Thrombogenic Performance of a St. Jude Bileaflet Mechanical Heart Valve in a Sheep Model

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The sheep model is preferred for chronic evaluation of prosthetic heart valves, surgical techniques, and endocardiographic studies. A bileaflet mechanical heart valve (MHV) was implanted into a sheep model to study its *in vivo* performance and to evaluate the thrombogenic potential of the valve. Transesophageal echocardiography and transcranial Doppler ultrasonography measurements were conducted before and after the valve implantation. Platelet activity state (PAS) assay measurements were also conducted before and after the implantation surgery. After sheep euthanasia, the MHV was explanted and scanning electron microscopy (SEM) was performed on the explanted valve to examine changes to the MHV surface. Tissue blocks were taken from the sheep brain, left ventricle, aorta, spleen, and lung lobes for histological examination. Our results indicated that after the MHV implantation, more embolic signals were detected in the sheep carotid artery, increasing monotonously as a function of implantation time. Echocardiographic parameters including blood aortic velocity, transvalvular pressure gradient, and velocity time integral increased. PAS increased significantly after valve implantation. SEM pictures demonstrated calcium and phosphate deposition on the valve surfaces. Histological examination demonstrated hemorrhage in the lung tissue, pulmonary thrombosis, and osteogenesis in heart tissue. *ASAIO Journal* 2006; 52:28–33.

Valve replacement is sometimes an essential treatment for patients with severe heart valve diseases. About 60% of the prosthetic heart valves being used are mechanical ones. St. Jude Medical bileaflet mechanical heart valves (MHVs) are widely used in the United States, and clinical studies indicate that they have good hemodynamic performances *in vitro* and *in vivo*.[1] However, the nonphysiologic flow patterns induced by MHVs may cause platelet activation and lead to thromboembolism and the attendant cardioembolic stroke, which are among their major complications.[2] The most common complications related to MHVs are major bleeding[3,4] and thrombosis.[5,6] Interestingly, cardiovascular osteogenesis is not uncommon after cardiovascular surgery.[7,8] In this study, sheep were chosen because they tolerate cardiac surgery well, and their cardiovascular anatomy is similar to that of humans. In recent years, many research groups have demonstrated that the sheep model is for chronic evaluation of prosthetic heart valves (PHVs), surgical techniques, and endocardiographic studies.[9–14] Compared to dogs, pigs, and calves, sheep have a relatively large aorta, which makes them technically better animal models for MHV studies. Anesthetic doses for sheep are similar to those for humans, and general anesthesia is well tolerated without any respiratory complications after exudation. Sheep also have a blood coagulation profile similar to that of humans,[15–17] which was another important consideration with regard to the sheep platelet activity measurements performed in our study.

Ruel *et al.*[18] used a sheep model to study the knotless suture welding techniques for the mitral valve replacement and concluded that the sheep model was very reliable, and it provided a promising method to model valve surgery procedures in human patients. Wheatley *et al.*[19] chose sheep to study and compare the *in vivo* performance of MHVs, tissue valves, and polymeric heart valves. They implanted ATS MHVs in 10 sheep, polyurethane valves in 8 sheep, and Carpentier-Edwards porcine valves in 10 sheep, and found that the porcine valve and the polyurethane valve had lower thrombogenicity than MHVs. Hofman *et al.* [20] used sheep to study the safety issues related to silicone-polyurethane valve implantation. They implanted polyurethane mitral valves in six sheep and studied the biodegradation and failure rate of the valve, demonstrating again that sheep were good *in vivo* models for mitral valve replacement studies.

The aim of this study was to establish the thromboembolic risk and the thrombogenic potential of a St. Jude Medical bileaflet MHV implanted in the aortic position in a sheep model. Transcranial Doppler (TCD) ultrasonography was conducted to measure the microembolic signals (MES) in the sheep carotid artery. Transesophageal echocardiography (TEE) was performed to study the changes in cardiac parameters in the sheep before and after MHV implantation. Blood samples from sheep were taken before and after MHV implantation and platelet activity state (PAS) was measured using a modified prothrombinase assay.[21,22] Preoperative to postoperative comparison established the platelet activity in the presence of MHV. Tissue samples were taken for histological and pathological examinations after the sheep euthanasia. Scanning electron microscopy (SEM) pictures were taken to study the surface changes of the MHV.

Materials and Methods

The animal studies conducted and described herewith were approved by the Stony Brook University Institutional Animal Care and Use Committee.

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The MHV implanted in the sheep model was a St. Jude Medical standard bileaflet MHV. The candidate sheep were female, weighing approximately 150 lb. Before the valve implantation surgery, TEE was conducted to measure the size of the sheep aortic root to determine the size of the MHV to be implanted. A Hewlett Packard 5 MHz Omni-plane probe and an HP Sonos 1500 machine were used. Arterial peak velocity, arterial mean velocity, aortic peak pressure gradient, aortic mean pressure gradient and ventricular time integration were measured.

On the day of the implantation surgery, the candidate sheep was sedated with ketamine/xylazine for induction and then intubated in the preoperating room. Halothane or isoflurane was used to anesthetize the sheep. Intravenous access was gained through the external jugular vein. Sheep blood was purchased from Hemostat Laboratories (Dixon, CA) for transfusions. Thoracotomy was favored over sternotomy as a less invasive surgical technique, with the additional benefit of better and less painful recuperation. (Sheep lie on their sternum when recovering from surgery, and are also prone to infection following sternotomy.) A standard left thoracotomy was performed through the fourth interspace to expose the chest cavity and the heart, followed by the MHV implantation. Briefly, the aorta was cross-clamped between the antegrade catheter and aortic cannulae, and the heart was arrested with hyperkalemic cardioplegia. An arteriotomy was created in the aorta, the aorta was sized for the valve replacement, and the native aortic valve was removed. The MHV was sutured with subannular pledgets, and the aorta was closed. The cross-clamp was removed from the aorta. After the heart regained function, the animal was slowly weaned from cardiopulmonary bypass. The whole procedure took approximately 4 hours.

Immediately after the surgery, another TEE was performed to measure the changes in the cardiac parameters and transvalvular gradients. Postoperative TEE and TCD ultrasonography were conducted regularly after the implantation surgery, and the recordings were compared with the preoperative measurements.

Transcranial Doppler ultrasonography was performed before and after the implantation surgery. The TCD measurements were designed to capture the high-intensity transient signals (HITS, aka microembolic signals [MES]) associated with emboli generated by prosthetic valves. The sheep cerebral circulation differs from that of humans: it has a single carotid artery on each side, distributing blood to the territories which are supplied by both the internal and external carotid arteries in humans. The presence of retia mirabile in sheep, intricate arterial capillary plexuses within the cranial cavity, is known to frequently trap emboli. This may limit the ability to measure emboli transcranially. Accordingly, the TCD probe was not placed in the traditional transcranial positions. Instead, HITS measurements were performed in the distal carotid artery of the sheep. A Nicolet TCD machine with 2 MHz transducer (EME Pioneer TCD system, Nicolet Biomecial, Madison, WI) was used to record HITS.

Artifacts, caused by movement of the probe or the animal, appeared as bi-directional signals with maximum intensity at the lower frequency range and an increased intensity spread over a wide range of frequencies. In contrast, emboli induce an intensity increase over a small band of frequencies in the Doppler spectrum, showing a bell-shaped distribution. After each measurement, artifacts were marked by a neurologist and removed from the recordings manually. The criteria for HITS was a duration of less than 50 milliseconds and an amplitude at least 9 dB higher than that of the background blood flow signal. The signals were recorded for 30 minutes before any surgical procedure to establish a baseline HITS level, and periodically after implantation.

Since particulate emboli, *i.e.,* thromboemboli, are clinically riskier than gaseous emboli, their resulting HITS were differentiated using their acoustic impedance disparity. The difference in acoustic impedance between blood and solid emboli is much less than that between blood and gaseous emboli, with solid emboli likely to generate lower-intensity signals as compared with gaseous emboli. Intensity was used accordingly as the parameter to distinguish between gaseous and particulate emboli, with threshold intensity of 20 dB and above indicating gaseous emboli, and vice versa.

A major advantage of the prothrombinase-based assay of platelet activity, *i.e.,* our PAS assay, is its applicability to platelets of other species. In contrast, the measurement of activation-dependent platelet-membrane antigens with monoclonal antibodies, *e.g.,* in flow cytometry, is species specific, because the antibodies are directed against human platelets. We have confirmed that our assay, using human factor Xa and acetylated prothrombin, is applicable to the measurement of the activity states of platelets from four mammals we have so far studied (humans, mice, cattle, and sheep).

Sheep platelet activities were measured before and after the MHV implantation using the PAS assay. For three months before the sheep surgery, sheep blood samples (5 ml from the sheep jugular vein) were taken every 2 weeks and PAS assays were conducted. Gel filtration was used to separate the platelets from plasma proteins, and platelets were assayed according to the methods described before.[21,22] Platelet activation was normalized by PAS/full activation achieved with Ca^{2+} Ionophore A23187. Normalized PAS before and after MHV implantation was compared, and Student's *t* test was used to analyze the data.

One sheep with a St. Jude Medical standard bileaflet MHV (19 mm) was euthanized 3 years after the valve implantation with an overdose of pentobarbital. A detailed autopsy was conducted for detection of thrombus formation and its downstream effects. Histological blocks were taken from anterior, lateral, posterior left ventricle, the interventricular septum, and the adjacent right ventricle. Tissue samples were stained with hematoxylin and eosin. The lungs were checked for visible lesions and histological blocks were taken from the lobes. The brain was taken out and external examination was conducted. Abnormalities, such as hemorrhages and atrophy, were recorded. Internal examination of the brain was conducted in locations with suspected lesions. Histological blocks were taken from the brain and the spleen. The carotid arteries were removed, and external examinations were conducted to check if there was thrombus formation.

The MHV was removed from the tissue and washed with a large amount of deionized water. The explanted MHV was visually examined, and SEM images were taken.

Table 1. Transesophageal Echocardiographic Measurements in Sheep

Sheep AV Parameters	Before Surgery	Immediately After Surgery	2 Weeks After Surgery	6 WeeksAfter Surgery
Maximum velocity (cm/s)	73.55	277	307	321.3
Mean velocity (cm/s)	48.6	181	200	233.7
VTI (cm-area/s)	12.5	38.05	46.1	54.9
Peak gradient (mm Hg)	2.17	30.7	37.7	41.3
Mean gradient (mm Hg)	1.11	15.65	18.7	24.9

AV, atrioventricular; VTI, ventricular time integration.

Results

Transesophageal Echocardiography

The TEE measurements were conducted before and after valve implantation. The postop results indicated a significant increase in all parameters measured, including the peak blood velocities and transvalvular pressure gradients as compared with the native valve, indicating a collar stenosis generated by the implanted valve. The TEE results are summarized in **Table 1**. The values were similar to those published by Salerno *et al.*[11] for sheep implanted with bileaflet MHV.

Transcranial Doppler Ultrasound Measurements

Transcranial Doppler measurements were taken in the sheep carotid artery. Before the surgery, in all sheep, very few HITS (aka MES) were detected, and no HITS were detected in the particular sheep shown in **Figure 1**. After valve implantation, the number of HITS rose sharply, increasing as a function of implantation time. Gaseous emboli appeared during first two months after implantation, with their number remaining relatively constant thereafter, while the number of thromboemboli kept increasing as a function of postimplantation time.

Sheep Platelet Activity State Study

Sheep platelets were obtained before and after MHV implantation, and PAS was measured as described above. To study the effect of MHV on platelet activities, PAS was measured periodically in a sheep with MHV (for 1.5 years after MHV implantation) and compared with PAS measurements performed in a control sheep. Blood was drawn from both sheep on the same days by the same person. One and a half years after MHV implantation (**Figure 2**), the platelet activity (PAS ± SEM) of the sheep with the MHV (0.054 ± 0.007) was much higher than the sheep without the MHV (0.038 ± 0.004). The difference between the two was significant (p = 0.008, n = 4).

Pathological and Histological Findings

Pulmonary thrombosis was detected in the lungs. Variable amounts of organized fibrin were found in the pulmonary muscular arteries, which were associated with the local hemorrhage and peripheral congestion. Osseous metaplasia was detected in the cardiac muscle and osteocytes could be seen within lacunae. Mild congestion was found in splenic sinusoids, and around the periarteriolar lymphoid area a mild hyperplasia was detected. No lesions were found in the cerebrum, cerebellum, or arteries.

Scanning Electron Microscopy of Explanted Valve

After valve explantation and removal from the sheep aorta, the MHV appeared clean of debris and deposits to the naked eye. There was no thrombus observed on or around the valve.

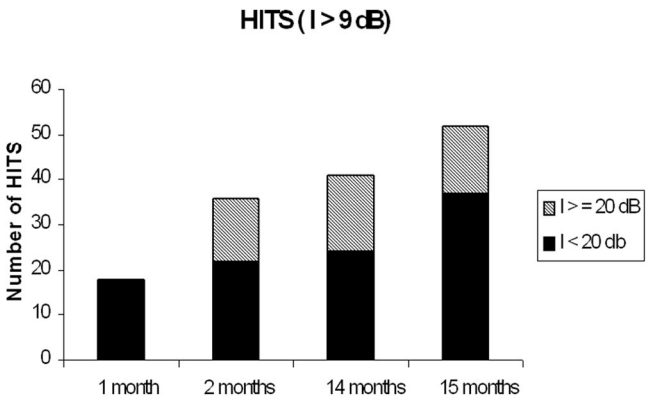

Figure 1. High-intensity transient signals (HITS) detected as a function of postimplantation time. The number of HITS increased monotonously as a function of postimplantation time. Differentiation between gaseous and thromboemboli was determined by the intensity of the reflected signal, with intensity greater than 20 dB indicating gaseous emboli and below 20 dB as thromboemboli.

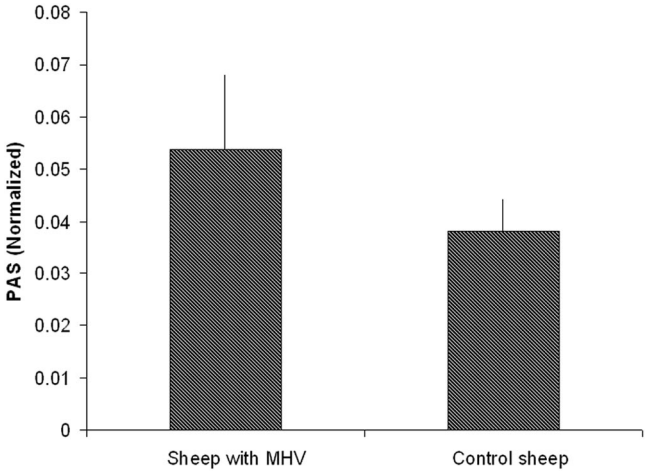

Figure 2. Platelet activity state (PAS) measurements obtained from sheep with MHV (1.5 years after implantation) and compared with a control sheep without MHV. PAS was measured periodically in both sheep on the same dates (for one and half year following MHV implantation). The sheep with MHV had a much higher PAS level (0.054 ± 0.007) than the sheep without the MHV (0.038 ± 0.004). The difference between the two was significant (p = 0.008, n = 4).

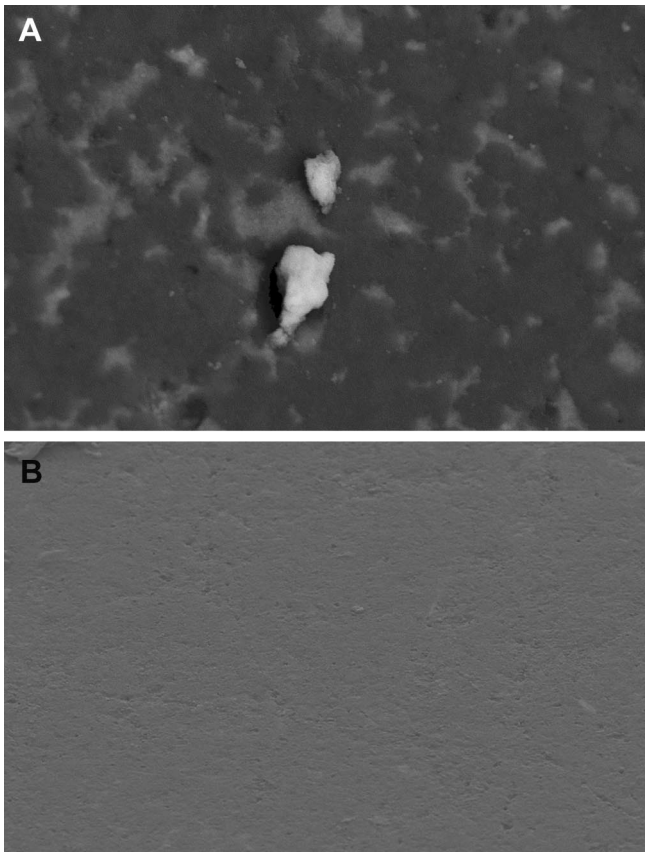

Figure 3. a: Explanted MHV (3 years after implantation, 10,000×) – the valve hinge area. Significant amounts of crystal deposit could be seen. ×-ray examination indicated large amount of Ca^{2+}. **b:** A picture of the control MHV at the valve hinge area. Not much crystal was detected. The magnification is 10,000×.

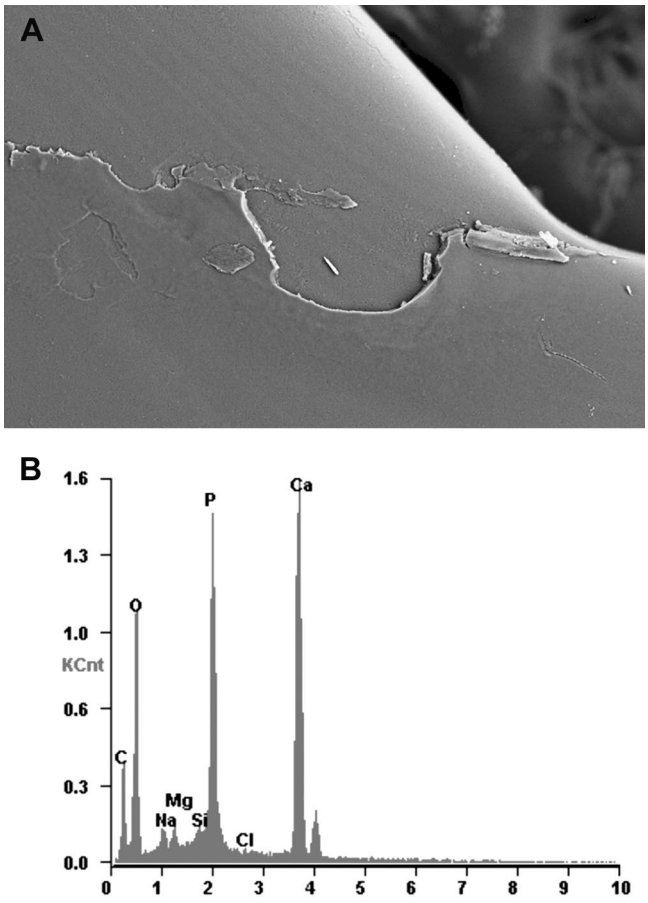

Figure 4. a: A thin film developed on the outflow surface of the implanted valve leaflet. The magnification is 679×. **b.** X-ray analysis indicating that calcium and phosphate are the major elements in the thin film of **Figure 4a**. Carbon, silicon and oxygen represent the material composition of the valve leaflet. Sodium, magnesium and chloride possibly come from the salt solution used to wash the valve leaflet.

However, some calcified tissue was observed around the aortic root.

The explanted MHV was cleaned and SEM images were taken and compared with SEM images of a control valve taken at the same time. The SEM images at the valve hinge area showed large amounts of Ca^{2+} deposited on the explanted valve, as compared with the control valve (**Figures 3a and 3b**). The SEM image of the outflow surface of the explanted valve appears in **Figure 4a**. A thin film which formed close to the leaflet's edge was examined further by x-ray. Calcium and phosphate were the major components of this deposited film (**Figure 4b**).

Discussion

We conducted extensive pre- and postimplantation measurements a sheep implanted with a St. Jude bileaflet MHV. This study provided very valuable information on the changes in flow parameters that may contribute to the thrombogenic potential of the MHV, and its apparent indicators: the appearance of microembolic signals, their frequency and constituency, and sheep platelet activity after implantation. After the sheep euthanasia, detailed pathological and histological examinations and SEM measurements on the explanted valve further provided evidence of the deleterious effects of the pathological flow fields that implantation of MHV induces, which in turn enhance thrombosis and thromboembolism rates.

Transcranial Doppler ultrasonography was conducted before and after the implantation surgery to measure MES (or HITS). No HITS were detected before the implantation. After valve implantation, the number of HITS rose sharply, increasing monotonously as a function of implantation time. Gaseous emboli, which are considered less risky because they mainly dissolve, appeared during first 2 months after implantation; however, their number remained relatively constant thereafter. In contrast, the number of thromboemboli, which are definitely riskier and may induce a cardioembolic stroke, kept increasing as a function of postimplantation time. Combined with the observed enhanced platelet activity following the valve implantation, this may indicate that the mechanism underlying the increased number of HITS (aka MES) measured is the formation of platelet aggregates in the wake of the MHV.

The PAS measurements were conducted before and after the MHV implantation in the sheep. For 3 months before surgery, sheep blood samples were taken every 2 weeks and PAS assays were conducted. Due to various bleeding problems, the sheep platelet level was abnormally high (more than 7%) and these

results were deemed invalid. Accordingly, in order to study the effects of the implanted MHV on sheep platelet activity, the PAS measurements were compared with those conducted in a control sheep without the MHV (chosen randomly). The results indicated that the platelet activation level in the sheep with the MHV was significantly higher than that in the control sheep. This validated our hypothesis that the implantation of MHV would enhance the platelet activation level. We assume that sheep have similar base platelet activation level; however, in order to minimize any potential variability in platelet base activity between various sheep, or even in the same sheep in various time points, all PAS results were normalized (as described in Materials and Methods). Although the comparison was not conducted in the same sheep, the comparison to a control sheep with a native valve was a valid measure of the increase in platelet activity after valve implantation.

After the sheep euthanasia, the pathological and histological studies detected pulmonary thrombosis, which is a valid marker of platelet activation after MHV implantation. Tissue calcification was noticeable by naked eyes on the aortic root endothelium. The major component of calcium deposit in the vessel wall is the calcium phosphate, and it is usually in the form of hydroxyapatite. This matches our findings from SEM pictures. Osteocytes were observed in the heart tissue. Demer *et al*.[23] report that it is not unusual to have both blood vessel wall calcification and osteogenesis at the same time in atherosclerotic lesions. But the mechanism behind this is not yet known.

The SEM images indicated a certain amount of calcium and phosphate deposition on the valve leaflets. Studies of the calcium deposition on MHV leaflets are unfortunately unavailable. Most calcification studies in mechanical valves were focused on the aortic tissue calcification after MHV implantation.[24] However, the numerous studies of dysfunction due to calcification[25,26] in bioprosthetic valves may indicate that potential calcification problems may exist for MHV.

The results obtained from this study provided very useful information with regard to the MHV-related changes *in vivo* after implantation. Previous *in vitro* and numerical studies by our group [27–30] demonstrated that the nonphysiologic flow patterns generated by MHVs can induce platelet activation and increase the risk of thromboembolism. This *in vivo* study validated the predictions of our numerical models and our *in vitro* findings. The results obtained from this study help in identifying the underlying mechanisms that are responsible for the enhanced hemostatic response, hence the increased cardioembolic risk, seen in patients with implanted MHV. In summary, by measuring the clinical end points of the effects of MHV implantation *in vivo*, a more accurate depiction of thromboembolism may arise and be clinically substantiated. Such methodology, combined with our previous *in vitro* and numerical modeling, may facilitate better device design by reducing the risks that patients implanted with these devices face, lowering the ensuing health care costs, and possibly offering viable long-term solutions for these patients.

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