

Proof of Concept of Surface Anticoagulation in Electrically Active Mechanical Mitral Valve Using Biomimetic Glycocalyx: In Vivo Swine Model Study

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Abstract

Prosthetic mechanical heart valve (MHV) implantation requires lifelong anticoagulation. Overdosage of anticoagulation increases the risk of bleeding, while suboptimal therapy may induce thromboembolism. Inspired by the negative charge of the endothelial glycocalyx, which repels platelets and anionic proteins, we developed an MHV assembly that applies a negative potential to the valve's blood-contacting surfaces. Early in vitro work showed that imposing ~ 0.5 V across a bileaflet MHV reproduces the anti-thrombotic behavior of the endothelium. Here we test this MHV assembly in vivo using a swine model. Four Pigs were implanted in the standardization phase with the first design of the MHV assembly; In the experimental phase, three pigs (test group) received the redesigned MHV assembly, and three control pigs received the MHV without electrical activation. Pigs were monitored for 20 weeks after cessation of dual antiplatelet therapy. All four standardization phase test animals succumbed to valve thrombosis. Following the implantation of the redesigned electrically activated MHV assembly, the three test animals survived to the end of the study without valve thrombosis, while two of the three controls died with valve thrombosis. These findings support electrical neo-endothelialization as a viable strategy to eliminate the need for long-term systemic anticoagulation after MHV implantation.

1 Introduction

The Global Burden of Disease (GBD) 2021 study revealed an alarming escalation in the impact of valvular heart disease on morbidity and mortality across all the surveyed world regions^{1,2}. The age-standardized prevalence of rheumatic heart disease (RHD) was high in underdeveloped countries, while the burden of calcific aortic valvular disease (CAVD) and degenerative mitral valvular disease (DMVD) was higher in developed countries. However, a 30-year trend (1990–2020) showed a significant increase in the prevalence of DMVD in the underdeveloped world, while the prevalence of RHD and CAVD increased in the developed world. These trends indicate that the need for heart valve implantation is expected to increase dramatically worldwide. When native valves become irreparably damaged as valvular heart disease progresses, they are replaced with either mechanical prostheses or bioprosthetic valves (xenografts or allografts). Mechanical valves offer unmatched durability and are therefore often recommended for patients younger than 50–60 years of age³; however, their synthetic surfaces trigger platelet adhesion and activation, causing thromboembolism that requires lifelong anticoagulation^{3,4}. Lifelong anticoagulant therapy increases the risk of major bleeding and burdens patients with frequent monitoring. Bioprosthetic valves do not require anticoagulation but undergo structural deterioration and calcification, which may necessitate reoperation. Consequently, there is an unmet need for a durable, non-thrombogenic prosthesis that does not require systemic anticoagulation.

The thromboresistance of native vascular surfaces arises partly from the electrochemical properties of the endothelial glycocalyx^{5–8}. Glycosaminoglycans carry sulfated and carboxylate groups that create a net negative surface charge and an electrical potential difference relative to the bulk plasma. This electrostatic barrier repels circulating platelets and anionic proteins, contributing to the non-thrombogenicity of healthy vessels. Titanium and pyrolytic carbon surfaces used in mechanical heart valves generally exhibit a mild negative surface charge under physiological conditions and rapidly become coated with adsorbed plasma proteins upon contact with blood. This protein layer mediates subsequent platelet adhesion, activation, and coagulation, which can contribute to thrombus formation^{9–11}. Early electrochemical studies showed that maintaining metal prostheses at negative potentials (-60 to -160 mV vs. normal hydrogen electrode) prevented thrombus formation in dogs, whereas identical prostheses with spontaneous positive potentials occluded within two weeks¹². These experiments indicated that an adequate negative interfacial potential is as important as the material composition in determining blood compatibility. Building on these insights, we designed a novel mechanical prosthetic heart valve assembly that generates the requisite negative electrostatic field (cathodic potential) on its blood-facing surfaces. The design, protected by United States patent US10736726B2, incorporates a mechanical bileaflet heart valve comprising pyrolytic carbon, a bipolar lead, and an implantable pulse generator (IPG)¹³. The IPG delivers programmable monophasic square pulses, transmitted via the bipolar lead to the valve's titanium housing, generating an electronegative field that acts as an electrical neo-endothelium.

We tested the concept of electronegativity-induced thromboresistance in vitro based on a 30-minute contact between a surface-charged valve and human platelet-rich plasma or whole blood. We have described the methodology and results of the in vitro experiments in a prior publication¹⁴. For the in vitro study, the antithrombotic effect was evaluated at two selected voltages, 0.25 V and 0.5 V, based on several key considerations. The anti-thrombogenic surface negative potential of the endothelium is ~ 0.3 V, making the chosen voltages relevant, as they lie close to this natural range. In addition, the valve assembly design incorporates a prosthetic mechanical heart valve integrated with commercially available IPG systems, whose minimum output voltages are typically 0.25 V (e.g., Abbott) and 0.5 V (e.g., Medtronic), thereby ensuring practical applicability. Higher voltage levels were avoided because they may increase the risk of unintended myocardial stimulation as pacing thresholds are approached, which could disrupt normal cardiac electrical activity, and they would also accelerate battery depletion, reducing device longevity and increasing the need for more frequent replacements in clinical use. Control valves (0 V) were compared with valves activated at 0.25 V and 0.5 V. Scanning electron microscope (SEM) images demonstrated that the deposit-free

area was highest (~ 96%) at 0.5 V, intermediate (~ 86%) in controls and lowest (~ 58%) at 0.25 V. Platelet counts, coagulation markers and hemolysis were similar across groups, confirming that the electrical field did not injure blood components¹⁴. These findings also indicated that 0.5 V is a safe and potentially effective voltage range for thromboresistance.

Here, we report a proof-of-concept in vivo study in a swine model to evaluate the feasibility, long-term safety and efficacy of electrical neo-endothelialization for the mitral position. Swine hearts are anatomically similar to human hearts and are widely used for preclinical valve testing^{15–18}. The study consisted of standardization and experimental phases. The standardization phase had two-fold objectives: 1. To customize the study protocols for the novel valve assembly in swine animal models, 2. To refine the design of the valve assembly before it is evaluated in the final experimental phase. The experimental phase aimed to compare long-term outcomes in test animals implanted with a valve assembly (valve-lead-IPG) and in control animals implanted with a standard bileaflet mechanical valve (without IPG connection) in the mitral position of swine.

2 Results

A total of ten healthy adult Ankamali pigs were selected for the study. Four pigs were implanted with the first design of the valve assembly in the standardization phase; the experimental phase implanted the modified design of the valve assembly in three pigs (test), while three pigs received the valve without any IPG connection (controls). The standardization phase results are summarized in Table 1, while the experimental phase results are shown in Table 2 (test) and Table 3 (control). **Table S1** (Supplementary information) shows pre-operative hematological parameters in the test (standardization and experimental phases) and the control animals.

2.1 Standardization phase

Animals in this phase received a valve welded to the lead, with the tip cut proximal to the ring electrode. One animal (ID: 280750) survived > 20 weeks of follow-up period, but died before elective euthanasia. The three other animals did not complete the follow-up period due to premature death (Table 1). Autopsy was performed in only three animals, as one animal (ID: 20833) died intraoperatively. Significant valve thrombosis was observed in all three animals, with thrombi present on both the inflow and outflow regions of the valve, resulting in immobilization of the valve leaflets (Fig. 1); all animals showed pulmonary edema. In addition, focal infarcts on the diaphragmatic surface of the liver (ID: 282666), an infarct in the inferior pole of the left kidney (ID:280750), and petechial hemorrhages on the cortical surface of both kidneys (ID: 20830) were found, indicating chronic thromboembolism. Explanted IPG batteries were found to be completely discharged with an estimated remaining longevity of zero.

We conjectured that the death of all animals with valve thrombosis and complete discharge of the IPG battery was consistent with an electrical short between the tip and ring conductors through the prosthetic valve material at the welded point. This could lead to an accelerated battery discharge, premature loss of anti-thrombotic efficacy, and thrombotic complications. Based on these insights, we redesigned the valve–lead junction within the assembly to preserve the original electrode tip geometry. In the first iteration of the valve, the lead tip was cut proximal to the ring electrode, and the resulting stump tip of the coaxial electrode was welded to the outer valve ring, possibly causing the shorting or near-shorting. In the subsequent valve assembly design, the helical tip was extended and inserted into the slit in the outer ring to establish electrical contact with the inner valve ring, as described previously; however, no portion of the tip was removed or otherwise damaged.

Table 1

Standardization phase results for Test animals. In this phase, the lead wire tip was cut proximal to the resistance band, and the tip was welded to the valve's outer ring surface. All the animals received DAPT (aspirin 75 mg and clopidogrel 75 mg) once daily for 30 days after surgery or until death, whichever came first, except for animal 2 (ID: 20833, intra-operative death).

S.No	Animal ID/ Other details	Valve/IPG/ Lead configuration	Implantation date	Date of death/ autopsy/ cause of death	Survival period	IPG Battery status after explantation
1	20830/ Female, weight at implantation = 57 Kg	Valve: SJM 25mm, mitral SN 31398139, Ref 25MECJ-502 IPG: Sphera TM SR MRI SureScan, SN FNG085864G/ SPSR01	19-08-2024	Animal found dead on 05-10-2024; Thrombi at mitral prosthesis annulus, both hinge regions at inflow and outflow region, valve leaflets were immobile, Blood entry into lead insertion point in pulse generator	47 days (17 days after DAPT cessation)	Battery completely discharged
2	20833/ Female, weight at implantation = 57Kg	Valve: ATS 25 mm Mitral V.681458-0018 IPG: Sphera TM SR MRI SureScan, SN FNG086370G/ SPSR01	20-09-2024	The animal fibrillated during the shifting from the operation theatre (OT). The animal was immediately brought to OT and intubated, but defibrillation was not successful. Hence, declared dead on the table, and no autopsy was done.	0 days	The battery was not explanted as an autopsy wasn't done.
3	280750/ Male, weight at implantation = 62 Kg	Valve: Medtronic open pivot 25 mm Mitral, SN 970544, Ref 500 DM25 IPG: Sphera TM SR MRI SureScan, SN FNG086030G/ SPSR01	15-03-2025	The animal was found dead on 14-09-2025. Pale thrombi were noted at the inflow and outflow hinge regions and on the disc surface. Excessive pannus was observed on the inflow surface, resulting in immobility of the valve surface. Blood entry into the lead insertion point in the pulse generator and the highly vascular capsule covering the pulse generator was seen.	183 days (153 days after DAPT cessation)	Battery completely discharged
4	282666/ Male, weight at implantation = 67 Kg	Valve: Medtronic open pivot 25 mm Mitral, SN 970544, Ref 500 DM25 IPG: Sphera TM SR MRI SureScan, SN FNG086030G/ SPSR01, 0.75 V was given to the valve in this animal, instead of 0.5 V used in other animals.	17-03-2025	An elective autopsy was performed on 19-03-2025 after the animal showed acute dyspnea. Small pale thrombi were noted at inflow and outflow hinge regions, adhering to the disc surface, and the valve leaflet mobility was partially compromised.	2 days	Battery completely discharged

2.2 Experimental phase

Three test animals received the redesigned valve assembly, which preserved electrode tip integrity. The results are summarized in Table 2. All the test animals in this phase survived for > 20 weeks after the cessation of DAPT without any salient clinical events. Elective euthanasia revealed that the valves were free of thrombi on the inflow and outflow sides, with no restriction of leaflet mobility (Fig. 2). Explanted IPG battery showed a mean estimated remaining longevity of ~ 7.2 years (6.5, 7.2, and 8 years for three test animals), indicating that the modified valve assembly did not exhibit the electrical shorting-induced accelerated battery drainage seen with the first version of the valve assembly.

Table 2

Experimental phase results for Test animals. In this phase, the lead tip was intact, and a helical-shaped tip was inserted into a potential space between the inner and outer rings through a pre-existing slit (Fig. 2) in the outer valve ring. All the animals received DAPT (aspirin 75mg and clopidogrel 75mg) once daily for 45 days after surgery and completed more than 20 weeks of uneventful DAPT-free follow-up period before elective autopsy was done. There was no blood entry at the IPG lead insertion site in any of the test animals during the experimental phase.

S.No	Animal ID/ Other details	Valve/IPG/ Lead configuration	Implantation date	Autopsy findings	Survival period	IPG Battery status after explantation
1	286528/ Male, weight at implantation = 76 Kg, Weight before autopsy = 96 Kg	Valve: Medtronic open pivot 24 mm, mitral AP360™, SN 1020815/RF:505DM24 IPG: Sphera™SR MRI SureScan, SN FNG086328G/ SPSR01	26-05-2025	Elective autopsy on 19-12-2025; No visible thrombi on disc surface, cage, hinge region at inflow and outflow side, no paravalvular leak, free leaflet movement was seen	207 days (162 days after DAPT cessation)	Estimated remaining longevity was 7.2 years
2	281430/ Male, weight at implantation = 58 Kg, Weight before autopsy = 75.5 Kg	Valve: Medtronic open pivot 24 mm, mitral AP360™, SN 1021448/RF: 505DM24 IPG: Sphera™SR MRI SureScan, SN FNG086369G/ SPSR01	28-05-2025	Elective autopsy on 18-12-2025; No visible thrombi on valve leaflets, cage, hinge region at inflow and outflow sides, no paravalvular leak, free leaflet movement was seen. At the outflow, focal thrombi arising between the cage rim and the sewing ring annulus were noted.	204 days (159 days after DAPT cessation)	Estimated remaining longevity was 6.5 years
3	286049/ Female, weight at implantation = 60 Kg, Weight before autopsy = 80 Kg	Valve: Medtronic open pivot 24 mm Mitral AP360™, SN 1021448/RF:500M25 IPG: Sphera™SR MRI SureScan, SN FNG115450G/ SPSR01	18-06-2025	Elective autopsy on 14-01-2026; No visible thrombi were seen on the valve leaflet surface, cage, and hinge regions at the inflow and outflow sides. However, focal thrombi were noted between the sewing ring and the cage rim on the outflow side. No paravalvular leak was seen, and valve leaflet movement was free.	210 days (165 days after DAPT cessation)	Estimated remaining longevity was 8 years

The results from three control animals are shown in Table 3. One control animal (ID: 284998) died prematurely (67 days after surgery), while another animal (ID: 20829) survived > 20 weeks (165 days) but died prior to elective euthanasia. Autopsy of both animals showed prosthetic valve thrombosis on the inflow and outflow sides, restricting leaflet mobility (ID-20829: Figs. 3a and 3b; ID-284998: Figs. 3d and 3e), despite receiving DAPT throughout the follow-up period. Focal Hepatic and renal infarcts were observed in both control animals, consistent with thromboembolism. The third control animal (ID: 291354) received DAPT for 45 days post-operatively and survived > 20 weeks after implantation. Autopsy after elective euthanasia showed that the valve was free of thrombus on the inflow and outflow sides, and the leaflets were mobile (Figs. 3c and 3f).

Table 3

Experimental phase results for control animals. A prosthetic valve was implanted in the mitral position without any IPG. The first two control animals received DAPT (aspirin 75mg and clopidogrel 75 mg) once daily throughout the follow-up period, while the third animal (ID: 291354) received DAPT for only 45 days after surgery.

S.No	Animal ID/ Other details	Valve	Implantation date	Autopsy findings	Survival period
1	20829/ Female, weight at implantation = 52 Kg	Valve: SJM 24 mm, mitral SN31449588/ Ref 25MECJ-502 Rf:505DM24	16-08-2024	Animal found dead on 28-01-2025; Thrombi on hinge regions at inflow and outflow sides, and valve leaflets were immobile.	165 days
2	284998/ Female, weight at implantation = 54 Kg	Valve: SJM 25 mm, mitral SL: 31413650/ 25MECJ-502	23-09-2024	Animal found dead on 29-11-2024. Thrombi on hinge regions at inflow and outflow sides, and valve leaflets were immobile.	67 days
3	291354/ Male, weight at implantation = 58 Kg, Weight before autopsy = 80 Kg	Valve: Medtronic open pivot 24 mm Mitral, SN 1020033/RF:505DM24.	16-06-2025	Elective autopsy on 05-01-2026. No visible thrombi were seen on the valve leaflet surface, cage, and hinge regions on the inflow and outflow sides. No paravalvular leak or pannus formation was seen, and valve leaflet movement was free.	203 days (158 days after DAPT cessation)

Red cell mass and platelet counts did not change substantially in test animals during the 20-week post-implantation follow-up period (**Tab. S2**: Supplementary information). Invasive hemodynamic assessment performed prior to elective euthanasia (3 tests, 1 control) demonstrated low trans-mitral pressure gradients in all animals (**Tab. S3**: Supplementary information). The pressure difference (pulmonary artery wedge pressure minus left ventricular pressure) was 4.6, 2.6, and 5.2 mmHg in the test-valve animals, compared with 3.4 mmHg in the control animal. Overall, the test-valve group showed a mean gradient of 4.13 mmHg, consistent with preserved mitral hemodynamic function without evidence of severe valvular obstruction.

2.3 SEM analysis

Only one explanted valve from each of the following conditions—the standardization phase test group (ID: 280750), experimental phase test group (ID: 286528), and control group (ID: 29154)—was subjected to SEM analysis; additionally, one unimplanted valve was examined as a negative control. Valve from the standardization phase showed changes indicating surface thrombogenicity, including extensive RBC-rich thrombotic deposition, platelet aggregates, fibrin-like meshwork formation, and surface roughening (**Fig. S1**: Supplementary information). The redesigned final test valve demonstrated substantially improved surface characteristics compared with the first version of the valve assembly (**Fig. S2**: Supplementary information). Valve shows preservation of smooth leaflet morphology, reduced cellular adhesion, and minimal thrombotic deposition. SEM images of the control valve showed limited focal deposits of RBC, platelets, and amorphous material (**Fig. S2**: Supplementary information). Except for sputter-coating artifacts, the unimplanted negative control was free of any organized deposits (**Fig. S1**: Supplementary information).

3 Discussion

The long-term hemocompatibility and valve thrombosis-free survival were examined for the novel valve assembly, in which the blood-contacting surfaces were imparted electronegativity by connecting a lead to an IPG. In the Standardization phase, all the test animals (n = 4) implanted with the first iteration of the valve assembly prematurely succumbed to valvular thrombosis, possibly due to electrical shorting and IPG battery discharge leading to loss of anti-thrombotic efficacy. For the experimental phase, the redesigned valve assembly with preserved lead tip geometry was implanted in test animals; in contrast, the lead electrode tip was cut proximal to the ring electrode in the first iteration of the valve assembly. All experimental phase test animals (n = 3) survived > 20 weeks of DAPT-free follow-up without salient hemolysis or thrombosis on the valve surface. Two of three control animals died with valvular thrombosis despite DAPT coverage for the entire duration of follow-up.

Lack of valve thrombosis with gross pathological examination in all the experimental phase test animals, and minimal deposits on SEM examination (1 test valve) at the end of a 5-month-long follow-up, indicates the potential efficacy of the concept of surface electronegativity-induced thromboresistance implemented by the modified version of the novel valve assembly. Red blood cell parameters and platelet counts varied within physiological limits across the three test animals during the experimental phase, indicating sustained hemocompatibility of the valve assembly. In contrast, substantial valve thrombosis was observed in 2 of the 3 control animals (despite DAPT) and in the standardization-phase test animals, where electrostatic thromboresistance failed due to the preliminary design's interrupted lead-tip geometry. These early-phase test animals thereby functioned effectively as “sham controls” by serendipity. We found

qualitatively similar results in in vitro experiments¹⁴, where valves activated at 0.5 V were virtually free of deposits after 30 minutes of exposure to blood or platelet-rich plasma. The thromboresistance of the valve assembly can be explained by electrostatic repulsion between the negatively charged blood cell surface and the negatively charged valve surface¹⁹. This design feature is analogous to, and inspired by, the physiology of the normal vascular endothelium, which is lined by a negatively charged, glycosaminoglycan-rich glycocalyx^{6–8}. It acts as an electrostatic barrier, preventing the blood cells from adhering to the endothelium.

To our knowledge, we are the first to demonstrate the potential of long-term anti-thrombotic effects via electrical polarization of valve surfaces in swine models. However, there is a substantial body of work supporting the use of surface electronegativity for thromboresistance. Sawyer and Pate (1953)²⁰ first demonstrated the role of bioelectric phenomena in intravascular thrombosis, showing preferential thrombus deposition near the anode, with minimal formation at the cathode. Subsequent studies^{12,21–23} established key principles: vascular endothelium and circulating blood elements carry a net negative charge; this charge is reduced or reversed at sites of injury; acidic conditions diminish surface negativity; antithrombotic agents enhance negative charge while prothrombotic conditions have the opposite effect; and positively charged biomaterials exhibit increased thrombogenicity compared with negatively charged surfaces. Furthermore, greater magnitude and uniformity of negative charge correlate with improved thromboresistance. Sawyer et al. achieved cathodic polarization by applying direct current ($\sim 200 \mu\text{A}$) or high-frequency alternating current ($\sim 10 \mu\text{A}$ at 100 kHz) to metallic grafts. In contrast, the present study employs a programmable cathodic potential delivered as a square-wave pulse (0.5 V at 70 pulses/min) via a widely used IPG-based system applied to pyrolytic carbon mechanical heart valves. Our findings indicate that modest electrical polarization can reproduce endothelial-like antithrombotic behavior on mechanical valve surfaces. A potential of 0.5 V was sufficient to achieve consistent thromboresistance and hemocompatibility in our sample. The associated energy consumption at this voltage, pulse duration (0.4 milliseconds), and frequency (70 pulses/min) is minimal, as corroborated by a mean estimated remaining longevity of ~ 7.2 years in explanted IPG batteries, supporting the feasibility of long-term anticoagulation with the valve assembly. Our strategy offers a potential non-pharmacological alternative to systemic anticoagulation, with the prospect of reducing lifelong drug dependence, improving patient quality of life, and expanding access to durable mechanical valve therapy.

Our study has the following limitations. The sample size is small, allocation to test and control groups was not randomized, blinding was not implemented at any stage of the study, and there was no explicit control for the confounders. Formal statistical comparisons and effect size estimations were not performed due to the sample size constraints. Only 4 animals (3 test and 1 control) completed the 20-week follow-up. Hematological and hemodynamic trends are available for only one control animal. These factors cumulatively limit the generalizability of our findings. Nevertheless, the results from our proof-of-concept study support the potential of safe long-term surface anticoagulation with electrical polarization of the mechanical prosthetic heart valve. The first two control animals received DAPT throughout the follow-up period, while the third control received DAPT for only 45 days. Although the inconsistency of DAPT duration in the control group is a salient limitation, valve thrombosis in the first two control animals despite uninterrupted DAPT and the lack of thrombosis in all the experimental phase test animals with only a 45-day DAPT (and > 20 weeks DAPT-free follow-up period) strengthen the evidence for the anti-thrombotic efficacy of our novel valve assembly. The third control survived for > 20 weeks without DAPT or electrical surface anticoagulation. Although the valve from this animal was grossly free of thrombus, SEM imaging revealed surface deposits of RBCs, scattered platelets, and focal amorphous deposits, whereas no such cellular deposits were observed on the experimental phase test valve. However, these findings cannot be generalized, as SEM imaging was performed only on a single explanted valve from each condition. The differences in the role of platelets and the coagulation cascade between pigs and humans during thrombus formation may limit the applicability of these findings in a clinical context. While our study demonstrates that surface electronegativity contributes to antiplatelet effects, it remains unclear whether the electrostatics-based mechanism explored here can beneficially modulate the coagulation cascade and reduce valve thrombosis in humans.

Future work should focus on replicating these findings in larger preclinical studies and, if feasible, in first-in-human clinical trials, given the far-reaching implications of the proposed valve assembly. The valve-lead junction and the pulse waveform properties could also be optimized to maximize the therapeutic efficacy, battery longevity, and safety.

4 Methods

4.1 Valve assembly for surface anticoagulation

The device assembly for the surface anticoagulation in electrically active mechanical mitral valve using biomimetic glycocalyx⁽¹³⁾ comprises of the following three components (Fig. 4): 1. Valve: A rotatable open pivot 24 or 25 mm bileaflet mechanical heart valve consists of an inner ring (with pivot mechanism and leaflets; the blood contacting surfaces of the valve), made of pyrolytic carbon, a titanium-based outer ring or housing, and the sewing cuff, a polyester fabric surrounding the outer ring and the space between inner and

outer rings like a doughnut. Both the inner and outer rings are made of electrically conductive material. The outer ring has a ~ 6 (length) $\times$ 1 (width) mm slit-like opening on its surface (Fig. 4c) through which the potential space between the inner and outer rings can be accessed.

2. Lead: In the first iteration of the valve assembly design, the terminal region of a pacemaker lead was welded to the outer valve ring through a fenestration made in the sewing cuff, and the valve-lead junction is covered with a PTFE tube graft. For the in vitro experiments¹⁴, Medtronic's CapSure Sense MRI™ SureScan™ lead (with small flexible tines at the tip) was used, while in vivo experiments used CapSureFix Novus MRI SureScan 5076 lead (with extendible/retractable helical tip) to connect the valve to the IPG, due to the greater availability and usage of the helical tip electrode.

3. IPG: The lead connects the valve housing to an IPG (Medtronic's Saphair™ MRI SureScan) that delivers monophasic square pulses at programmable amplitudes (0.5 V in this study) and 70 pulses min⁻¹. The IPG is used only to impart electronegativity to the valve surface, not for pacing the heart. It is customary to use a voltage of ~ 1.5 – 3.5 V for pacing applications²⁴, while we have used a fraction of that voltage for this proof-of-concept study. To evaluate the device's thromboresistance, we conducted in vitro experiments¹⁴ followed by in vivo experiments as outlined in Fig. 5. We report the results of the in vivo study in this work.

4.2 In vivo study

The in vivo study was conducted at the Biomedical Technology Wing, Sri Chitra Tirunal Institute of Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, India. The center is the apex institute in India for conducting large-animal experiments, particularly for the development and testing of prosthetic endovascular devices such as stents and valves in swine models. It has extensive experience, standardized protocols, and in-house animal breeding facilities for prosthetic valve implantation in large-animal models. The study was approved by the Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Fisheries, Animal Husbandry and Dairying, Govt. of India (No. V-11011(13)/6/2024-CPCSEA-DADF, dated 08-07-2024), for the proof-of-concept study. The Animal handling in the study conformed to the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines, 1998, of the Ministry of Environment, Forest, and Climate Change, Government of India²⁵.

Healthy adult Ankamali pigs were selected from the Large Animal House, Biomedical Technology Wing, SCTIMST, and housed in separate holding pens at ambient temperature for 1 week prior to surgery for acclimatization. Pregnant animals, echocardiographically detected cardiovascular anomalies, and those with any significant abnormality revealed during initial veterinary physical examination were excluded from the study. Animals were fed once daily with broiler finisher feed containing a minimum digestible energy of 3.3 Mcal/Kg, metabolizable energy of 3.1 Mcal/Kg, and crude protein level of 14%. Potable water suitable for drinking (as per IS 10500: 2012) was made available ad libitum. Animals were mostly provided with natural lighting, except during the acclimation and post-operative periods, when artificial lighting was provided for 12 hours daily. Animals were dewormed and vaccinated against hemorrhagic septicemia; tetanus toxoid was also administered during the holding period. Preoperative hematological investigations were performed on all animals. The in vivo study design (shown in Fig. 5) consisted of standardization and experimental phases. The standardization phase included only the test animals, while the final experimental phase compared test and control animals.

4.3 Standardization phase

Four healthy adult pigs (2 Females; weight = 60.5 ± 4.15 kg) received the novel test valve assembly with IPG. The tip of the pacemaker lead (with helical tip) was cut proximal to the ring electrode, and a conducting junction was established between the terminal region of the electrode and the outer surface of the valve ring using micro resistance spot welding in an end-to-side (for the first two animals) or side-to-side configuration (for the third and fourth animals) (shown in Fig. 4e). For the end-to-side configuration, the underlying coaxial conducting electrode was exposed by removing the silicone sheathing, and the “end” was welded to the outer surface of the valve ring (“side”). The valve assembly with an end-to-side junction was used in the in vitro experiments¹⁴. Due to mechanical stability concerns at the welded region, a side-to-side configuration was adopted later, where the “side” of the coaxial electrode was welded to the outer surface of the valve ring (side-to-side configuration). The IPG was programmed to deliver square pulses at an amplitude of 0.5 V for the first three animals. The last animal received a voltage amplitude of 0.75 V (ID: 282666, Table 1). The delivery of electrical current to the valve's blood-contacting surfaces was verified with a multimeter prior to ethylene oxide (ETO) sterilization. Following surgery, all the animals in this phase received dual antiplatelet therapy (DAPT; 75 mg aspirin + 75 mg clopidogrel) for 30 days.

4.4 Experimental phase

Three control animals underwent mitral valve implantation without an IPG, while three other animals received the modified valve assembly with an IPG (test animals). The animals were allocated to the test and control groups without randomization, and blinding was not implemented at any stage of the experiment. Due to the small sample size, confounding variables were not explicitly controlled. The prosthetic valve-lead junction was redesigned based on the insights derived from the standardization phase. The lead helical tip and ring electrodes were preserved in this phase, in contrast to the valve design in the standardization phase, where the removal of the lead tip

beyond the ring electrode was associated with an accelerated IPG battery discharge and valve thrombosis (elaborated in the results section). The final design of the valve assembly is described as follows. A fenestration of ~ 3 mm was made in the sewing cuff at the outer surface of the housing of the valve in the vicinity of the valve leaflet hinges. The valve is rotated within the sewing cuff to align the slit on the outer valve ring (shown in Fig. 4c) with the fenestration made in the sewing cuff. The first two turns of the helical tip were unwound and inserted into the slit on the outer surface of the valve (Fig. 4f) through the fenestration. The electrode was advanced until the entire helical tip entered the slit, ensuring firm locking of the tip between the inner and outer rings. The mechanical stability of the valve-lead connection was verified by tugging the electrode wire. The delivery of current to the blood-contacting surfaces of the valve was checked with a multimeter before the device was ETO-sterilized and packed for implantation. The delivery of the electricity to the valve discs after implantation was also confirmed intraoperatively prior to closure of the left atrium. The test animals in this phase received DAPT for 45 days postoperatively and were then followed up for 20 weeks. The control animals, on the other hand, were implanted with the mitral mechanical prosthetic valve without any IPG-induced electrical activation. The first two control animals received post-operative DAPT throughout the follow-up period, while the third control animal received DAPT for 45 days.

4.5 Surgical procedure

Animals were premedicated with atropine (0.03 mg kg⁻¹), xylazine (1.5 mg kg⁻¹), midazolam (0.5 mg kg⁻¹), followed by ketamine (15 mg kg⁻¹). After intravenous access, deep surgical anesthesia was induced with propofol (2 mg kg⁻¹) and maintained with 1–1.8% isoflurane delivered via positive pressure ventilation. Prophylactic antibiotics included ceftriaxone–sulbactam (30 mg kg⁻¹). Pre-operative 2D-echocardiography was done, a left thoracotomy through the fourth intercostal space was performed to access the heart, and animals were placed on cardiopulmonary bypass under moderate hypothermia (core temperature 28°C) using cannulation of the descending thoracic aorta and right atrium. Heparin (300 IU kg⁻¹) was administered to maintain an activated clotting time above 400 s. The native mitral valve was excised, and the mechanical valve assembly was sutured into the annulus with pledgeted interrupted Ethibond sutures (**Fig S3: Supplementary information**). In the test animals, the bipolar lead was attached to the IPG, which was anchored to the endothoracic fascia at the apex of the left hemithorax. At the valvular end, the lead-PTFE sheath transit through the left atrium was buried in the endocardium using polypropylene continuous sutures until it exited the left atrium (through the appendage) to eliminate blood exposure. In control, the IPG was not connected to the valve. The animals were weaned off cardiopulmonary bypass and decannulated. The chest was closed in layers. Post-operative 2D echocardiography was performed to assess prosthetic mitral valve function. Postoperative care included antibiotics, analgesics (tramadol and non-steroidal anti-inflammatory drugs) and multivitamin injections.

4.6 Monitoring and follow-up

Animals were housed in the acute care unit and monitored daily for any clinical abnormalities for 10 days postoperatively. Thereafter, they were transferred to experimental rooms, where they were managed under the supervision of animal care staff. In the event of any clinical abnormality, a comprehensive veterinary examination was performed, and findings were documented. Following surgery, both test and control animals received DAPT as discussed before. After cessation of DAPT, all animals were followed for at least 20 weeks or until death, whichever occurred first. Animals that survived beyond 20 weeks were electively euthanized using intravenous 5% thiopentone sodium. Prior to euthanasia, pre-explantation hematological investigations and cardiac catheterization were performed to assess prosthetic valve function and the trans-mitral pressure gradient. Following death or euthanasia, a complete autopsy was conducted. The heart and other organs were subjected to pathological examination to identify thrombosis, embolism, infarction, and any associated abnormalities.

One explanted valve from each of the standardization, test, and control groups, along with one unimplanted valve serving as a negative control, was subjected to SEM analysis. The explanted pacemaker battery was evaluated for estimated remaining longevity. The primary endpoints were survival without major valve-related complications and absence of thrombus formation on the valve. Secondary endpoints included normal hemodynamic performance, absence of hemolysis, and adequate residual IPG battery longevity. Because of the small sample size, results are presented descriptively, and no formal statistical comparisons or effect size estimations were performed.

Declarations

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Author contributions

Lokeswara Rao Sajja: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing - Original Draft, Writing - Review & Editing; **Thomas Mathew:** Investigation, Methodology, Project administration, Resources, Validation, Writing - Review & Editing; **Aditya Koppula:** Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing.

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None

Competing interests

The corresponding author (Lokeswara Rao Sajja) is the inventor and patent holder of the device evaluated in this study and may benefit financially from its commercialization. This constitutes a potential conflict of interest. The other authors declare no competing interests. All efforts were made to ensure that the study was conducted and reported objectively.

Data availability statement

All the data used in the preparation of this manuscript have been archived in Zenodo (<https://doi.org/10.5281/zenodo.20606956>). It is publicly accessible with the following link (https://zenodo.org/records/20606956?token=eyJhbGciOiJIUzUxMiJ9.eyJpZCI6IjZiNmZDImLWI1MDgtNDcxZi05ZDMyLTl0Yjc2Nzg3ZDQ4ZCIsImRhdGEiOnt9LCJyYW5kb20iOiI1NDVjNTQ3NjJIM2YxMzcwYzU1ZGI5YzUwNTU3MGU1YyJ9.fsGdeqn9jb5tlg_CEMiMaaOcNrLeQBJFxarve1-zPljv-JGuWe81tUIIR-H_DufMb1yvW3hrksQwSYpGQ61OQ).

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Figures

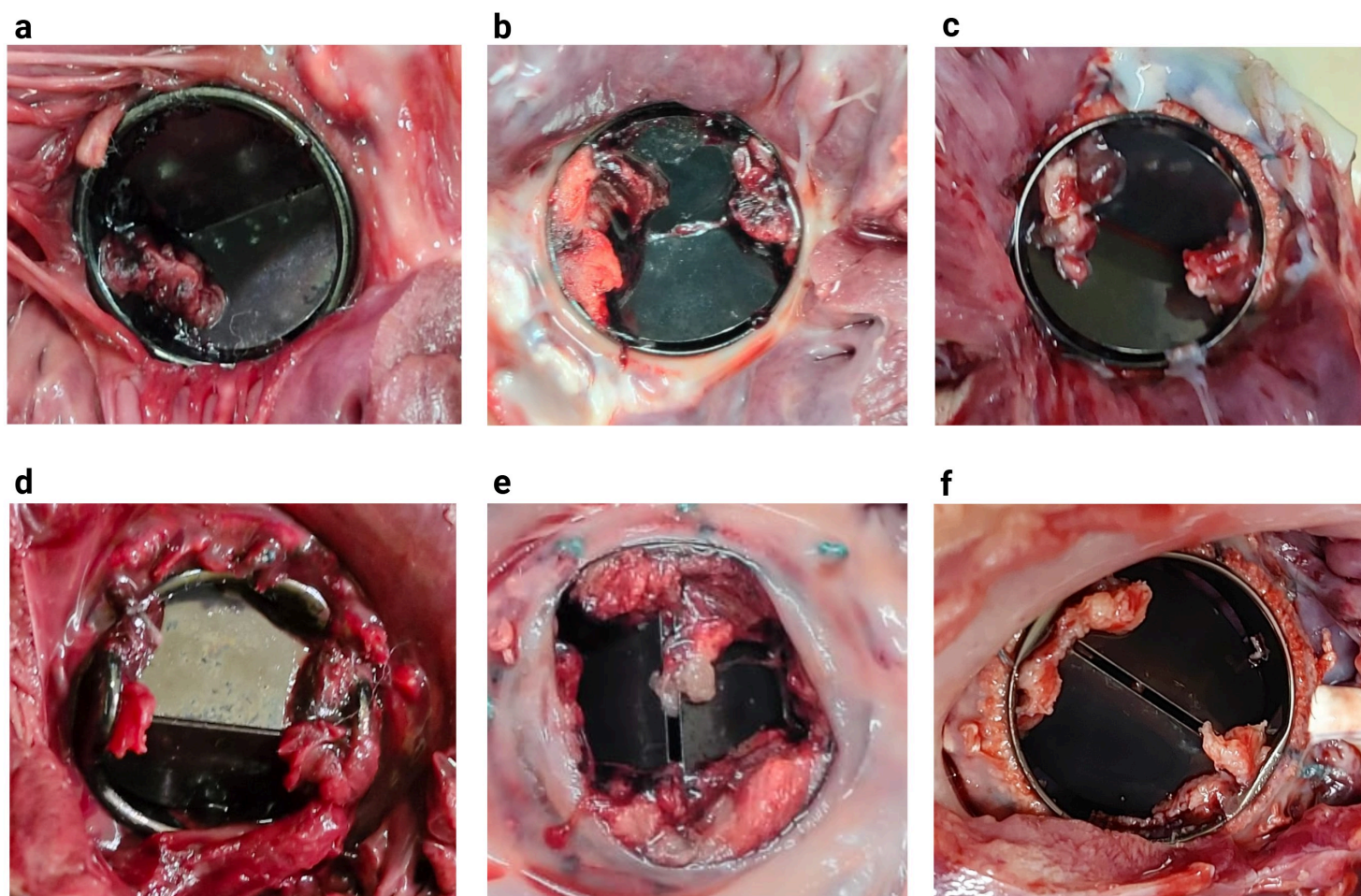


Figure 1

Gross photographs of valves from standardization phase test animals at autopsy. The images show extensive valvular thrombosis on both the inflow (a-c) and outflow (d-f) sides. **a** and **d**: Animal ID- 20830; **b** and **c**: Animal ID- 280750; **c** and **f**: Animal ID- 282666.

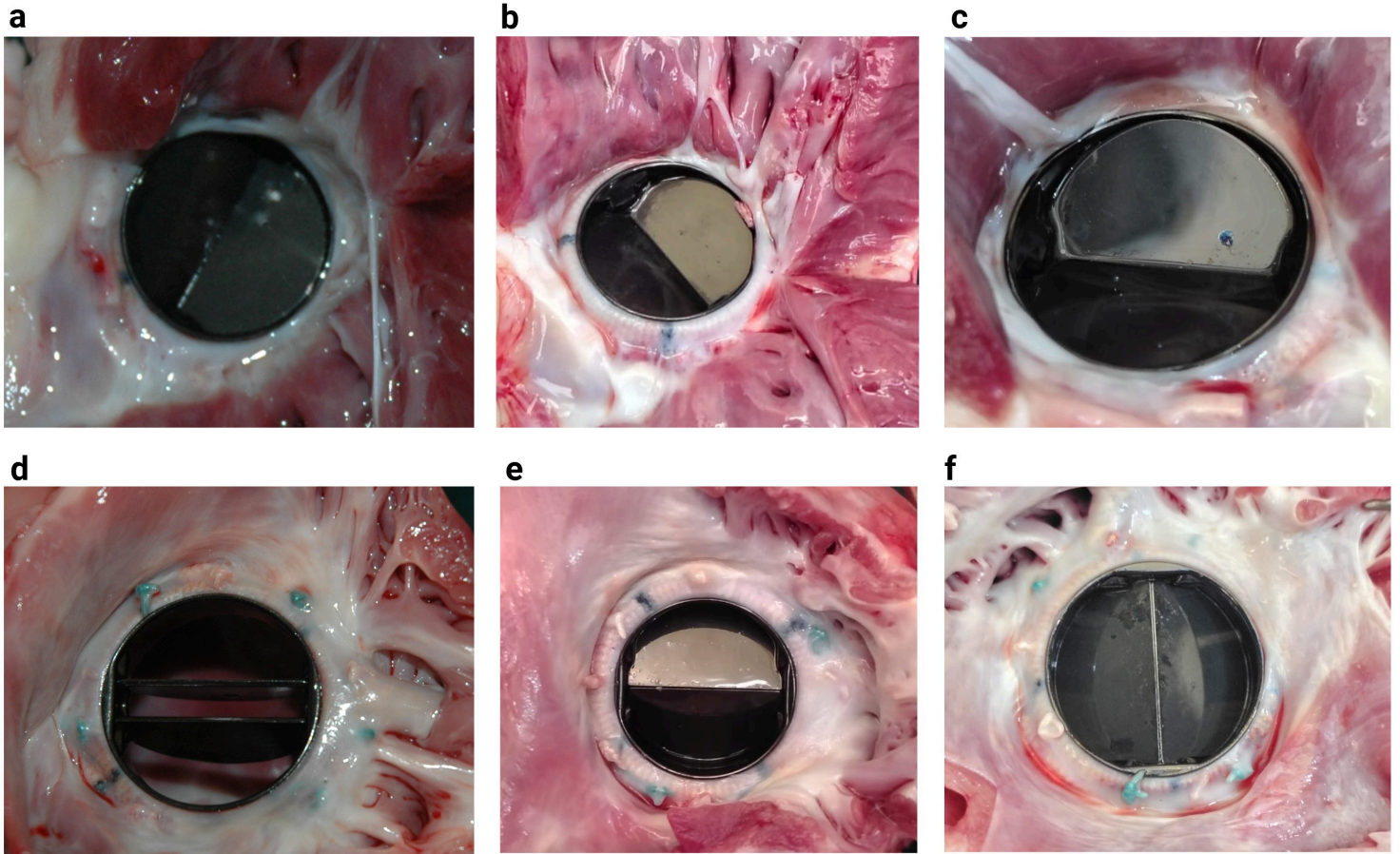


Figure 2

Gross photographs of valves from experimental phase test animals at autopsy. The valves are “clean,” and there is no discernible thrombosis on the inflow (a-c) and outflow (d-f) sides of the valves. a and d: Animal ID- 286528; b and e: Animal ID- 281430; c and f: Animal ID- 286049.

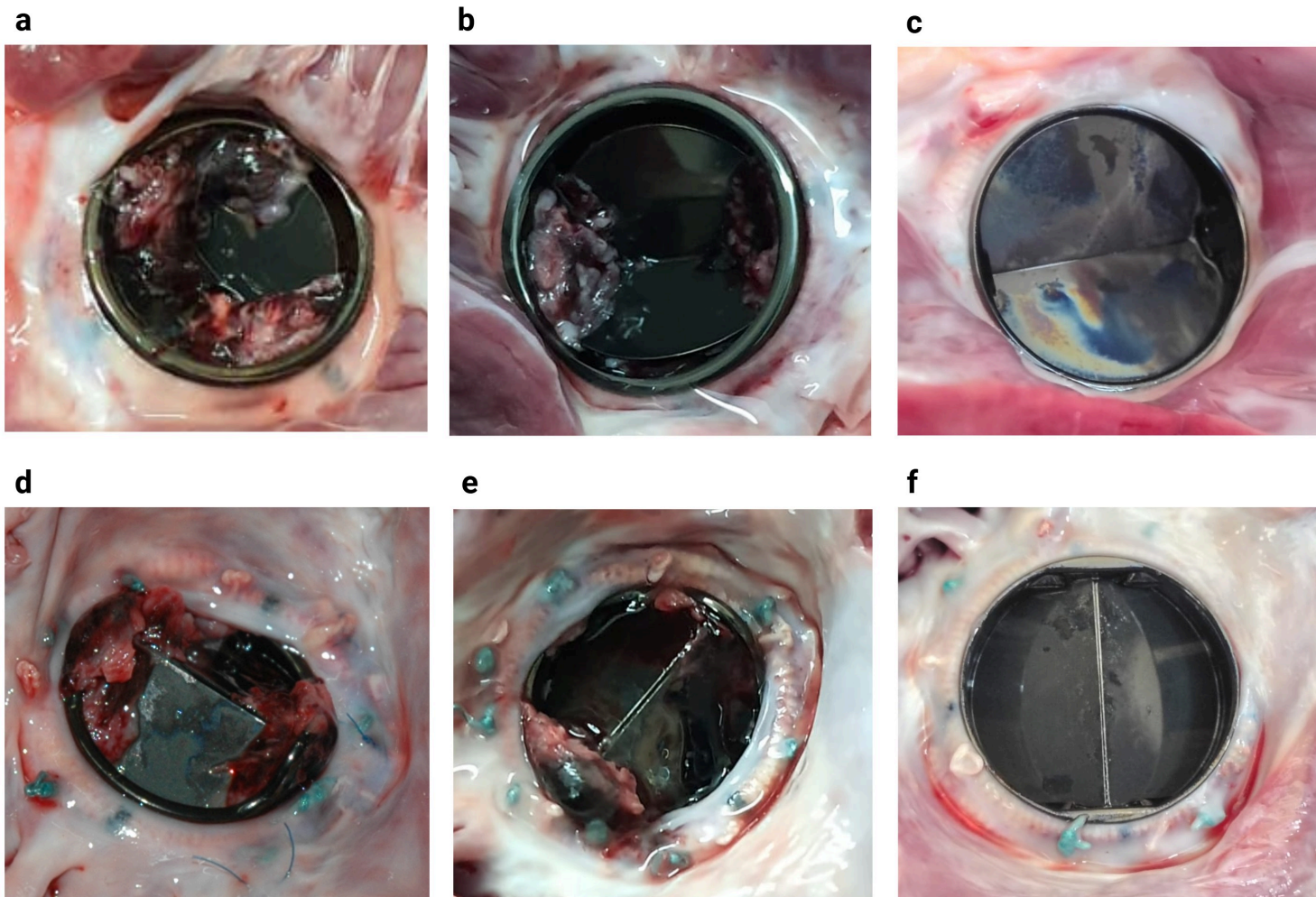


Figure 3

Gross photographs of valves from the control animals at autopsy. Thrombosis is seen on both the inflow (**a-b**) and outflow (**d-e**) sides of the valves for the first two controls. No gross thrombus deposition is discernible on the inflow (**c**) and outflow (**f**) sides of the third control valve. **a** and **d**: Animal ID- 20829; **b** and **e**: Animal ID- 284998; **c** and **f**: Animal ID- 291354 (3rd control).

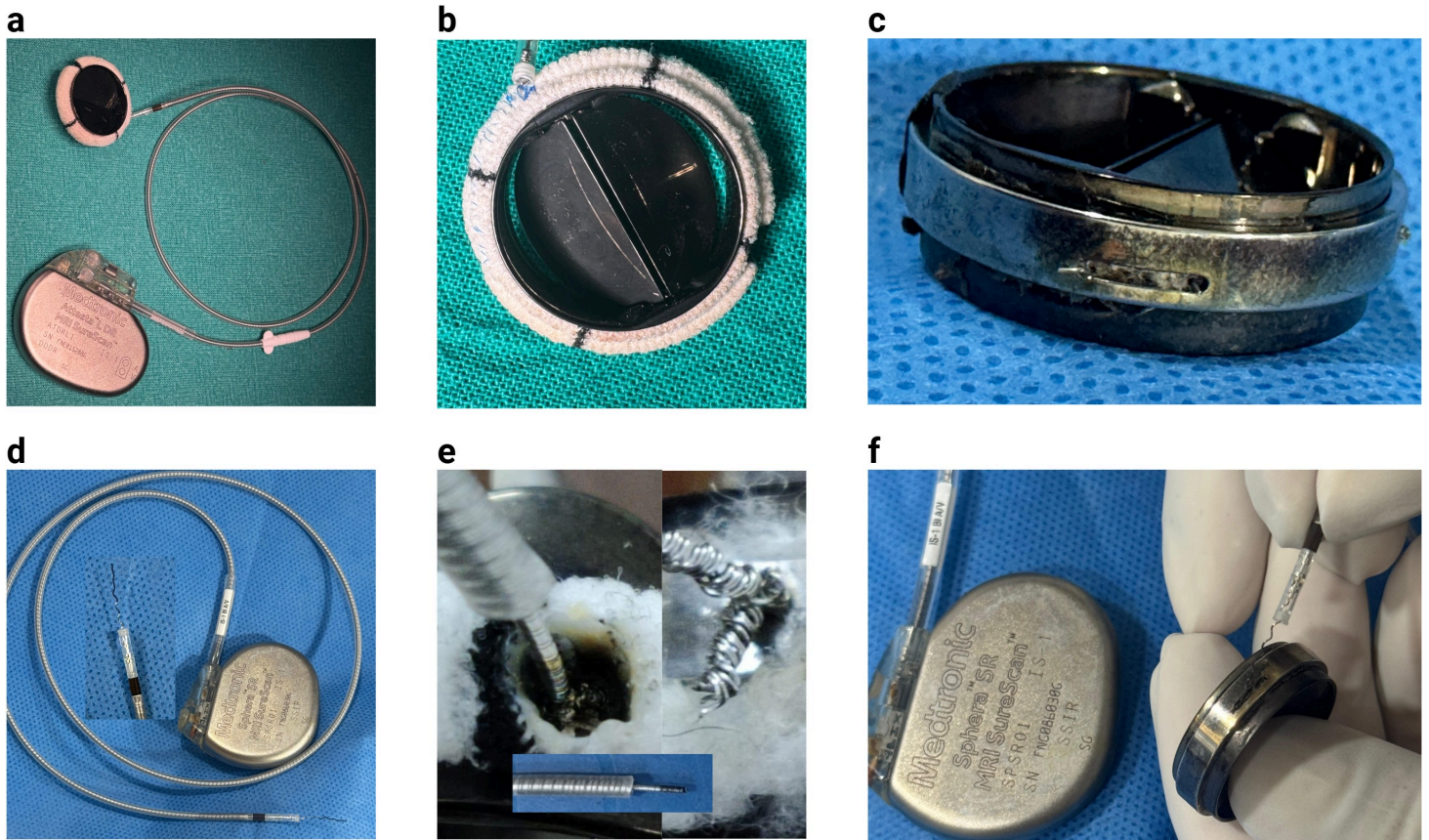


Figure 4

Novel prosthetic mechanical valve assembly design. **a-** The valve assembly has a valve, a lead wire, and the IPG. **b-** Bileaflet valve with outermost sewing cuff and inner ring with leaflets is shown in outflow-side view. **c-** The sewing cuff is removed to show the outer ring (titanium) with a slit and the inner ring, including leaflets (pyrolytic carbon). **d-** The helical tip (tip electrode-cathode) of the lead wire and the black-colored ring electrode (anode) are shown. **e-** Valve-lead junction in the first design of valve assembly is shown where the tip (cut proximal to the ring electrode) is welded to the outer ring (end-to-side and side-to-side junction). **f-** Valve-lead junction in final valve assembly design is shown; sewing cuff is removed for illustration, and helical tip is advanced into the slit on the outer ring.

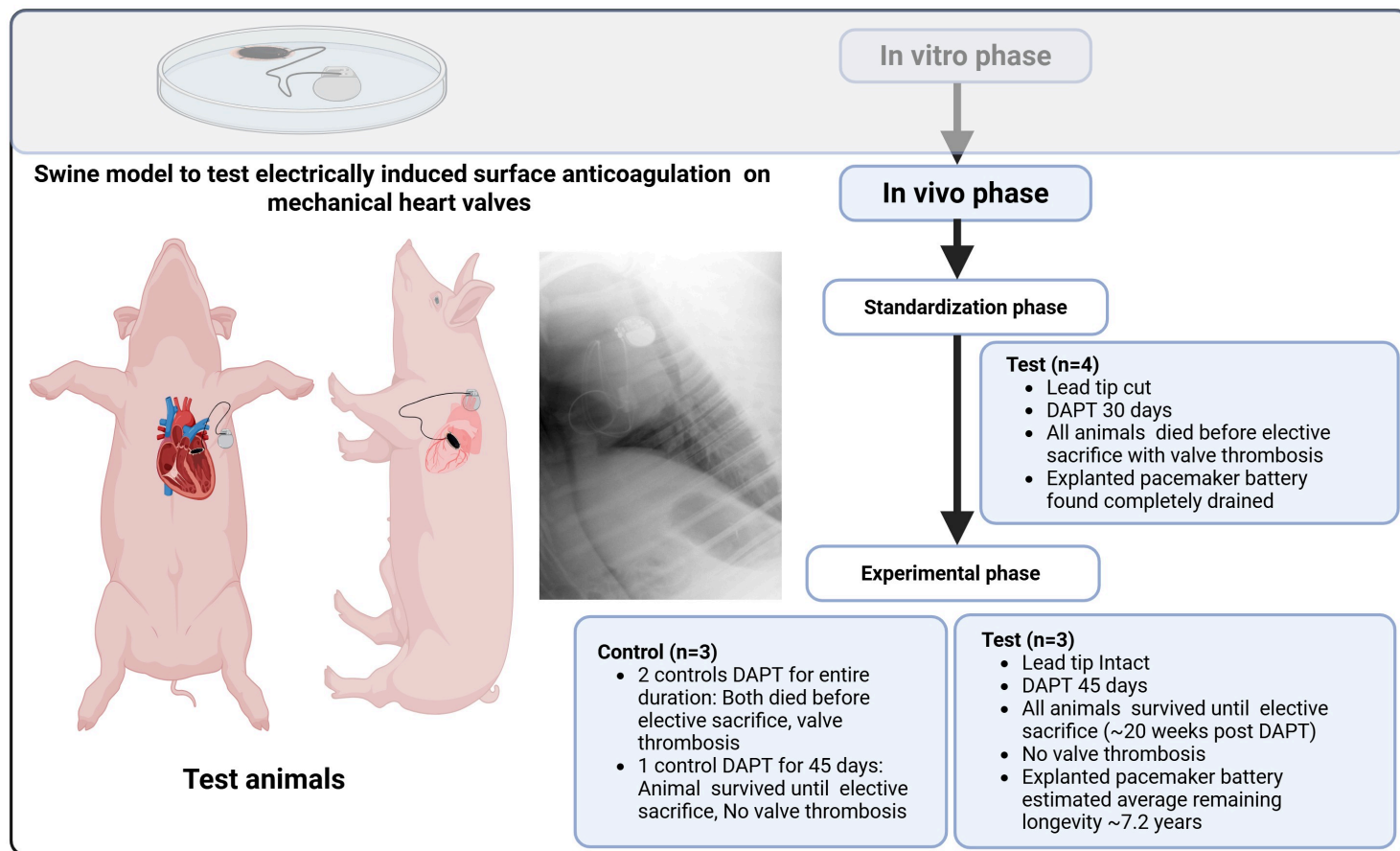


Figure 5

The study design with in vitro and in vivo stages. A schematic representation of valve-lead-IPG in a test animal heart is shown. The inset shows a radiograph of the test animal after implantation, depicting the IPG, lead wire, and prosthetic valve. We also show the outcomes at the standardization and experimental phases of the in vivo study.

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