in 1 sheep. Central trace mitral regurgitations were rare.

Leaflet Motion and Valve Thrombosis on Echocardiography

Because the outer part of the valve is composed of metal, echo artifacts did not allow for proper evaluation of the leaflet motion in some cases. However, if imaging quality was sufficient, leaflet motion of the replacement valve as well as the adjacent native mitral valve appeared normal for all animals at each time point, and thus no obvious signs of valve thrombosis were observed.

Macroscopic Evaluation

All implanted valves showed nice pannus integration at the level of the suturing ring. All leaflets were free of pannus and thrombosis. In the animals at 90 days, there were no abnormal macroscopic findings at the level of the devices (Figure 4). Pannus/tissue integration/ingrowth was observed all around the devices at the level of the suturing ring. Macroscopic findings at the level of systemic organs (lungs, liver, spleen, trachea bronchial lymph mode, kidneys, and brain) consisted of only rare, small infarcts on the right and/or left kidney. All other organs were free of findings. The findings in the animals followed up at 1 year were similar to those evaluated at 90 days, with a more important tissue ingrowth identically localized mainly on the suturing ring (Figure 5). No strokes or bleeding were reported during follow-up.

Histologic Evaluation

Animals killed at 3 months after implantation. A mild, completely reendothelialized fibrous pannus (ie, collagen fibers and fibroblasts with mild vascularization) completely embedded the fabric of the suturing ring and suture material on the abluminal side of the device. The fibrous pannus and the mitral leaflet were folded partially over the ventricular edges of the annular body from both animals (Figure 4).

TABLE 1. Hemodynamic parameters of the 4 sheep at different time points

| | Sheep 71270 | | | Sheep 81030 | | | Sheep 30187 | | | Sheep 71204 | | |
| | | EOA, cm^2 | | | EOA, cm^2 | | | EOA, cm^2 | | | EOA, cm^2 | |
Day	PTG, mm Hg	VTI	V$_{max}$	PTG, mm Hg	VTI	V$_{max}$	PTG, mm Hg	VTI	V$_{max}$	PTG, mm Hg	VTI	V$_{max}$
Preop	1.00	3.00	3.10		3.30	4.10		3.90	3.40		2.86	2.56
Postop	3.00	2.50	2.60	2.67	2.99	2.75	2.67	3.30	3.06	6.00	1.80	1.69
30	10.33	2.52	1.86	4.00	3.75	3.98	10.00	2.55	1.84	7.00	1.29	1.33
60				3.67	2.80	2.84	12.33	0.91	0.74	19.67	0.71	0.55
90	11.67	1.54	1.31	9.00	0.99	0.99	8.00	1.07	0.93	10.67	1.05	1.26
120	7.00	2.05	2.32	5.67	1.18	1.10						
180	6.00	1.88	1.94	6.33	3.25	2.43						
240	2.33	2.62	2.84	2.67	3.01	2.92						
300	8.33	2.87	2.83	3.00	1.35	1.35						
1 y	4.67			5.33								

PTG, Peak aortic transvalvular gradient; *EOA*, effective orifice area; *VTI*, velocity time index; *V$_{max}$*, maximum velocity.

FIGURE 4. Macroscopic aortic (*upper*) and ventricular view of the explanted novel mechanical trileaflet heart valve (*lower*) (*left column*, sheep #30187; *right column*, sheep #71204) after 90 days.

The luminal aspect of the annular ring, as well as the surface of the struts and ball heads, were devoid of any tissue ingrowth, fibrin deposits, or endothelial cells (Figures 5 and 6).

Eosinophilic deposits (likely plasma insudation) were noted within the interstices of the fabric of the suturing ring. Minimal fibrin deposits were focally observed within the pannus between the annular body and the fabric of the suturing ring in 2 of 6 sections.

Minimal to mild mononuclear inflammatory cells (ie, macrophages and lymphocytes) were noted within the fibrous pannus. Minimal multinucleated foreign body–type giant cells were also observed, mostly around polyester fibers of the suture material and the fabric of the suturing ring.

With the exception of very thin layer (approximately 6 μm) of eosinophilic deposits (likely plasmatic proteins) on the inflow surface of one leaflet of 1 animal, the microscopic features of the leaflets were similar to those of the control nonimplanted material. There were no fibrin deposits, no tissue ingrowth (fibrous pannus formation), and no endothelial cells on the inflow or outflow surfaces of the leaflets.

There were no relevant microscopic findings in the examined sections from the heart, lung, liver, spleen, tracheobronchial lymph nodes, and brain that could be related to the device implantation.

Renal infarcts were noted in all additional samples from macroscopic findings. These infarcts were focal and minimal and relatively old lesions (ie, cone-shaped areas of fibrosis, mononuclear inflammation with eosinophilic tubular casts extending from the kidney capsule deeply in the cortex).

Animals killed at 12 months after implantation. A mild, completely reendothelialized fibrous pannus completely embedded the fabric of the suturing ring and stitches on the abluminal side of the device. In 1 anamial, the fibrous pannus and the mitral leaflet were folded over the ventricular edge of the annular body in a small section (Figure 5). The luminal aspect of the annular ring, as well as the surface of the struts and ball heads, were devoid of any tissue ingrowth (fibrous pannus), fibrin deposits, or endothelial cells, similar to explanted devices after 3 months (Figure 6).

The microscopic features of the poly-ether-ether-ketone leaflets were similar to those of the control nonimplanted material. There were no fibrin deposits, tissue ingrowth
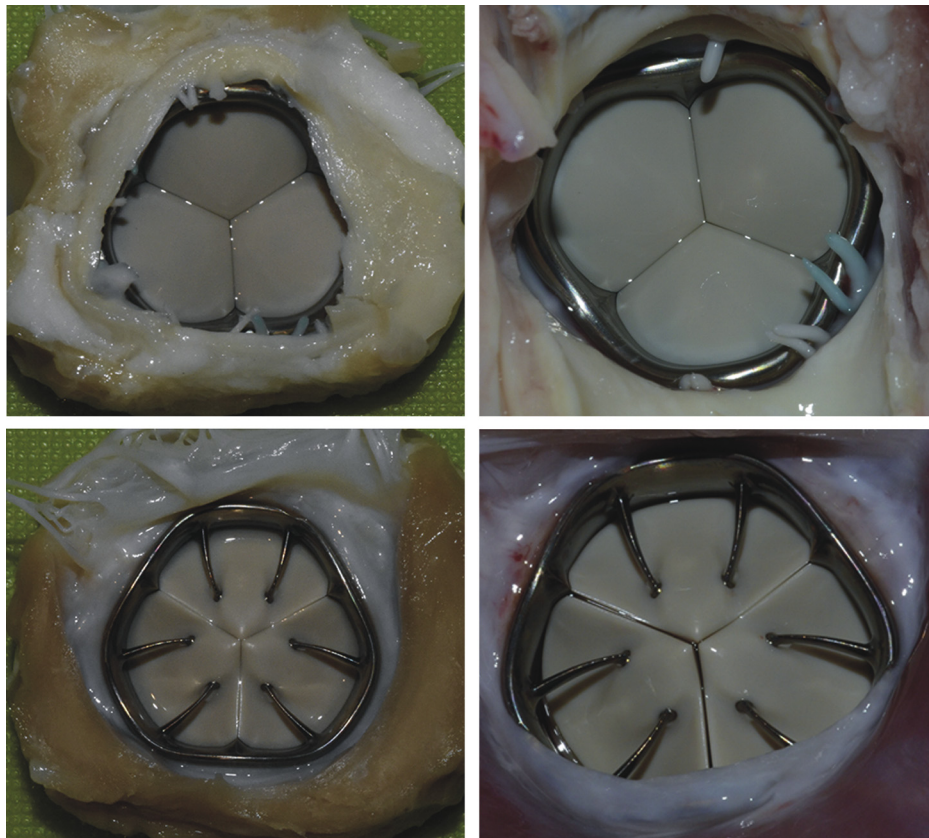
FIGURE 5. Macroscopic aortic (*upper*) and ventricular view of the explanted novel mechanical trileaflet heart valve (*lower*) (*left column*, sheep #71270; *right column*, sheep #81030) after 407 and 375 days, respectively.

(fibrous pannus formation), or endothelial cells on the inflow or outflow surfaces of the leaflets.

The hearts showed microscopic findings consisting of minimal to mild fibrosis of the epicardium and, rarely,

minimal inflammation of the myocardium. All changes were most likely related to the surgical procedure. There were no relevant microscopic findings in the lung, liver, spleen, trachobronchial lymph nodes, and brain that could

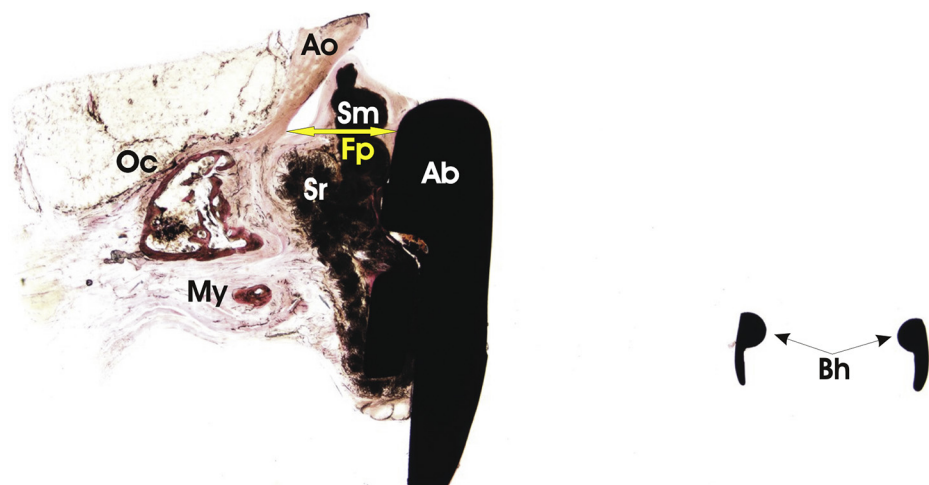**FIGURE 6.** Microscopical vertical section of the novel trileaflet mechanical heart valve through the annular body, after the surute ring and the ball heads of the joints in sheep #81030, explanted at day 375, showing no thrombosis and pannus at the device. *Ao*, Aorta; *Sm*, suture material; *Oc*, os cordis; *Fp*, fibrous pannus; *Ab*, annular body; *Sr*, suture ring; *My*, myocardium; *Bh*, ball head.

be related to the device. The findings in the kidneys were similar to those in the sheep that were explanted at 3 months.

Ultrastructural Analysis
Animals killed at 3 months after implantation. The samples from the right coronary leaflets of both animals were similar to the control sample from nonimplanted material, with the exception of wavelets and some rod-shaped deposits in 1 animal.

Animals killed at 12 months after implantation. In both samples from the right coronary leaflet, the material was intact and similar to the control with regular-shaped cavities. In the sample from the animal that had gone an additional month without anticoagulation and without aspirin, many blood cells as well as numerous organic/inorganic deposits that contained fibrin were noted at the surface, including platelets. In the sample from the other animal, few blood cells as well as organic/inorganic deposits covered with bacteria were noted at the surface. These bacteria were likely incidental contamination at the time of explantation and sampling (Figure 7).

DISCUSSION

As formulated by Rahimtoola,[5] an ideal heart valve prosthesis should have the properties of lifelong durability, off-the-shelf availability, excellent hemodynamics, easy implantability, no need for anticoagulation therapy, and no risk of thromboembolic events. However, even after decades of intensive research, no such ideal heart valve prosthesis is available. The commonly used bioprostheses or mechanical valves are burdened by reduced durability, thromboembolism, need for anticoagulation, and imperfect hemodynamics. To ameliorate these shortcomings, we developed a novel trileaflet mechanical heart valve without the need for anticoagulation and with only continuous aspirin as an antiplatelet therapy. The important findings of this in vivo experimental study with the novel prosthesis without anticoagulation are a low transvalvular gradient, no or trace regurgitation, a low rate of thrombotic events, and no hemolysis.

EOA and pressure gradients are important commonly used parameters to evaluate the systolic performance of aortic valve substitutes, because they may influence left ventricle function, exercise capacity, and survival.[6-9] At the latest follow-up of 1 year, the mean EOA was 2.11 cm^2, comparing nicely with that measured for the On-X mechanical heart valve of 2.0 cm^2 in a 21-mm device but considerably exceeding the values of novel bioprotheses.[10] In some measurements EOA was higher, but this seems to be an overestimation of echocardiographic data. Mazine and colleagues[11] reported mean aortic pressure gradients in adults after mechanical heart valve replacement between 9.3 and 17 mm Hg in a systemic review and meta-analysis. Interestingly, Milewski and colleagues[12] found an increase in the mean aortic transvalvular gradient over time even in mechanical valves, reaching 20 mm Hg at 9.5 years postoperatively. The mean

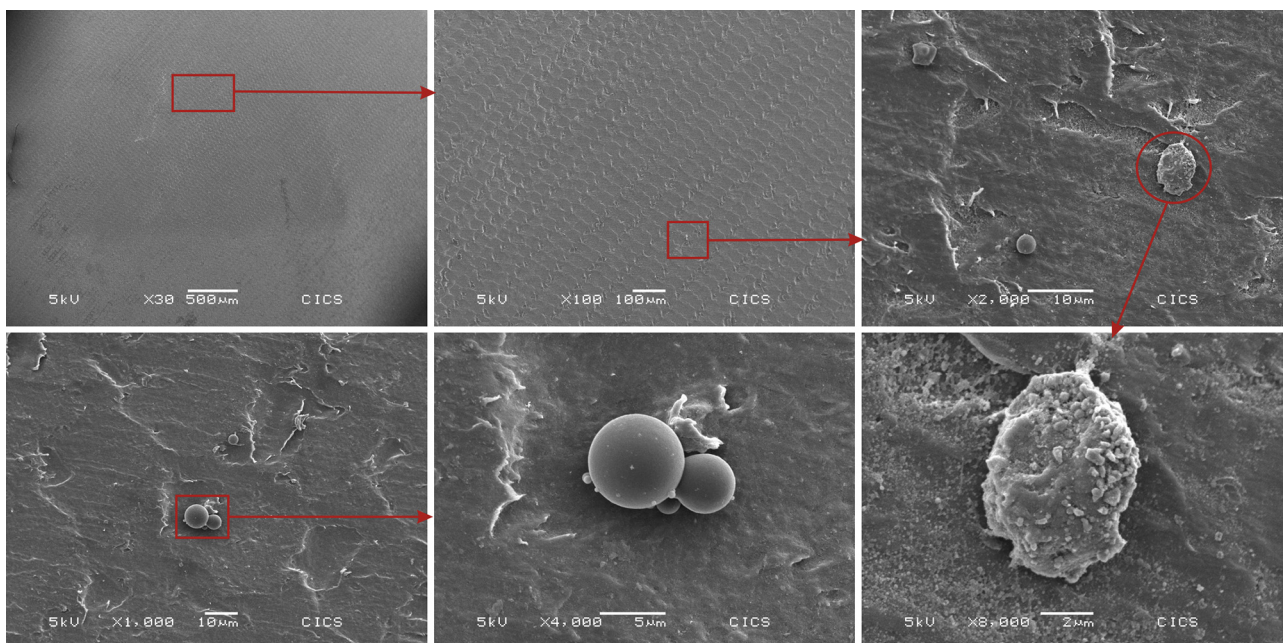**FIGURE 7.** Scanning electron microscope picture of a leaflet of the novel valve from sheep #81030 explanted after 375 days. Row 1: From *left to right*, overview at 30× magnification, sectional view at 100× magnification, and possible deposit at 2000× magnification (*red circle*) and also at 8000× magnification. Row two: from *left to right*, lipids or solvent trace at 1000× and 4000× magnification.

VIDEO 1. Prof Dr Hans H. Sievers on the development and the latest results of an anticoagulation free mechanical heart valve. Video available at: https://www.jtcvs.org/article/S2666-2736(21)00118-2/fulltext.

transvalvular pressure gradient over time was low (<6 mm Hg) in this study, comparable to the in vitro data for this valve.[3] These results are favorable, particularly because the valve size was only 21 mm and the weight of the animals increased, with the expectation of a higher gradient. Most likely the design of the leaflets, imitating flying wings, along with the confusor/diffusor shape of the ring, had positive effects on undisturbed flow, reducing flow resistance.[4]

An essential design feature of this novel mechanical valve is the fact that theoretically no regurgitation occurs in the closed position, which also may contribute to potentially less hemolysis and thrombus formation by diastolic jets through the pivots inherent in currently available bileaflet mechanical valves.[13] Furthermore, whether residual aortic regurgitation (eg, after transcatheter aortic valve replacement) has an impact on mid-term and long-term survival remains a matter of debate.[14,15] In this study, aortic regurgitations were trivial, central traces were observed only rarely, and only a trace paravalvular leak was visible at day 30 in 1 sheep.

A considerable shortcoming of mechanical valves is the risk of thromboembolism despite anticoagulation therapy. The linearized rate of thromboembolic events is reported to be 1.5% to 2% per patient-year, and that of bleeding is approximately 3% per patient-year.[16,17] In this study, the luminal aspect of the annular ring, the surface of the struts, the pivot ball heads, and the leaflets were devoid of fibrin deposits. This is reflected by the absence of device-related microscopic changes in the examined sections from the heart, lung, liver, regional lymph nodes, and brain. In the kidney, focal, some relatively old and minimal infarcts were noted. These infarcts were likely postischemic events of thromboembolic origin rather than related to the device implantation procedure. Despite the statistically small number of animals, these very low rates of thrombotic and

thromboembolic events even after a period of 1 year with aspirin only and without any anticoagulation in 1 sheep for the last month are very promising and need further evaluation in comparison with standard devices. This impressive finding is basically related to the central position of the pivots. In a flow cross-section, the fluid velocity decreased sharply toward the housing. Thus, positioning of the pivots in this area, as in bileaflet mechanical heart valves, seems to be critical. Several investigations not only have indicated thrombotic potential in the diastolic hinge flow of bileaflet mechanical heart valves but also have revealed irregular flows and possible platelet aggregation along the near-wall leaflet edges in systole.[18,19] Interestingly, elder monoleaflet mechanical valves, in which the pivot mechanisms are located more centrally, also show less platelet activation.[20] In this regard, positioning of the pivots in the systolic high-flow area seems to be beneficial, as implemented in the novel trileaflet valve.

The fabric of the suturing ring and stitches were completely embedded by a mild reendothelialized pannus. The fibrin pannus formation around the suture lines was extensive, but there was no interaction with the leaflet function, because the leaflets move away from the ring during opening. This pannus and the mitral leaflet were occasionally folded over the edges of the annular body. Whether this is related to an imbalance between the height of the suture ring and the annular body or to intrinsic anatomic characteristics of the sheep's left ventricular outflow tract remains speculative and needs further evaluation.

Although organic/inorganic deposits that contained fibrin were noted at the surface of the leaflets samples from sheep killed at 12 months after implantation, no fibrin deposits were found at the microscopic level on the metallic elements or leaflets in the examined histologic sections. In the sheep treated with aspirin for 1 year and without any antithrombocytic or anticoagulative medication in the last month until sacrificed platelets could be observed on scanning electron microscopy pictures. This may be related to the complete lack of antithrombocyte therapy in that last month, but it should be kept in mind for further studies.

In another trileaflet mechanical heart valve study by Gallegos and colleagues[21] in sheep using no anticoagulation, a 20% per year risk of thromboembolism was observed at necropsy. This confirms our findings and supports the policy that at least antithrombocyte therapy seems necessary, which in combination with a lifelong durability would already represent considerable progress in the treatment of heart valve disease.

Limitations

The number of animals in this study was rather low and the duration of follow-up was too short to allow a completete evaluation of the valve's performance. Because of limited resources, a comparison group is missing. Thus, the present

work is not a full study but rather a proof-of-concept study. Nevertheless, this 1 year in vivo test provides a reliable basis for further evaluation.

The physiology of sheep and humans are different overall, impeding transfer of our results to the clinical situation. Limitations also concern the detection of valve thrombosis during follow-up because echocardiographic observation of leaflet motion as a sole parameter was difficult owing to the metal valve ring. However, postexplantation inspection of the valves showed no macroscopic signs of valve thrombosis.

In conclusion, the preliminary results presented here show favorable hemodynamics of the novel valve and a very low rate of thrombotic events during exclusive antiplatelet therapy. In this respect, the special design of the novel mechanical heart valve prosthesis could represent progress on the way to an anticoagulation-free and durable heart valve prosthesis (Video 1).

Conflict of Interest Statement
Dr Sievers is patent holder of the novel mechanical heart valve (US 9,775,708 B2) described in this article. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: animal study, mechanical heart valve, anticoagulation, aortic valve replacement

TABLE E1. Biochemistry panel for sheep #71270

Parameter	D0	D30	D60	D90	D120	D180	D240	D300	D407	Normal concentration Low	Normal concentration High
Urea, mmol/L	7.6	7.7	7.4	7.6	6.5	4.1	8.8	7.3	6.8	2.3	10.2
Creatinine, μmol/L	119	113	122	119	116	125	110	123	90	68	150
Glucose, mmol/L	6.8	3.4	4.5	3.8	3.6	3.6	3.8	4.1	3.1	2.4	9.5
Sodium, mmol/L	147	154	143	147	147	148	145	147	148	142	155.5
Potassium, mmol/L	4.1	5.4	4.3	4.5	4.5	4.5	4.3	4.6	4.2	3.9	6.3
Chloride, mmol/L	103	104	106	105	109	105	102	108	109	98	112
Bicarbonate, mmol/L	20	28	21	21	21	27	25	24	27.6	19	32
Plasma proteins, g/L	83	78	79	78	79	80	81	79	77.6	60	86
Calcium, mmol/L	2.42	2.59	2.79	2.64	2.55	2.73	2.7	2.59	2.7	2.02	2.81
Phosphorus, mmol/L	2.06	1.8	*0.74*	1.3	1.11	1.18	1.25	1.12	*1.06*	1.09	3.27
Magnesium, mmol/L	0.88	0.95	0.92	0.89	0.92	0.97	0.92	0.83	0.87	0.64	1.11
Total bilirubin, μmol/L	4	<2.5	<2.5	<2.5	<2.5	4	<2.5	3	<2.5	0	6
Alkaline phosphatase, U/L	118	140	144	151	135	113	133	94	169	37	299
Gamma glutamyl transferase, U/L	49	66	56	51	53	57	58	51	45	33	84
Aspartate aminotransferase, U/L	103	107	123	145	138	125	118	138	149	67.5	253.5
Alanine aminotransferase, U/L	18	15	21	21	20	19	21	22	42	9	46.5
Creatine phosphokinase, U/L	272	211	435	457	411	377	617	815	505	154	2588
Lactate dehydrogenase, U/L	509	564	784	750	728	720	763	783	729	335.15	870.5

The biochemistry panel was normal except for phosphorus, which was low at day 60 but returned to normal thereafter. Phosphorus was also very slightly low on the day of death. This finding is incidental, unrelated to the device, and without clinical significance. Data out of normal rangetration are in bold italic type.

TABLE E2. Biochemistry panel for sheep #81030

Parameter	D0	D30	D60	D90	D120	D180	D240	D300	D375	Normal concentration Low	Normal concentration High
Urea, mmol/L	4.4	5.7	7.3	7	6.1	5.4	5.4	4.3	4.8	2.3	10.2
Creatinine, μmol/L	132	115	109	116	114	120	120	106	99	68	150
Glucose, mmol/L	4.4	3.1	4.1	3.4	3.7	3.5	3.6	3.9	3	2.4	9.5
Sodium, mmol/L	146	148	145	147	147	144	148	149	147	142	155.5
Potassium, mmol/L	4.9	4.8	4.2	4.4	4.6	4.6	4.8	4.6	4.4	3.9	6.3
Chloride, mmol/L	103	104	101	107	103	104	107	111	110	98	112
Bicarbonate, mmol/L	24	24	24	23	27	24	22	25.5	25.5	19	32
Plasma proteins, g/L	77	77	77	77	78	76	84	75.9	77.5	60	86
Calcium, mmol/L	2.61	2.61	2.34	2.43	2.44	2.35	2.56	2.34	2.47	2.02	2.81
Phosphorus, mmol/L	2.38	2.62	2.18	1.69	2.36	1.91	2.4	2.19	2.16	1.09	3.27
Magnesium, mmol/L	0.87	0.98	0.9	0.87	0.91	0.98	0.85	0.91	0.93	0.64	1.11
Total bilirubin, μmol/L	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	0	6
Alkaline phosphatase, U/L	118	80	99	89	69	88	51	48	48	37	299
Gamma glutamyl transferase, U/L	70	77	67	64	71	72	74	67	70	33	84
Aspartate aminotransferase, U/L	100	95	109	106	125	114	123	145	141	67.5	253.5
Alanine aminotransferase, U/L	14	19	18	17	15	17	18	33	30	9	46.5
Creatine phosphokinase, U/L	179	294	316	239	208	337	351	381	410	154	2588
Lactate dehydrogenase, U/L	493	652	668	721	571	587	638	634	576	335.15	870.5

TABLE E3. Biochemistry panel for sheep #30187

Parameter	D0	D30	D60	D90	Normal concentration	
					Low	High
Urea, mmol/L	3.7	7	7.8	6.6	2.3	10.2
Creatinine, μmol/L	103	129	112	109	68	150
Glucose, mmol/L	3.8	3.4	4	3.6	2.4	9.5
Sodium, mmol/L	149	145	147	147	142	155.5
Potassium, mmol/L	4.7	4.5	4.5	4.6	3.9	6.3
Chloride, mmol/L	101	101	102	105	98	112
Bicarbonate, mmol/L	29	24	27	25	19	32
Plasma proteins, g/L	73	71	73	74	60	86
Calcium, mmol/L	2.5	2.66	2.39	2.48	2.02	2.81
Phosphorus, mmol/L	2.29	2.13	1.91	1.74	1.09	3.27
Magnesium, mmol/L	1.01	0.98	0.97	0.94	0.64	1.11
Total bilirubin, μmol/L	<2.5	<2.5	<2.5	<2.5	0	6
Alkaline phosphatase, U/L	48	63	***36***	37	37	299
Gamma glutamyl transferase, U/L	40	55	44	42	33	84
Aspartate aminotransferase, U/L	106	92	222	152	67.5	253.5
Alanine aminotransferase, U/L	17	22	44	31	9	46.5
Creatine phosphokinase, U/L	161	383	1003	902	154	2588
Lactate dehydrogenase, U/L	445	584	***1095***	806	335.15	870.5

The biochemistry panel is normal. Data out of normal range are in bold italic type.

TABLE E4. Biochemistry panel for sheep #71204

Parameter	D0	D30	D60	D90	Normal concentration	
					Low	High
Urea, mmol/L	5.7	6.4	6.9	5.6	2.3	10.2
Creatinine, μmol/L	131	124	119	113	68	150
Glucose, mmol/L	6.5	3.9	3.8	3.6	2.4	9.5
Sodium, mmol/L	148	146	146	151	142	155.5
Potassium, mmol/L	4	4.3	4.4	4.9	3.9	6.3
Chloride, mmol/L	105	100	104	109	98	112
Bicarbonate, mmol/L	22	24	24	24	19	32
Plasma proteins, g/L	***87***	86	81	82	60	86
Calcium, mmol/L	2.66	2.64	2.53	2.63	2.02	2.81
Phosphorus, mmol/L	1.4	1.56	1.4	1.41	1.09	3.27
Magnesium, mmol/L	0.83	0.94	0.92	0.92	0.64	1.11
Total bilirubin, μmol/L	4	<2.5	<2.5	<2.5	0	6
Alkaline phosphatase, U/L	69	71	71	76	37	299
Gamma glutamyl transferase, U/L	50	59	54	53	33	84
Aspartate aminotransferase, U/L	113	97	94	115	67.5	253.5
Alanine aminotransferase, U/L	25	26	23	26	9	46.5
Creatine phosphokinase, U/L	230	528	507	866	154	2588
Lactate dehydrogenase, U/L	548	862	644	668	335.15	870.5

The biochemistry panel is normal. Data out of normal range are in bold italic type.

TABLE E5. Complete blood count panel for sheep #71270

Parameter	D0	D30	D60	D90	D120	D180	D240	D300	D407	Normal concentration Low	High
Red blood cells, $\times 10^{12}$/L	13.3	13.4	13.4	12.4	10.8	12.2	13	11.6	11.8	9.1	14.7
Hemoglobin, g/dL	14.1	*15.4*	*15.4*	13.5	11.6	12.8	14.2	12.3	13	9.6	14.8
Hematocrit, L/L	0.43	*0.48*	*0.45*	0.42	0.34	0.4	*0.44*	0.38	0.4	0.27	0.43
White blood cells, 10^9/L	5.7	*10.9*	6.8	6.5	5.8	5.5	6	5.4	4.8	3.4	10.8
Neutrophil ratio, $\times 10^9$/L	1.94	*7.41*	2.31	2.02	1.8	1.87	2.46	2.27	1.78	1.03	5.44
Eaosinophil ratio, $\times 10^9$/L	0.17	0.44	0.82	0.59	0.52	0.55	0.36	0.27	0.29	0.05	1.32
Basophil ratio, $\times 10^9$/L	0.11	0.11	0.07	0.07	0	0	0	0	0	0	0.12
Lymphocyte ratio, $\times 10^9$/L	2.74	2.62	3.4	3.64	3.42	2.92	2.52	2.65	2.45	1.49	5.89
Monocyte ratio, $\times 10^9$/L	*0.74*	0.33	0.2	0.2	0.06	0.17	*0.66*	0.22	0.29	0.05	0.56
Platelets, $\times 10^9$/L	422	*878*	451	555	300	581	480	333	196	119	765
Reticulocytes, %	0.17	0.21	0.15	0.15	0.07	0.05	0.08	0.03	0.09	0	0.4
Free hemoglobin, g/L	0.16	0.02	0.06	0.46	0.14	0.06	0.15	0.06	NA	0	1.0

Hemoglobin, hematocrit, neutrophils, and platelets were slightly increased at D20 to D60 but returned to nomral after D60. These clinically nonsignificant findings were most certainly related to the surgery performed at D0 or to the implanted device with no clinical significance. Data out of normal range are in bold italic type.

TABLE E6. Complete blood count panel for sheep #81030

Parameter	D0	D30	D60	D90	D120	D180	D240	D300	D375	Normal concentration Low	High
Red blood cells, $\times 10^{12}$/L	*15.6*	12.7	12.1	12.2	13.1	12.5	13.1	11.8	13.6	9.1	14.7
Hemoglobin, g/dL	14.6	13.2	12.7	13.2	13.2	12.6	13.1	11	12.7	9.6	14.8
Hematocrit, L/L	*0.47*	0.42	0.39	0.39	0.4	0.39	0.4	0.36	0.41	0.27	0.43
White blood cells, $\times 10^9$/L	8.1	8.5	7.4	8	6.2	7.3	5.8	6.8	5.7	3.4	10.8
Neutrophil ratio, $\times 10^9$/L	1.7	1.96	1.92	1.44	1.12	1.75	2.49	1.63	1.77	1.03	5.44
Eaosinophil ratio, $\times 10^9$/L	*1.46*	1.02	1.26	1.76	0.74	0.8	0.52	0.95	0.86	0.05	1.32
Basophil ratio, $\times 10^9$/L	0.08	0.09	0.07	*0.16*	0.06	0.07	0	0.07	0.06	0	0.12
Lymphocyte ratio, $\times 10^9$/L	4.86	5.36	4.07	4.48	4.15	4.6	2.73	4.08	2.91	1.49	5.89
Monocyte ratio, $\times 10^9$/L	0	0.09	0.07	0.16	0.12	0.07	0.06	0.07	0.11	0.05	0.56
Platelets, $\times 10^9$/L	432	745	707	489	449	452	471	369	385	119	765
Reticulocytes, %	0.17	0.14	0.14	0.22	0.04	0.03	0.03	0.08	0.05	0	0.4
Free hemoglobin, g/L	0.04	0.1	0.11	0.07	0	0.08	NA	NA	NA	0	1.0

Red blood cells, hematocrit and eosinophils were slightly increased at D0 but returned to normal at D30. Eosinophilis and basophils were slightly increased at D90 but returned to normal at D120. These findings were unrelated to the implanted device and without clinical significance. Data out of normal concentration are in bold italic type.

TABLE E7. Complete blood count panel for sheep #30187

Parameter	D0	D30	D60	D90	Normal concentration	
					Low	High
Red blood cells, $\times 10^{12}$/L	12.1	11.6	11.7	11.1	9.1	14.7
Hemoglobin, g/dL	13.2	13.8	13.7	13.4	9.6	14.8
Hematocrit, L/L	0.4	0.42	0.41	0.39	0.27	0.43
White blood cells, $\times 10^9$/L	5.6	5	6.5	7.1	3.4	10.8
Neutrophil ratio, $\times 10^9$/L	2.91	2.45	2.86	3.2	1.03	5.44
Eaosinophil ratio, $\times 10^9$/L	0.11	0.5	1.3	1.14	0.05	1.32
Basophil ratio, $\times 10^9$/L	0.06	0.05	0.07	0.07	0	0.12
Lymphocyte ratio, $\times 10^9$/L	2.3	1.85	2.21	2.49	1.49	5.89
Monocyte ratio, $\times 10^9$/L	0.22	0.15	0.07	0.21	0.05	0.56
Platelets, $\times 10^9$/L	440	521	495	399	119	765
Reticulocytes, %	0.17	0.13	0.16	0.14	0	0.4
Free hemoglobin, g/L	0.06	0.29	0.06	0.12	0	1.0

TABLE E8. Complete blood count panel for sheep #71204

Parameter	D0	D30	D60	D90	Normal concentration	
					Low	High
Red blood cells, $\times 10^{12}$/L	13.2	13.3	12.3	12.5	9.1	14.7
Hemoglobin, g/dL	14.2	14.5	13.4	13.7	9.6	14.8
Hematocrit, L/L	0.42	*0.45*	0.42	0.42	0.27	0.43
White blood cells, $\times 10^9$/L	3.5	7.1	6.3	6.6	3.4	10.8
Neutrophil ratio, $\times 10^9$/L	1.82	2.98	2.71	2.57	1.03	5.44
Eaosinophil ratio, $\times 10^9$/L	0.11	0.99	0.63	0.73	0.05	1.32
Basophil ratio, $\times 10^9$/L	0.04	*0.14*	0.06	0.07	0	0.12
Lymphocyte ratio, $\times 10^9$/L	*1.47*	2.7	2.84	3.1	1.49	5.89
Monocyte ratio, $\times 10^9$/L	0.07	0.28	0.06	0.13	0.05	0.56
Platelets, $\times 10^9$/L	178	579	286	175	119	765
Reticulocytes, %	0.25	0.17	0.18	0.24	0	0.4
Free hemoglobin, g/L	0.08	0.15	0.07	0.04	0	1.0

In this sheep, lymphocytes were very slightly low at D0 but returned to normal thereafter. Hematocrit and basophils were very slightly increased at D30 but returned to normal thereafter. These findings were unrelated to the implanted device and without clinical significance. Data out of normal range are in bold italic type.