



# *"Evaluation Of Safety And Performance Of WALL-NUT™ Left Atrial Appendage Occluder System (LAAOD): A Porcine Model Analysis Over Multiple Time Points"*

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## **ABSTRACT**

This study evaluated the thrombogenicity and biocompatibility of the WALL-NUT™ left atrial appendage (LAA) occluder device in three animals (porcine model). No device-related thrombogenic events, embolization, or adverse outcomes were observed throughout the study period. Transesophageal echocardiography (TEE) revealed stable device positioning without evidence of thrombus formation, device migration, or pericardial effusion. Clinical assessments showed no signs of morbidity, abnormal weight loss, or significant changes in hematology or clinical chemistry between pre and post-implantation. Histopathological evaluations indicated a mild inflammatory response around the implant, with progressive endothelialization of the device surface. Two animals exhibited up to the 75% endothelial coverage, while one achieved complete coverage by 90 days. The presence of inflammatory cells was noted, but without escalation of the response over time. These findings demonstrate the LAA occluder's strong safety profile and biocompatibility, supporting its potential for clinical application in preventing stroke in patients with atrial fibrillation. Future studies with longer follow-up are recommended to confirm long-term efficacy.

## **KEYWORDS**

WALL-NUT™ Left atrial appendage (LAA) occluder Device, Porcine Model, Safety Evaluation, Performance Assessment, radiography and angiographic findings, Histopathological Findings.

## INTRODUCTION:

The Left Atrial Appendage (LAA) is a key source of thromboembolism in patients with atrial fibrillation, making its occlusion a promising strategy for stroke prevention. This study aims to evaluate the safety and performance of the WALL-NUT™ Left Atrial Appendage Occluder Device (LAAOD) in a preclinical porcine model, which closely mimics human cardiac anatomy and physiology. The research focuses on device performance, integration into the endothelial lining, and overall biocompatibility. The findings of this study will provide valuable insights into the WALL-NUT™ LAAOD's potential for future clinical applications.

Three female swine were selected for the study, with all animals meeting the pre-defined acceptance criteria following an overnight fasting period. The WALL-NUT™ LAAOD was implanted in each swine, the device was successfully deployed in all animals. Scheduled transthoracic echocardiographic evaluations revealed no significant complications, such as pericardial effusion, device-related mitral valve dysfunction, or pulmonary venous obstruction. Postmortem pathological analysis confirmed that the device was appropriately positioned, with complete sealing of the LAA ostium in all cases.

Importantly, no adverse events such as device embolization, thrombus formation, or occluder failure were observed during the study's follow-up period. Macroscopic evaluations of the explanted hearts, as well as echocardiographic assessments, showed no signs of thrombus on the device. The healing process was marked by thin, laminar fibrin deposition on the membrane, which served as a matrix for inflammatory cells and fibroblasts, ultimately leading to complete endothelialization by 12 weeks. Histological analysis demonstrated secure anchoring of the device, with collagen embedment and infiltration of smooth muscle cells leading to a biocompatible blood-contacting surface that resembled normal endocardium. The study did not observe significant inflammatory reactions or necrosis in adjacent cardiac tissue, and all animals exhibited normal clinical outcomes, including weight gain, during the follow-up period. proper placement of the occluder, with full sealing of the LAA ostium, and no damage to the device during repositioning attempts.

Importantly, no thrombus formation or occluder embolization was observed at follow-up, and histological analysis demonstrated robust endothelialization after 12 weeks. The device's healing process was deemed safe, with minimal inflammatory reactions and no necrosis in adjacent cardiac tissue.

## MATERIALS AND METHODS

### DEVICE DESIGN

The Left Atrial Appendage (LAA) is a small, ear-shaped sac in the muscle wall of the left atrium (top left chamber of the heart) commonly associated with thrombus formation. The WALL-NUT™ Left Atrial Appendage (LAA) Occluder System is indicated to reduce the risk of Thrombus Formation. The WALL-NUT™ Left Atrial Appendage (LAA) Occluder System is a self-expanding nitinol frame structure with fixation anchors and permeable polyester (PET) fabric covering the Left Atrial Appendage (LAA) Occluder System. The duration of contact is permanent (lasting beyond 30 days). The total dimensions of the Occluders are mentioned in the table 1. Sterilization methods include Ethylene Oxide (ETO).

**SIZE MATRIX**

Table 1: Size matrix of Device

Device Diameter (mm)	Delivery Sheath Diameter (F)	Access Sheath Diameter (F)
18	12	14
20		
24		
28		
32		
36		
38		

**PRODUCT IMAGE**



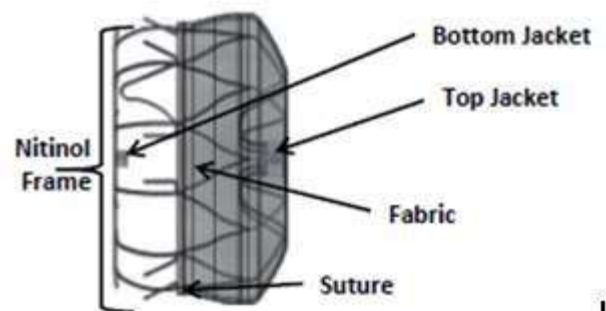
(A)



(B)



(C)



(D)

Figure 1. WALL-NUT™ Left Atrial Appendage (LAA) Occluder Device.

- A. Front View of WALL-NUT™ Left Atrial Appendage (LAA) Occluder Device,
- B. Top View of WALL-NUT™ Left Atrial Appendage (LAA) Occluder Device,
- C. Side View of WALL-NUT™ Left Atrial Appendage (LAA) Occluder Device,

## D. Schematic Diagram of WALL-NUT™ Left Atrial Appendage (LAA) Occluder Device

The WALL-NUT™ Left Atrial Appendage (LAA) Occluder System is a self-expanding nitinol frame structure with fixation anchors and permeable polyester (PET) fabric covering the Left Atrial Appendage (LAA) Occluder System.

**DEVICE COMPONENT DESCRIPTION**

Table 2: Device components description

Sr. No.	Parameters	Specifications
<b>Implant Details:</b>		
1.	Frame	Nitinol
2.	Fabric	Polyethylene terephthalate
3.	Bottom Jacket	Stainless steel
4.	Top Jacket	
5.	Suture	Polyester
6.	Device size; mm	18, 20, 24, 28, 32, 36, 38
7.	Guidewire compability; inch	0.035"
8.	Access Sheath; F	14
9.	Delivery sheath; F	12
10.	Y- Hub	Polypropylene
11.	Delivery cable	Stainless steel

**MATERIALS REQUIRED**

- Introducer Sheath
- 2-3 Syringes
- Normal heparinised saline (HepNS)
- 0.035'' diameter guide wire
- Contrast diluted 1:1 with normal saline
- Inflation device
- Three-way stopcock
- Guide Wire Introducer

**Medication Details**

Animals were kept on anticoagulant treatment, Aspirin 300 mg/animal and clopidogrel 75 mg/animal (PO) at least 3 days prior to the procedural day, prior to implantation of test item and continued from day 1 to day 90 with reduced dose of aspirin i.e., 150 mg/animal. Animal was weighed, anesthetized, instrumented, and monitored using Ketamine 15 mg/kg (IM), Xylazine 2.5 mg/kg (IM), Propofol 0.5mg/kg (IV bolus) followed by inhalation of anesthesia 1-3% through facemask. Clinical signs were mentioned in table 3.

Table 3: Animal 01, 02 and 03 Clinical signs observation on Day 0 to Termination Day

Sr. No.	Animal No.	Treatment (Day 0-90)
1	P1 (30 Days)	Normal
2	P2 (60 Days)	Normal
3	P3 (90 Days)	Normal

## EXPERIMENTAL PROCEDURES

### Fasting:

The animal was fasted and deprived of water for at least 12 hours prior to the procedure. Post-procedural care involved maintaining the animals nil per os (NPO) for a duration of six hours following their recovery. This protocol was implemented to ensure optimal conditions for the procedures and recovery phases.

### Animal Preparation included:

Animals were kept on anticoagulant treatment, Aspirin 300 mg/animal and clopidogrel 75 mg/animal (PO) at least 3 days prior to the procedural day, prior to implantation of test item and continued from day 1 to day 90 with reduced dose of aspirin i.e., 150 mg/animal.

On the day of the procedure, each animal was weighed, anesthetized, instrumented, and monitored. The anesthetic protocol included an intramuscular administration of Ketamine 15 mg/kg (IM), Xylazine 2.5 mg/kg (IM), Propofol 0.5mg/kg (IV bolus) followed by inhalation of anesthesia 1-3% through facemask. List of drugs used during the study was mentioned in table 4. The neck, chest and thigh area was clipped free of hair for femoral vein and femoral artery approach and ECG leads application, respectively.

The animal was prepared and draped for aseptic procedure with medications as described in Atropine, at a dose of 0.05 mg/kg IM, was administered to control respiratory tract secretions that might obstruct the endotracheal tube used for the inhalation anesthesia. The animals were then prepared and draped for aseptic procedures, with appropriate medications administered throughout. List of drugs used in the study mentioned in table 4.

Table 4: List of Drugs used

Drug name	Manufactured by	Batch / Lot No.
Atropine	Atropin Vet	BY22001
Ketamine	Troikaa pharmaceuticals Ltd	K50514
Xylazine	IIL India	FHK1003
Isoflurane	Neon Lab	KPNP700013
Thiopentone Sodium	Neon Lab	173274
Heparin	Gland pharma Ltd	101109
Aspirin	USV Ltd	52001055
Clopidogrel	Cipla Ltd	SN91823

Enrofloxacin	ENROX	CM1433012
Thiopental	Thiozol	173274

## EXPERIMENTAL DESIGN OR ANIMAL TRIAL

**DAY 0** - The groin regions of the animal, both right and left, were shaved to allow for the procedure. Using a percutaneous approach via the Seldinger technique, an 8F sheath was inserted into the femoral vein, and a 6F sheath was placed into the jugular vein. Activated Clotting Time (ACT) was measured before and after heparinization, with heparin administered to maintain ACT values between 250 and 550 seconds. The initial bolus dose of heparin was 100 IU/kg IV, with subsequent doses titrated based on ACT levels.

A stiff 150 cm, 0.035-inch guidewire was employed alongside a catheter to anchor the foramen ovale at the septal wall within the right atrium. An 8F Mullins sheath was advanced from the groin to the right atrium to facilitate the passage of a trans-septal needle. Chest radiography and transthoracic echocardiography (TTE) were utilized to puncture the septum, and a non-compliant 6.0x40 mm balloon was used to create a shunt in the atrial septal wall for the passage of a long sheath (4.67 mm diameter). A 0.035-inch, 260 cm Amplatzer extra-stiff guidewire was advanced through the Mullins sheath into the left atrial appendage. A 14F long sheath was then passed over the Amplatzer guidewire into the left atrial appendage, and the LAA occluder device (LAAOD) was deployed using a delivery system navigated through the sheath. Chest radiography and TTE were performed both before and after the implantation to confirm placement and function. The animal was recovered post-procedure and monitored until the terminal day. Device implantation matrix outlined in table 5.

Table 5: LAA occluder device Implantation Matrix

Animal No	Left Atrial Appendage
01	28 mm (Waist size)
02	28 mm (Waist size)
03	28 mm (Waist size)

Table 6: Animal 01, 02, 03 ACT Values (Day 0 and Termination Day)

Animal Number	Time (min)	ACT Value
P1	0 Day	98
	30 Day	102
P2	0 Day	108
	60 Day	102
P3	0 Day	93
	90 Day	107

From Day 1 to Day 90, the animals were closely monitored for any signs of illness. Cage-side observations were conducted and recorded daily for each animal. Early elective euthanasia was performed based on humane endpoints, followed by gross and histopathological evaluations to determine the cause of mortality or morbidity.

Activated Clotting Time (ACT) is an important measure in animal studies that shows how long it takes for blood to clot. It is commonly used in research involving blood clotting, anticoagulant drugs like heparin, and surgeries that require blood thinners. Monitoring ACT helps ensure anticoagulants are working effectively during procedures like cardiopulmonary bypass and hemodialysis. In surgical models, ACT is used to maintain safe levels of anticoagulation during invasive procedures. Before starting any experiment, baseline criteria are set to provide a consistent starting point, making the results more reliable and easier to understand.

### Day 30

On Day 30, animal P1 underwent follow-up angiography and transthoracic echocardiography (TTE) to confirm the positioning of the occluder device. The animal was then euthanized for the harvesting of the heart, which was photographed in situ. Gross and histopathological evaluations were conducted, along with gross necropsy and additional photography.

### Day 60

On Day 60, animal P2 was subjected to follow-up angiography and TTE to verify the placement of the occluder device. The animal was euthanized, and the heart was harvested for in situ photography of the device, followed by gross and histopathological evaluations. Gross necropsy and photography were also performed.

### Day 90

On Day 90, animal P3 underwent follow-up angiography and TTE for device positioning verification. The animal was then euthanized, the heart was harvested and photographed in situ, and gross and histopathological evaluations were completed. Gross necropsy and additional photography were also conducted. Weight of the animals is illustrated in table 7.

Table 7: Animal 01, 02, 03 Body weights (Day 0 to Day 90)

Animal Number	Sex	Treatment (Day 0)	Terminal
P1 (30 Day)	Female	42.1 kg	43.4 kg
P2 (60 Day)	Female	43.5 kg	44.9 kg
P3 (90 Day)	Female	42.6 kg	51.6 kg

### Monitoring During Procedure: Day 0 to 90 days

During the procedure, electrocardiogram (ECG), respiration rate, heart rate, and oxygen saturation were continuously monitored.

### Pre-operative

Tramadol (2 mg/Kg IM) as an analgesic was administered to the animal once prior to anesthetic induction. Atropine (0.05 mg/Kg, IM) was also given as pre-anesthetic. Animal was sedated with Ketamine (15 mg/Kg, IM), Xylazine (2.5 mg/Kg, IM) subsequently delivering Isoflurane (1-3%) through face mask.

### Intra-operative and post-operative

Anesthesia was maintained using 1-3% Isoflurane through endotracheal intubation. All medications administered

to each study animal during the procedure were recorded in their individual records.

## OBSERVATION

The study monitored animal body weights, clinical signs of illness or distress, and the performance of test items. Body weights were recorded during acclimatization, procedure, and euthanasia. Animals were observed daily for signs of illness, which were promptly reported and documented. The performance of the test items included assessing the delivery system's access, handling, and visualization, as well as deployment accuracy, withdrawal ability, and hemostasis. Additionally, it involved evaluating implant position, integrity, and functionality, along with the histology and pathology of explants.

## PATHOLOGY

- Clinical Pathology

Blood was collected before the procedure and at euthanasia for hematological and biochemical analysis.

- Euthanasia

Animals were euthanized with thiopental sodium at 100 mg/Kg, IV. Death was confirmed by observing the heart/lungs, a systolic ECG, and zero oxygen saturation.

- Necropsy

Gross necropsy was performed on all animals. Organs were weighed and examined, with no lesions found in standard organs.

- Histopathological Evaluation of Implanted Device

The implanted device, along with the atrial septum and left atrial appendage, was harvested for photography and subjected to gross and histopathological evaluation in euthanized animals. A comprehensive necropsy of standard organs was conducted according to the sponsor's guidelines. The harvested WALL-NUT™ left atrial appendage occlusion device (LAAOD) was rinsed with normal saline and preserved in 10% formalin. For histological analysis, the device underwent resin embedding using the Technovit kit T9100, followed by tissue sectioning with a Struers Secotom 60, polishing with a Metco grinder/polisher, and staining with Hematoxylin and Eosin. Endothelialization and thrombosis were evaluated using Leica's inbuilt image analysis software. Additional sections were taken as necessary based on the gross examination of the left atrial appendage.

Original data was collected for each section, and comments on inflammation or other safety parameters were included.

## RADIOGRAPHY AND ANGIOGRAPHIC FINDINGS

Radiographic images for the thrombogenicity evaluation were provided. Among the three animals tested with the WALL-NUT™ left atrial appendage (LAA) occluder device, none exhibited device-related thrombogenicity or

embolization. A follow-up examination was conducted after a specified period, including a general health assessment and transesophageal echocardiography (TEE) evaluation of the device. TEE imaging was used to assess device position, potential leakage, thrombus formation, and the presence of pericardial effusions. No adverse events were recorded during the follow-up. Notably, no device-associated thrombus, dislocation, embolization, device failure, or clinically significant pericardial effusions were detected.

## **PATHOLOGY**

Blood collection was performed on day 0, prior to the procedure, and on the day of euthanasia. The following hematological and biochemical parameters were analyzed: complete blood count (including differential count and reticulocytes), as well as clinical evaluations of LDH, AST, creatinine, creatine kinase, urea, BUN, sodium, potassium, chloride, and calcium. These assessments were conducted both before and after the procedure.

Both baseline and termination day hematological and biochemical blood counts were within normal ranges on day 0 and at subsequent follow-up time points (days 30, 60, and 90). No abnormal findings were observed in the clinical biochemistry results at day 0, and at days 30, 60, and 90. Clinical chemistry data was mentioned in table 8 and data of Haematology Parameters were mentioned in table 9.

Table 8: Individual data of Clinical chemistry (Day 0 (baseline), Day 30, Day 60 and Day 90)

Animal No.	P1		P2		P3		Mean (Day 0)	SD (Day 0)
	Day 0	Day 30	Day 0	Day 60	Day 0	Day 90		
AST (U/L)	48	25	48	28	49	36	48.33	0.58
Ca (mmol/L)	2.57	2.58	2.56	2.70	2.52	2.72	2.55	0.03
CK (U/L)	2701	663	2578	451	2861	459	2713.33	141.90
Creat (µmol/L)	110	117	109	111	111	90	110.00	1.00
LDH (U/L)	959	350	982	581	992	626	977.67	16.92
BUN (mmol/L)	4.12	4.14	4.21	5.98	4.22	2.16	4.18	0.06
Na (mmol/L)	142.5	138.5	142.2	141.3	142.2	138.0	142.30	0.17
K (mmol/L)	3.74	3.73	3.76	3.35	3.77	4.17	3.76	0.02
Cl (mmol/L)	106.0	105.1	105.9	105.4	105.6	94.1	105.83	0.21

Table 9: Individual data of Haematology Parameters (Day 0 (baseline), Day 30, Day 60 and Day 90)

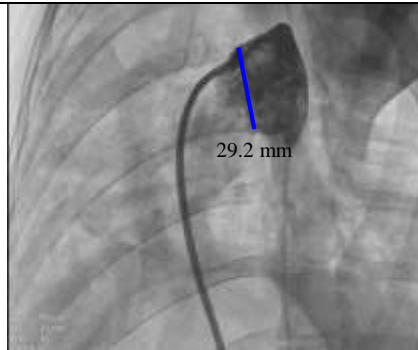


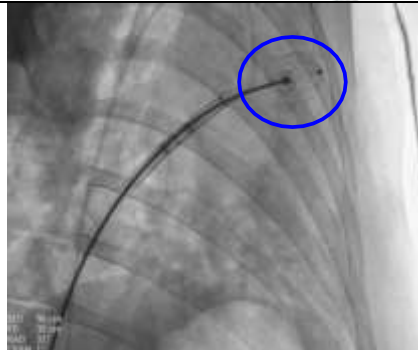
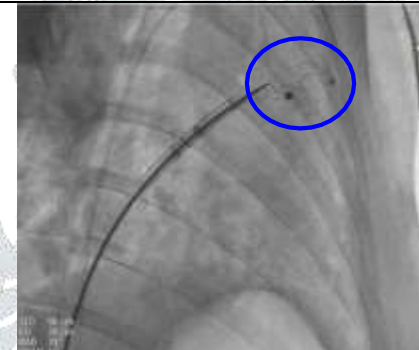
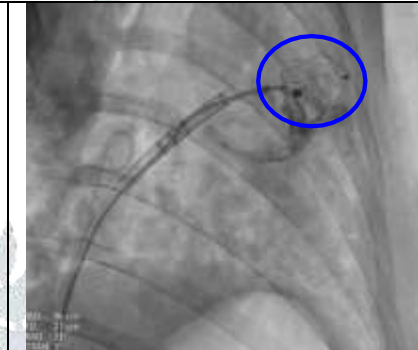
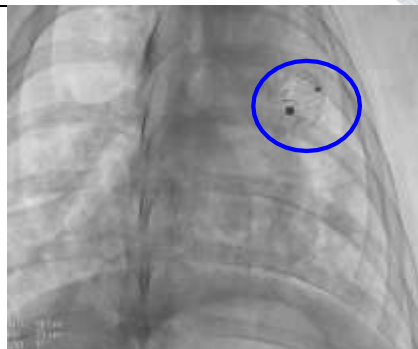
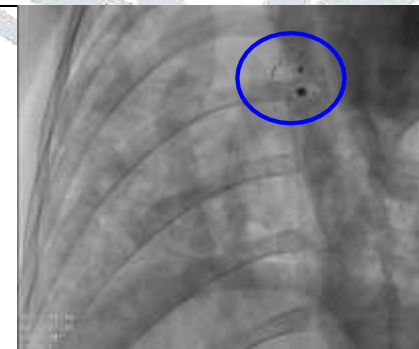
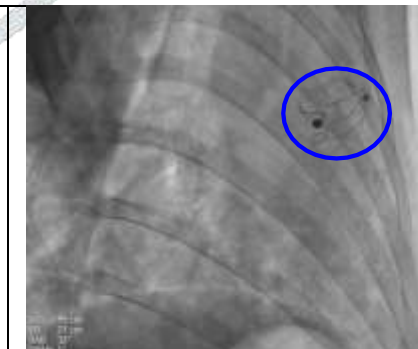
Animal No.	P1		P2		P3		Mean (Day 0)	SD (Day 0)
	Day 0	Day 30	Day 0	Day 60	Day 0	Day 90		
WBC (109/L)	19.89	14.34	19.27	22.08	19.83	32.84	19.66	0.34
RBC (1012/L)	6.00	5.49	5.89	7.92	5.90	6.85	5.93	0.06
HGB (g/L)	101	93	101	150	101	109	101.00	0.00
HCT (L/L)	0.350	0.301	0.345	0.529	0.346	0.346	0.35	0.00
MCV (fL)	58.4	54.8	58.5	66.8	58.6	50.4	58.50	0.10
MCH (pg)	16.8	16.8	17.1	18.9	17.1	15.9	17.00	0.17
MCHC (g/L)	288	308	293	283	291	316	290.67	2.52
RDW (%)	16.2	15.9	16.2	14.5	16.2	17.1	16.20	0.00
HDW (g/L)	16.3	15.9	15.7	24.4	16.7	19.9	16.23	0.50
PLT (109/L)	419	250	419	180	414	403	417.33	2.89
MPV (fL)	7.9	10.4	7.7	9.0	7.9	8.5	7.83	0.12
NEUT (%)	41.1	68.3	40.4	15.9	40.9	46.7	40.80	0.36
LYM (%)	54.8	23.0	54.3	75.7	53.9	47.1	54.33	0.45
MONO (%)	2.7	6.9	1.6	6.3	3.1	4.5	2.47	0.78
EOS (%)	0.5	0.2	0.4	0.8	0.5	0.9	0.47	0.06
LUC (%)	0.7	1.6	3.2	1.2	1.5	0.7	1.80	1.28
BASO (%)	0.1	0.0	0.1	0.1	0.1	0.0	0.10	0.00
Retic (%)	2.14	0.16	1.79	1.61	1.97	0.82	1.97	0.18

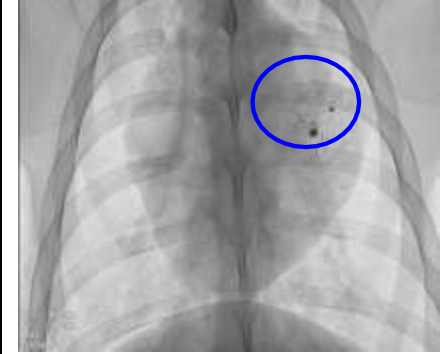


Table 10: Individual Animal Gross Pathology Findings - Female

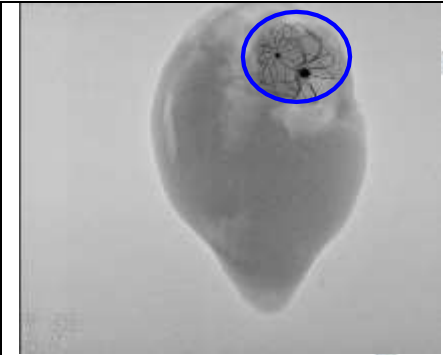
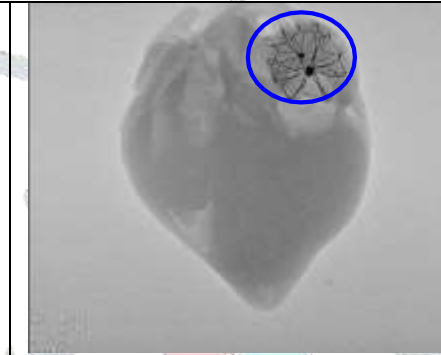
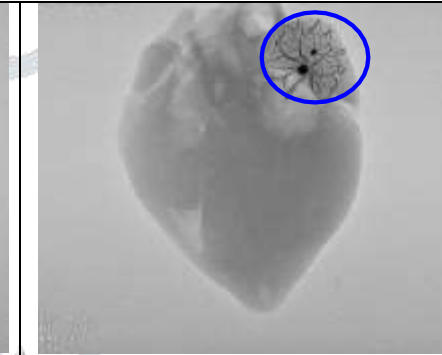
Animal Number	Sex	Mode of Death	Macroscopic/Gross pathological observation	
			External	Internal
P1	Female	Terminal Sacrifice	NAD	NAD
P2	Female	Terminal Sacrifice	NAD	NAD
P3	Female	Terminal Sacrifice	NAD	NAD

FLUOROSCOPY IMAGES

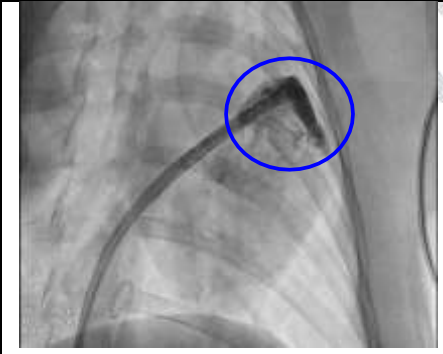


Animal No.P1: **Make clear visible remarks**

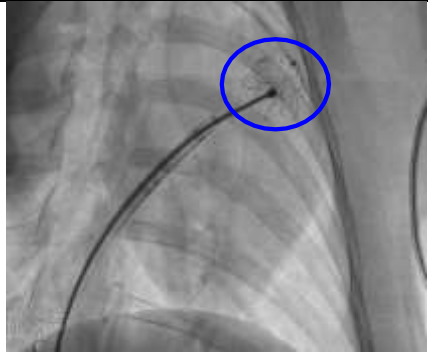
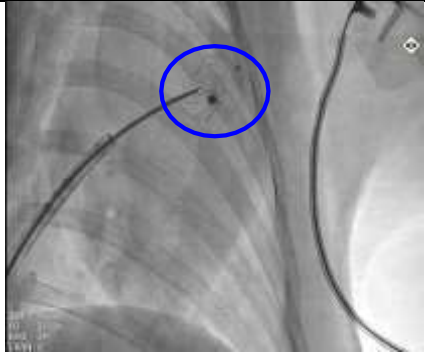
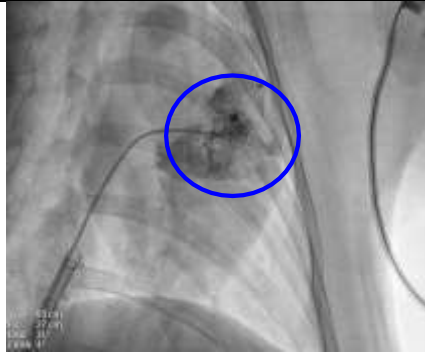
		
<p>On 0 day, animal P1 LA appendage baseline angio</p>	<p>On 0 day, animal P1 LA appendage size measurement</p>	<p>On 0 day, animal P1 guide wire passed into LA to descending aorta for support to passing the LA 14F of long sheath with dilator</p>
		
<p>On 0 day, animal P1 LAAOD device placed in LA appendage</p>	<p>On 0 day, animal P1 LAAOD device was released in LA appendage</p>	<p>On 0 day, animal P1 after released LAAOD device, post LA appendage check angio was done</p>
		
<p>On 0 day, animal P1 LAAOD device fluoroscopy was taken in AP view</p>	<p>On 0 day, animal P1 LAAOD device fluoroscopy was taken in LAO view</p>	<p>On 0 day, animal P1 LAAOD device fluoroscopy was taken in RAO view</p>




		
<p>On follow up day 30, animal P1 LAAOD device fluoroscopy was taken in AP view</p>	<p>On follow up day 30, animal P1 LAAOD device fluoroscopy was taken in LAO view</p>	<p>On follow up day 30, animal P1 LAAOD device fluoroscopy was taken in RAO view</p>

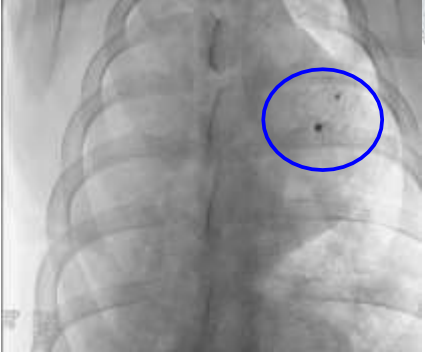
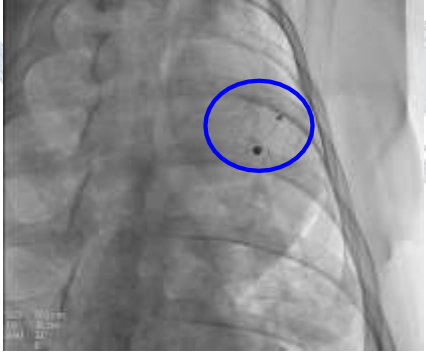

		
<p>On follow up day 30, animal P1 collected heart with LAAOD device fluoroscopy was taken in LAO view</p>	<p>On follow up day 30, animal P1 collected heart with LAAOD device fluoroscopy was taken in AP view</p>	<p>On follow up day 30, animal P1 collected heart with LAAOD device fluoroscopy was taken in RAO view</p>

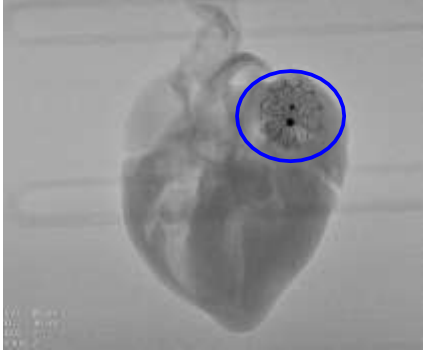
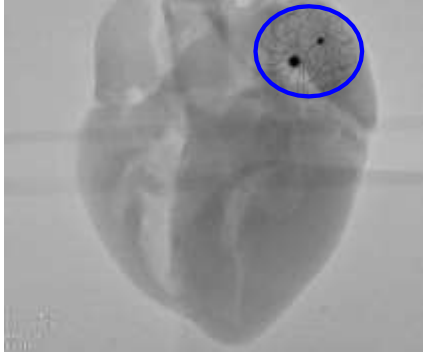
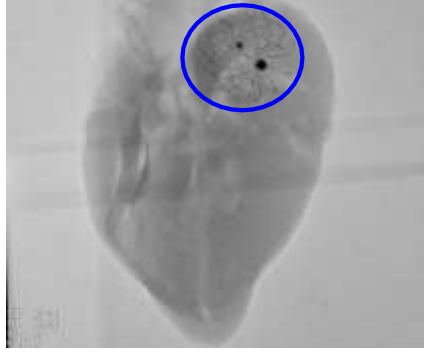
**Animal No. P2:**

		
<p>On 0 day, animal P2 LA appendage baseline angio</p>	<p>On 0 day, animal P2 LA appendage size measurement</p>	<p>On 0 day, animal P2 guide wire passed into LA to descending aorta for support to passing the LA 14F of long sheath with dilator</p>

		
<p>On 0 day, animal P2 LAAOD device placed in LA appendage</p>	<p>On 0 day, animal P2 LAAOD device released in LA appendage</p>	<p>On 0 day, animal P2 after released LAAOD device, post LA appendage check angio was done</p>

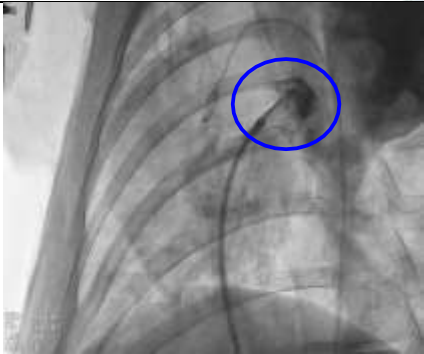

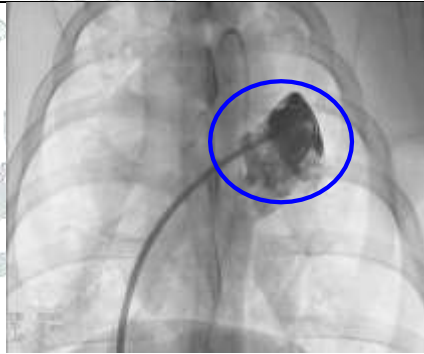
		
<p>On 0 day, animal P2 LAAOD device fluoroscopy was taken in RAO view</p>	<p>On 0 day, animal P1 LAAOD device fluoroscopy was taken in LAO view</p>	<p>On 0 day, animal P2 LAAOD device fluoroscopy was taken in AP view</p>

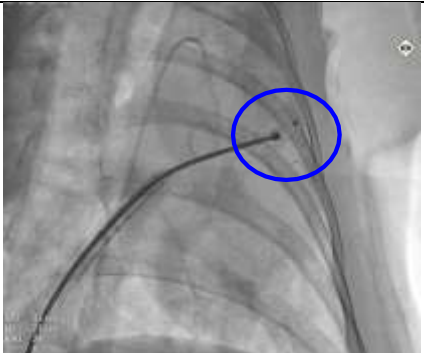
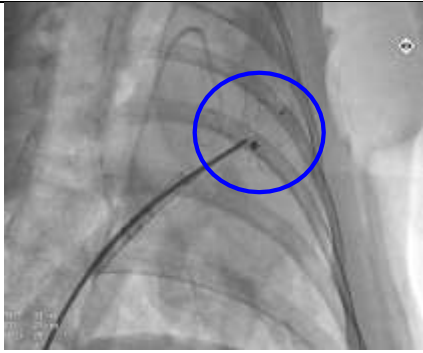
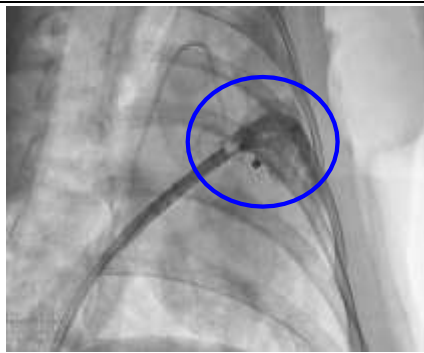
		
<p>On follow up day 60, animal P2 LAAOD device fluoroscopy was taken in AP view</p>	<p>On follow up day 60, animal P2 LAAOD device fluoroscopy was taken in RAO view</p>	<p>On follow up day 60, animal P2 LAAOD device fluoroscopy was taken in AP view</p>

		
On follow up day 60, animal P2 collected heart with LAAOD device fluoroscopy was taken in AP view	On follow up day 60, animal P2 collected heart with LAAOD device fluoroscopy was taken in RAO view	On follow up day 60, animal P2 collected heart with LAAOD device fluoroscopy was taken in LAO view



**Animal No. P3:**

		
On 0 day, animal P3 LA appendage baseline angio with mullins sheath	On 0 day, animal P3 LA appendage size measurement	On 0 day, animal P3 LA appendage baseline angio with long sheath

		
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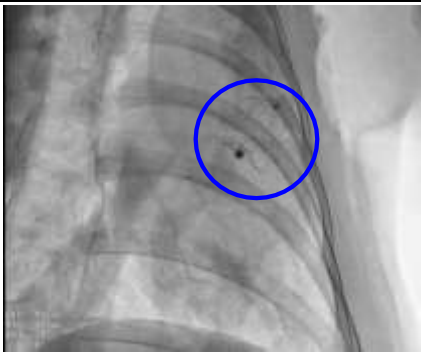
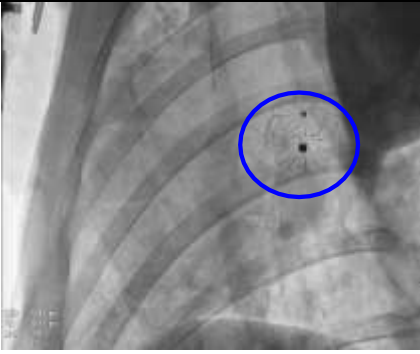


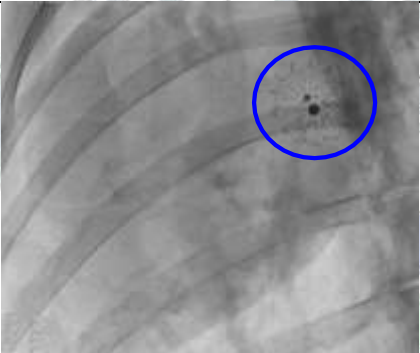
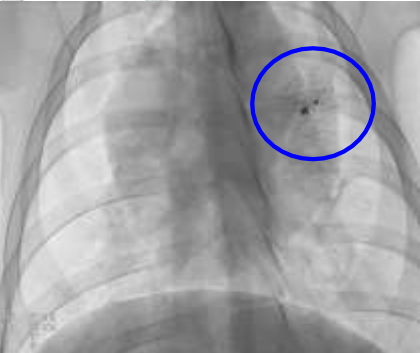

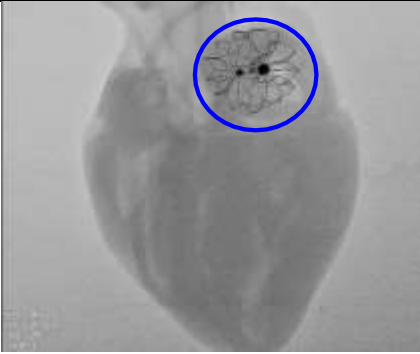
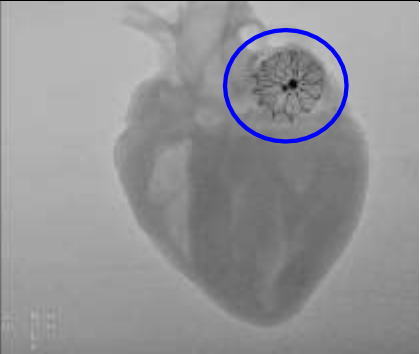
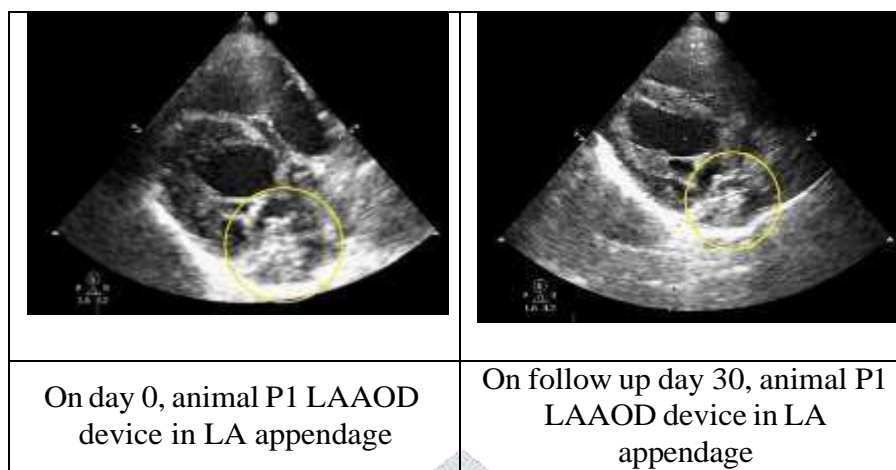
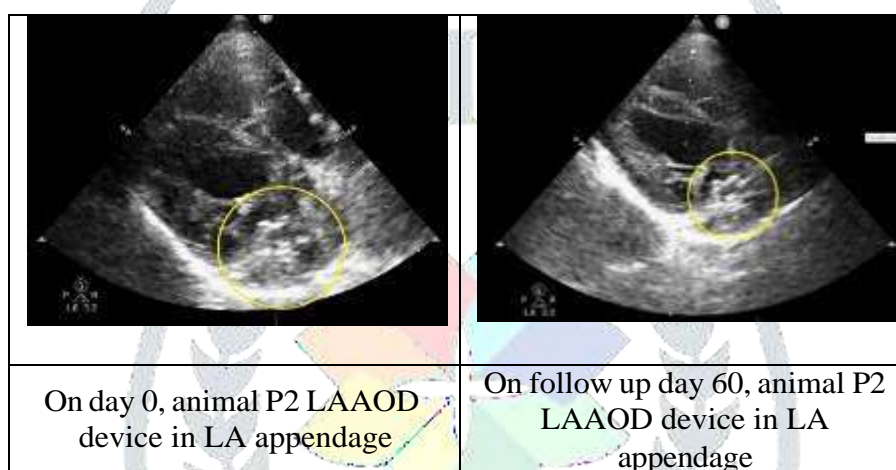
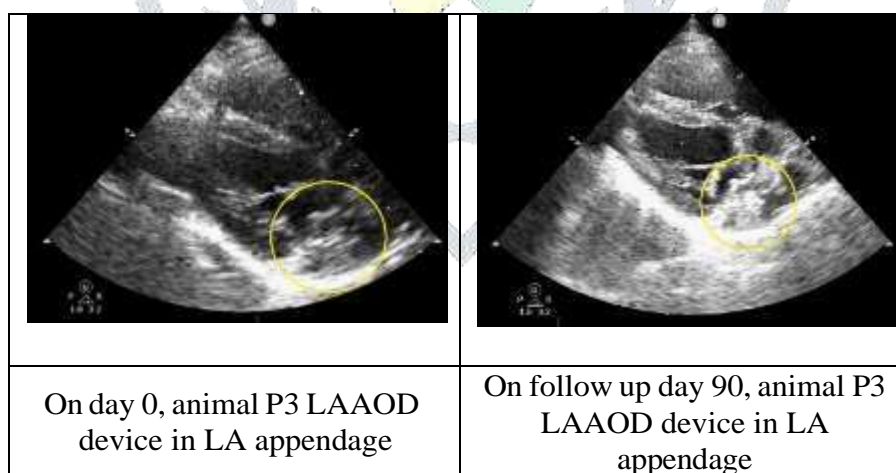
<p>On 0 day, animal P3 LAAOD device placed in LA appendage</p>	<p>On 0 day, animal P3 LAAOD device released in LA appendage</p>	<p>On 0 day, animal P3 after released LAAOD device, post LA appendage check angio was done</p>
		
<p>On 0 day, animal P3 LAAOD device fluoroscopy was taken in RAO view</p>	<p>On 0 day, animal P3 LAAOD device fluoroscopy was taken in LAO view</p>	<p>On 0 day, animal P3 LAAOD device fluoroscopy was taken in AP view</p>
		
<p>On follow up day 90, animal P3 LAAOD device fluoroscopy was taken in RAO view</p>	<p>On follow up day 90, animal P3 LAAOD device fluoroscopy was taken in LAO view</p>	<p>On follow up day 90, animal P3 LAAOD device fluoroscopy was taken in AP view</p>
		
<p>On follow up day 90, animal P3 collected heart with LAAOD device fluoroscopy was taken in RAO view</p>	<p>On follow up day 90, animal P3 collected heart with LAAOD device fluoroscopy was taken in LAO view</p>	<p>On follow up day 90, animal P3 collected heart with LAAOD device fluoroscopy was taken in AP view</p>

Figure 2: Fluoroscopy images of animal P1, P2 and P3

**Echo cardiography Images****Animal No. P1:****Animal No. P2:****Animal No. P2:****NECROPSY**

At the scheduled sacrifice dates (P1 on Day 30, P2 on Day 60, and P3 on Day 90), all animals were humanely euthanized via an overdose of Thiopental sodium. A pathologist then conducted a thorough examination for any external or internal gross pathological changes. According to the study protocol, the implanted devices were retrieved and preserved in 10% neutral buffered formalin. The left atrial appendage, along with the implanted device, was processed for resin embedding. Tissue sections, ranging from 100 to 200 microns in thickness, were

prepared using a Secotom cutting machine. Subsequently, the thickness of these sections was further reduced using a Bainpol VTD polishing machine to achieve the desired precision. The tissue sections were stained with Hematoxylin and Eosin (H&E) and evaluated under a light microscope by the study pathologist for histopathological lesions.

The external examination of all female animals revealed no lesions of pathological significance. Similarly, the internal examination showed no evidence of any lesions of pathological importance.

**HISTOPATHOLOGY:** The histopathology report for animals from P1 to P3 indicates the following findings

Table 11: Histopathology Scores for Left Atrial Appendage Occluder System

Device location	Animal number	Inflam mation	Septal wall injury	Smooth muscle cell loss	Fibrin deposition	Endoth elial loss	Total score
Left atrial appendage	P1(30 days)	3	1	0	1	0	5
	P2 (60 days)	3	1	0	0	0	4
	P3 (90 days)	3	1	0	0	0	4

**Note: Lesser the score is better histology**

**Keys:** Inflammation (0 - No or very few(<3); 1 - Few(4-10); 2 - (> 10)Many without circumference; 3(> 10) - Many with circumference) Septal wall injury (0- No injury,1- break in the endocardium,2- perforation of myocardium, 3- perforation of myocardium and endocardium) Smooth muscle cell loss (0- No; 1- Minimal (< 25%); 2- Mild (25-50%); 3- Moderate (51-75%); 4- Marked loss (>75%) Fibrin deposition (0- none;1- Minimal ; 2- Mild; 3- Moderate; 4- severe).

Table 12: Histopathological observations of the device deployed area

Device location	Animal number	Perforation/ pericarditis	Infection irritation	Endothelial growth	Nitinol PET covering
Left atrial appendage	P1(30 days)	No	No	50-75% (endothelialization)	Partially covered
	P2 (60 days)	No	No	50-75% (endothelialization)	Fully covered
	P3 (90 days)	Diffuse mainly ventricular vegetative growth	No	100% (endothelialization)	Fully covered

**Animal No.01:**

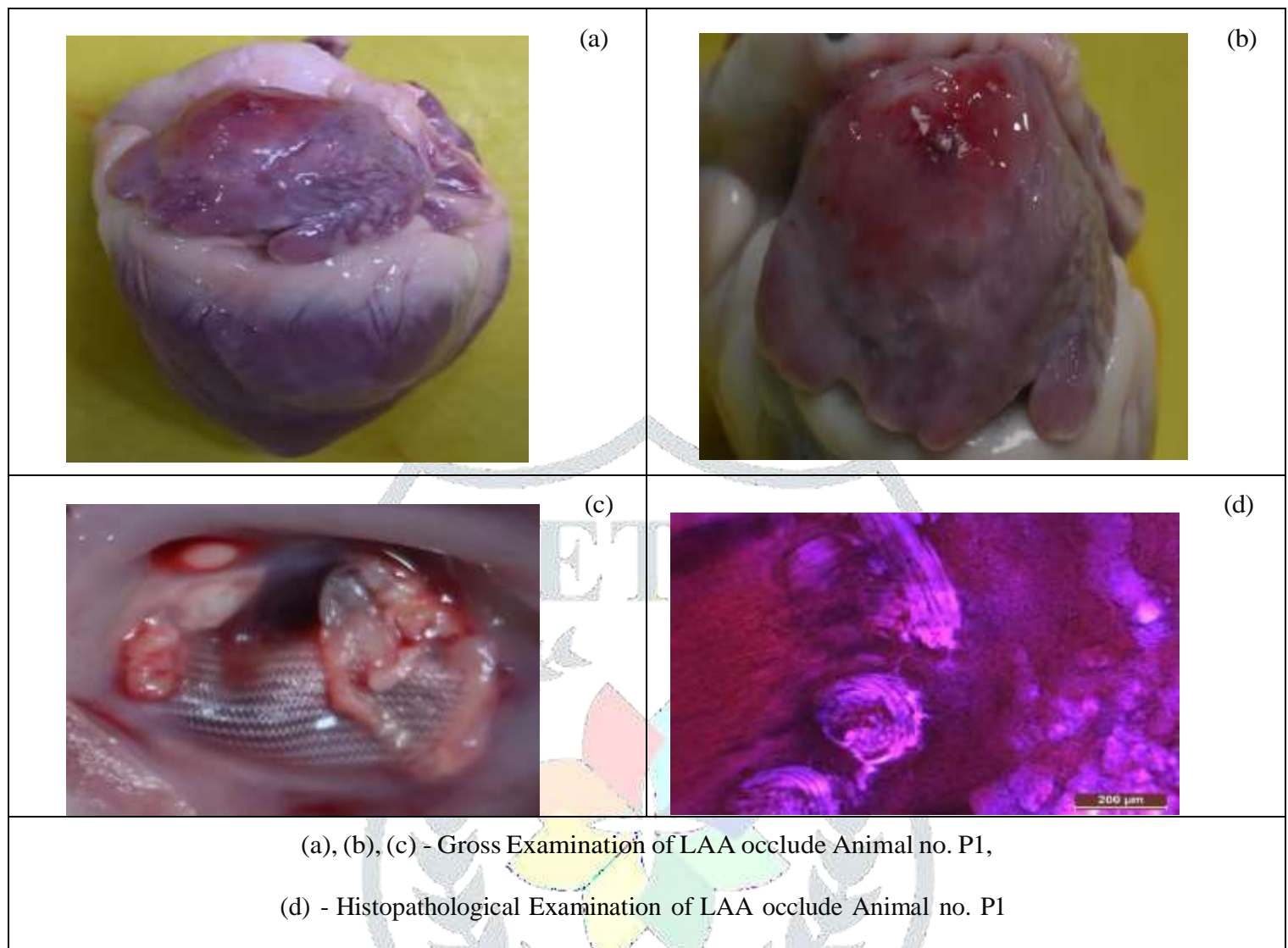
Microscopic examination revealed numerous inflammatory cells infiltrating and disrupting the surrounding tissue, with a break in the endocardium and minimal fibrin deposition. No loss of smooth muscle cells was noted, and the total histopathology score was 5. Endothelialization covered 50-75% of the intimal surface with epithelium.

Gross and Histopathological Findings at 30-Day Follow-Up:

Macroscopic evaluation showed the device effectively sealing the left atrial appendage (LAA) ostium. Connective tissue, covered by an endothelial layer, partially filled the device surface. The thrombus was fully organized by connective tissue. The membrane was completely covered with connective tissue, which in turn was covered by an endothelial monolayer. There was connective tissue development accompanied by mild diffuse inflammation, as observed through hematoxylin-eosin (HE) staining.



Table 13: Gross and Histopathological Examination of LAA occlude Animal no. P1, H&amp;E 1.25X and 40X.

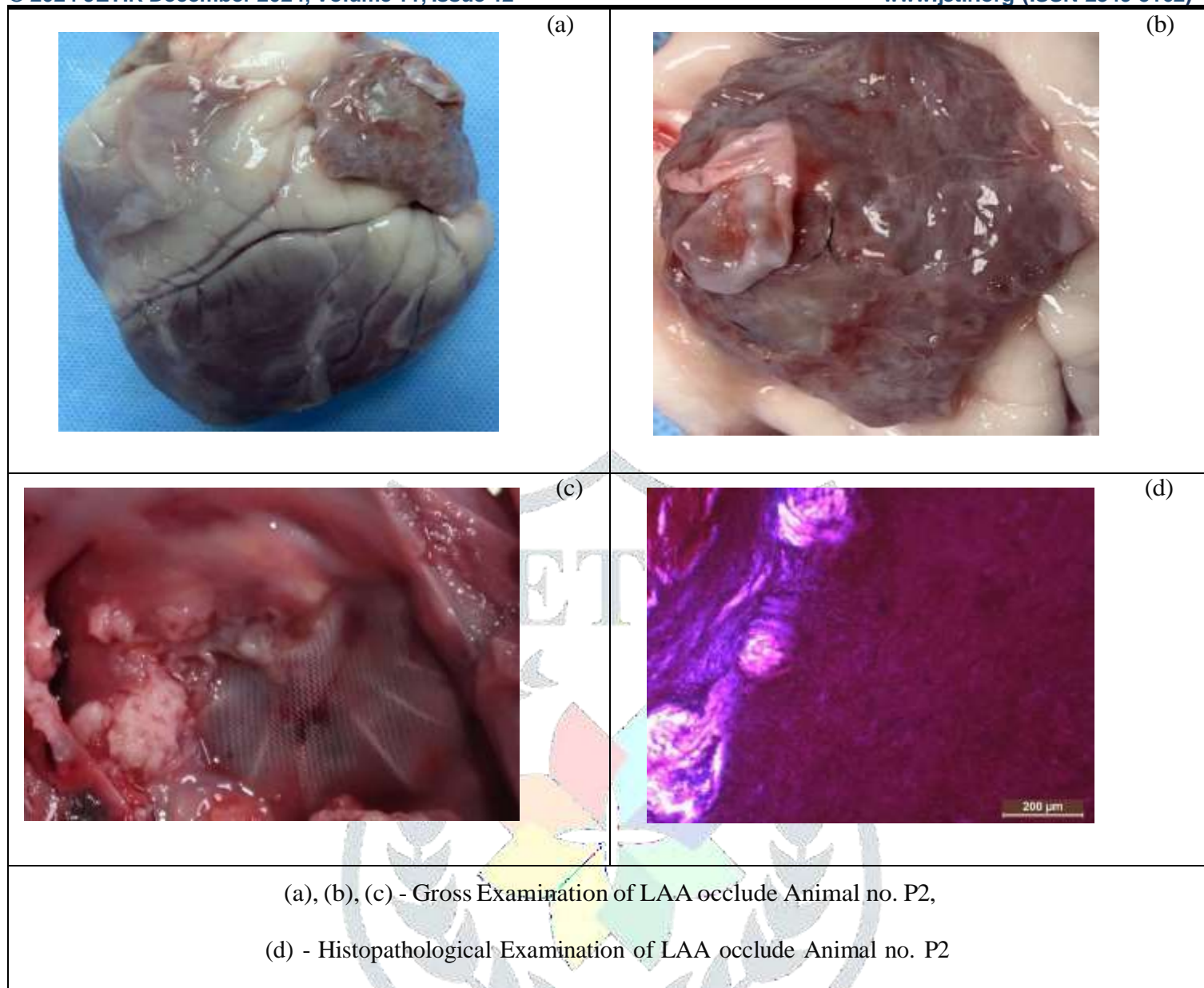
**Animal No.02:**

Microscopic examination demonstrated significant infiltration of inflammatory cells, disrupting the surrounding tissue. A break in the endocardium was observed, with no fibrin deposition or smooth muscle cell loss. The total histopathology score was 4, with 50-75% of the intimal surface covered by endothelialization.

**Gross and Histopathological Findings at 60-Day Follow-Up:**

Macroscopic evaluation showed the device sealing the LAA ostium, with connective tissue covered by an endothelial layer partially filling the device surface. The thrombus was fully organized by connective tissue. The membrane was completely covered with connective tissue, which was itself covered by an endothelial monolayer. A discrete, diffuse inflammatory response was observed in the connective tissue, as seen with HE staining.

Table 14: Gross and Histopathological Examination of LAA occlude Animal no. P2, H&amp;E 1.25X and 40X.



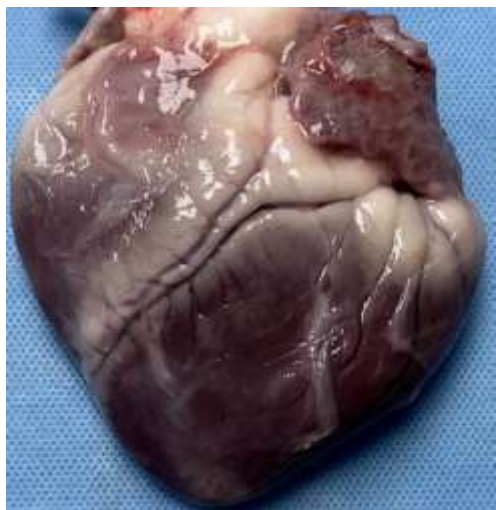
### Animal No.03:

Microscopic evaluation revealed extensive infiltration of inflammatory cells, causing disruption to the surrounding tissue, with a break in the endocardium. There was no fibrin deposition, but smooth muscle cell loss was noted. The total histopathology score was 4, with complete endothelialization (100%) of the intimal surface.

#### Gross and Histopathological Findings at 90-Day Follow-Up:

Macroscopic evaluation demonstrated the device sealing the LAA ostium, with connective tissue, covered by an endothelial layer, fully filling the device surface. The thrombus was fully organized by connective tissue. The membrane was completely covered by connective tissue, which was itself covered by an endothelial monolayer. Discrete diffuse inflammation was observed within the connective tissue, as indicated by HE staining.

Table 15: Gross and Histopathological Examination of LAA occlude Animal no. P2, H&E 1.25X and 40X.



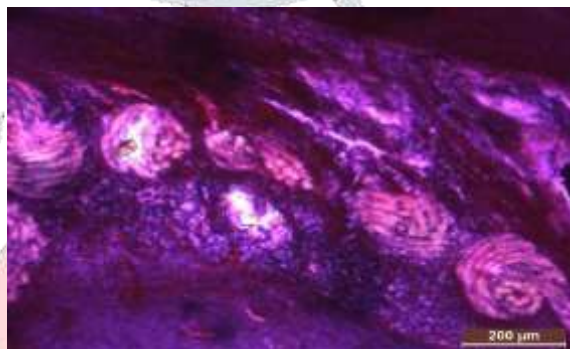
(a)



(b)



(c)



(d)

(a), (b), (c) - Gross Examination of LAA occlude Animal no. P3,

(d) - Histopathological Examination of LAA occlude Animal no. P3

## Results

The radiographic thrombogenicity evaluation of the WALL-NUT™ LAA occluder device revealed no device-related thrombogenicity or embolization in the three animals tested. Throughout the follow-up period, no adverse events, thrombus formation, pericardial effusion, or device dislocation were observed during transesophageal echocardiography (TEE) imaging. The animals showed no signs of morbidity or early death, and no abnormal clinical signs were noted throughout the study. Additionally, there was no significant weight loss in any of the animals, and hematology and clinical chemistry tests revealed no differences or abnormalities between pre- and post-implantation results.

Pathological evaluations, both gross and microscopic, did not reveal any external or internal lesions of pathological significance. Histopathological analysis showed the presence of inflammatory cells in all three animals, with varying degrees of endothelialization of the occluder surface. Inflammatory cells were noted around the implant site, with a break in the endocardium and minimal fibrin deposition in some animals.

Endothelialization progressed over time, with 50-75% coverage in two animals (P1 and P2) and complete coverage

in the third (P3).

## Discussion

The findings from this study indicate that the WALL-NUT™ LAA occluder device demonstrates excellent biocompatibility and safety. The absence of thrombogenic events or device-related complications suggests that the material and design of the device effectively minimize the risk of thrombus formation. The use of transesophageal echocardiography (TEE) for real-time monitoring further supports the reliability of the evaluation process, as it allowed for the identification of potential issues such as thrombus formation or device migration.

Histopathological evaluations revealed that the inflammatory response surrounding the device was consistent across all animals but remained mild and did not escalate over time. The presence of inflammatory cells suggests an appropriate tissue response to the implant, indicating that the body is recognizing the device while simultaneously promoting healing and tissue integration. The gradual endothelialization observed indicates that the device is well-tolerated, allowing for the development of a protective endothelial layer, which is crucial for long-term functionality and reduced risk of thrombosis.

The study's timeline illustrates a clear pattern of tissue ingrowth and integration, which is essential for the success of LAA occlusion procedures. The progression from initial inflammatory response to eventual complete endothelial coverage of the device demonstrates the dynamic healing process following implantation. Notably, the complete endothelialization achieved by 90 days post-implantation indicates a favorable environment for long-term stability of the device. The tissue response characterized by a thin layer of connective tissue and the presence of immune cells in the center of the occluder highlights the ongoing interaction between the implant and host tissues.

Despite the mild inflammatory response observed, the overall biocompatibility of the WALL-NUT™ LAA occluder device suggests that any potential risks associated with the implant are minimal. This low level of chronic inflammation, paired with the effective integration of the device into the surrounding tissue, supports the hypothesis that the WALL-NUT™ LAA occluder can be safely used in clinical settings.

## Conclusion

In conclusion, the WALL-NUT™ LAA occluder device exhibits excellent safety and biocompatibility as evidenced by the absence of significant thrombotic events and the favorable tissue response observed in the animal model. The study confirmed that the device integrates effectively into the left atrial appendage over time, with progressive endothelialization providing a protective barrier against potential thrombus formation. Furthermore, the stable and mild inflammatory response suggests that the device does not elicit an adverse reaction from surrounding tissues, minimizing the risk of complications associated with long-term implantation.

These findings indicate that the WALL-NUT™ LAA occluder device has a promising profile for clinical use, particularly in patients at risk for stroke due to atrial fibrillation. The successful tissue integration observed in this study may lead to improved outcomes in patients requiring left atrial appendage occlusion. Future studies, including longer follow-up durations and evaluations in larger animal models, would provide further insights into the long-term performance and safety of this device in clinical applications. Overall, the results of this study

support the continued development and potential clinical application of the WALL-NUT™ LAA occluder device in managing patients at risk for thromboembolic events.

## REFERENCES

1. Organization for Economic Co-operation and Development - Principles of GLP and Compliance Monitoring (as revised in 1997) ENV/MC/CHEM (98) 17.
2. Guidance Document on the Recognition, Assessment and Use of Clinical signs as humane endpoints for Experimental Animals used in Safety Evaluation. ENV/JM/MONO (2000)7. OECD, December, 2000.
3. Compendium of CPCSEA 2018; Guidelines for Laboratory Animal Facility 2015; 7: 61 – 96.
4. Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources. Commission on Life Sciences. National Research Council. National Academy Press. Washington, D.C. 1996.
5. Implants for surgery – Active implantable medical devices – Part 5: Circulatory support devices. (ISO 14708-5:2020).
6. CFR - Code of Federal Regulations Title 21- Part 58: Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58) 2019.
7. Kornowski et al (1998). In-Device Restenosis: Contributions of Inflammatory Responses and Arterial Injury to Neointimal Hyperplasia. *J Am Coll Cardiol.* 1: 224-230.
8. Schwartz et al (1992). Restenosis and the Proportional Neointimal Response to Coronary Artery Injury: Results in a Porcine Model *JACC* 19-2: 267-74.
9. Yazdani et al (2014). Vascular, Downstream, and Pharmacokinetic Responses to Treatment with a Low Dose Drug-Coated Balloon in a Swine Femoral Artery Model. *Catheterization and Cardiovascular Interventions.* 83 (1): 132-140.

