Animal experimental implantation of an atrial septal defect occluder system

A Bloch Thomsen, M Schneider, U Baandrup, E V Stenbøg, J M Hasenkam, J P Bagger, G Hausdorf

Abstract

***Objective*—To establish the implantation technique for the atrial septal defect occluder system (ASDOS) device in an experimental animal model and to determine long term mechanical stability of the device and its in vivo properties in terms of biocompatibility and tissue reaction.**

***Materials and methods*—An atrial septal defect was created and the device implanted in 17 pigs (mean weight 30 kg). The implantation technique was refined and modified because of initial technical and anatomical complications during nine acute pilot studies. The technique proved to be feasible in eight subsequent survival studies. Four pigs were electively killed three months after implantation (group 1). The remaining four pigs were killed six months after implantation (group 2).**

***Results*—Necropsy showed all devices were embedded in soft tissue three months after implantation. Microscopic examination of atrial septal tissue showed an acute granulomatous inflammatory reaction in group 1 and fibrosis in group 2. The intensity of the inflammatory reaction around the device was clearly milder in group 2, indicating a decline in the inflammatory response with time. Clinical and biochemical investigations indicated acceptable biocompatibility of the device.**

***Conclusion*—The implantation technique for the ASDOS device in a chronic pig model has been established. Biocompatibility of the device was acceptable.**

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Keywords: congenital heart disease; atrial septal defect; catheter technique; occluder device

Institute of Experimental Clinical Research, Department of Cardiothoracic and Vascular Surgery and Department of Cardiology, Skejby Sygehus, Aarhus University Hospital, Denmark
A Bloch Thomsen
U Baandrup
E V Stenbøg
J M Hasenkam
J P Bagger

Department of Pediatric Cardiology, Charitè Hospital, Humboldt Universität, Berlin, Germany
M Schneider
G Hausdorf

Correspondence to:
Dr A Bloch Thomsen, Department of Research in Cardiothoracic and Vascular Surgery, Skejby Sygehus, DK-8200 Aarhus N, Denmark.

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The atrial septal defect occlusion system (ASDOS) (Sulzer, Osypka; Rheinfelden, Germany) differs from other devices for closure of atrial septal defect (ASD).[1–6] The system consists of two individual umbrellas which are inserted separately and connected within the heart by a screwing mechanism to achieve a safe and firm connection. A guidewire circuit is created for implantation of the ASDOS device, which theoretically implies a safe implantation method with minimised risk of device embolisation and increased accuracy during implantation. Furthermore, it allows repeated repositioning of the device from both sides of the atrial septum until an adequate position is achieved. However, the implantation procedure of the ASDOS device is technically more complicated than implantation techniques of other devices. An experimental animal model was designed to establish the implantation procedure, evaluate potential complications of the technique, and to determine long term mechanical stability of the device and its in vivo properties in terms of biocompatibility and tissue reaction.

Materials and methods

PROTOCOL

Experiments were performed in 18 pigs (mixed Danish landrace and Yorkshire) of both sexes, weight range 28–33 kg. The pigs were sedated by an initial dose of azeperonum 40 mg/10 kg and midazolam 5 mg/10 kg. Intravenous access was obtained through an ear vein, and ketamine 150–200 mg was given to enable endotracheal intubation. During intervention, the pigs were intravenously infused with fentanyl 7.7 mg/kg/h, midazolam 0.2 mg/kg/h, and ketamine 5.2 mg/kg/h and ventilated on a Servo 900 ventilator. The experiments were performed under sterile conditions.

Cardiac catheterisation was performed through a 6 F sheath inserted in the femoral artery, and a 7 F sheath inserted in the femoral vein exposed by a cut down in the right groin just distal to the inguinal ligament. An ASD was created by transseptal puncture using Mullin's technique (Mullin's sheath 7 F; Bard, Galway, Ireland) followed by dilatation of the septal defect using a 6 F Opta (Cordis, Roden, the Netherlands) balloon catheter, inflated with diluted *x* ray contrast to a balloon diameter of 8–10 mm. The inflation pressure was 6–8 bar. Cineangiograms were obtained by injections of contrast material in the left atrium to verify the presence of a septal defect.

One dose of heparin 250 IU/kg was given intravenously after transseptal puncture. During survival experiments, ampicillin 1 g was given intravenously before and after operation, and prophylactic ampicillin 1 g was administered intramuscularly for the first six postoperative days.

Monitoring included continuous electrocardiography (ECG), intermittent arterial pressure measurement, rectal temperature, and blood gas analysis when required. Transoesophageal echocardiography was performed in the first studies, but proved to be of little value, as the porcine heart is dextroversed, and an extra lobe of lung tissue is interpositioned between the atria and the oesophagus, thereby impeding ultrasonic visualisation of the atria.

The ASD was occluded immediately in 17 pigs: a device was not implanted in one pig

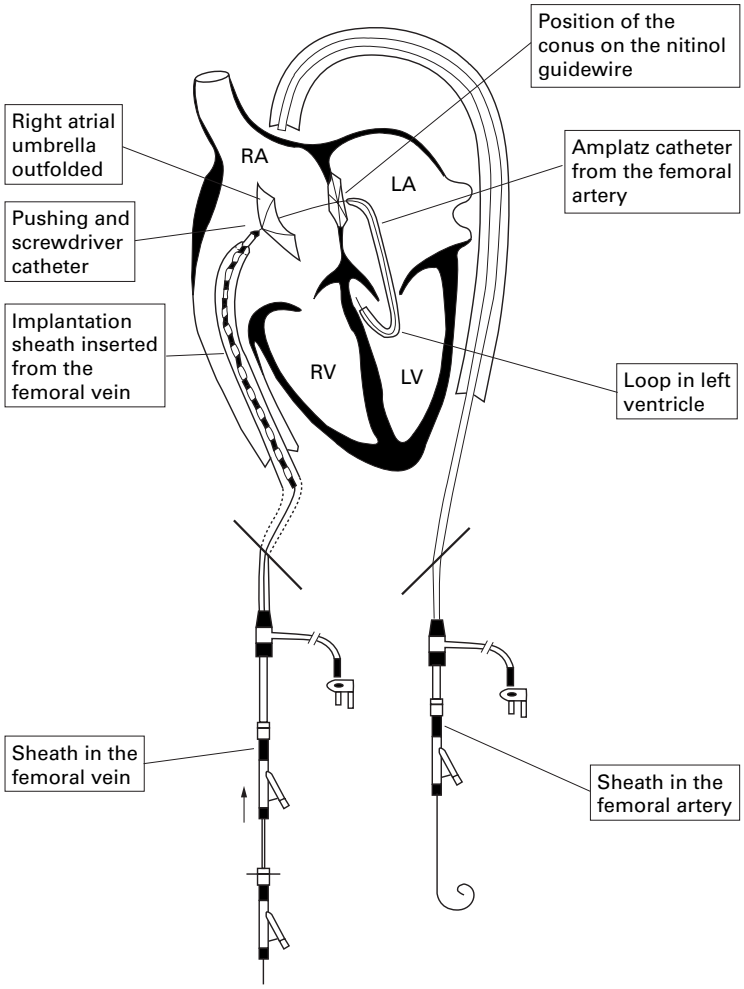

Figure 1 Implantation procedure. The guidewire circuit has been created and the distal (left atrial) umbrella is in place. The proximal (right atrial) umbrella is pushed towards the right side of the atrial septum. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

(control pig). The diameter of the implanted device varied from 30 mm to 45 mm.

ETHICS

The experiments were approved by the Danish Ethical Committee for Animal Experiments, Institute of Experimental Clinical Research, Skejby at the Sygehus, Aarhus University Hospital, Denmark.

PROCEDURE

The established implantation procedure for clinical use of the ASDOS device has recently been published.[7] A brief description is given here (fig 1). A nitinol guidewire circuit was established from the femoral vein through the ASD to the left ventricle and antegrade to the femoral artery. Both umbrellas were implanted through an 11 F long sheath in the femoral vein. A conus on the nitinol guidewire prevented the distal umbrella from embolisation and enabled retraction of the distal umbrella against the left side of the atrial septum. The umbrellas were connected by rotating the screwdriver catheter outside the sheath in the femoral vein (fig 1).

Angiography of the left atrium was performed before withdrawing the nitinol guidewire from the sheath in the femoral artery, using the guidewire loop through the device as a safety precaution.

Acute study

Nine acute experiments were performed to obtain experience of the implantation procedure, elucidate potential complications, and to establish the technique for device retrieval.

Chronic study

Chronic implantation of the device was performed in eight pigs. Four animals were electively killed three months after implantation (group 1): the remaining pigs were killed six months after implantation (group 2).

During follow up all pigs were observed by personnel trained to detect abnormal behaviour, signs of pain, dyspnoea, or infection. Haemolysis was assessed by measurement of β haemoglobin, plasma haemoglobin, haptoglobin, and lactate dehydrogenase before implantation, and three and six months after implantation.

A sternotomy was carried out at the end of the follow up period and epicardial cross sectional echocardiography of the beating heart was performed, to disclose the position of the device. Colour Doppler investigation was used to evaluate regurgitation of the mitral and tricuspid valves and interatrial shunting.

The animals were killed by exsanguination and the spleen, liver, lungs, and kidneys were examined for emboli. The heart, a section of the spleen, the liver, and kidneys were removed for further pathological examination. The hearts were fixed in buffered formaldehyde for microscopic examination. Sections (5 μm) were cut after dehydration and paraffin embedding of the tissue. The following staining and immunohistological reactions of the cardiac tissue were undertaken: haematoxylin and eosin, elastic van Gieson, CD 34, Ulex (endothelian marker), and Gram staining.

Haematoxylin and eosin stained sections of the spleen, liver, and one kidney were examined microscopically.

Control study

Device implantation was not performed in one pig with a septal puncture and dilatation of the septal defect. Epicardial echocardiography was carried out after a follow up period of four months and the pig was killed. The heart was examined macroscopically and microscopically, to assess the impact of transseptal puncture and balloon dilatation on atrial septal tissue.

Results

ACUTE STUDIES

Most initial problems were related to the anatomy of the fossa ovalis, the orientation of the heart—that is, dextroversion compared with human heart anatomy, and the steep angle of the catheterisation route to the heart passing under the femoral ligament (table 1).

Kinking of the loop in the left ventricle was prevented by changing the 7 F wedge catheter, used for creating the loop, to a 6 F coronary

Table 1 Acute studies

Pig number	*Complications*
Pig 1	Kinking of the loops in the LV. Haematoma in the annulus of the mitral valve. Device position too low, ie, located in the IVC
Pig 2	Kinking of the loop. Device caught by Eustachian valve
Pig 3	Pericardial effusion during puncture of the atrial septum. Kinking of the loop. Damage to aortic valve
Pig 4	Died from ventricular fibrillation
Pig 5	Separation of the two umbrellas a few seconds after implantation. Retraction of the right atrial umbrella. Embolisation of the left atrial umbrella to the aortic valve. Corresponding to a weld on the metallic skeleton, a fracture of one arm in this umbrella had occurred. This was the only pig in which we intended to implant a 45 mm oversized device
Pig 6	Pre-existing ASD. Too big for closure with a 35 mm device. Successful retrieval of the device was performed
Pig 7	Pre-existing ASD. Too big for closure with a 35 mm device. Successful retrieval of the device was performed
Pig 8	Laceration of the inguinal ligament and the femoral artery
Pig 9	Laceration of the inguinal ligament and the femoral artery

ASD, Atrial septal defect; IVC, Inferior vena cava; LV, Left ventricle

catheter with a 0.96 mm lumen, which was advanced from the femoral vein over a 0.89 mm guidewire until its tip was positioned in the ascending aorta. The nitinol guidewire was inserted through the coronary catheter and its floppy J tip caught by a 6 F Lasso catheter (Osypka) advanced from the femoral artery, performing capture in the descending aorta without damaging the valve. A 5 F Amplatz catheter (Cordis) was inserted over the nitinol guidewire from the femoral artery immediately after creation of the guidewire circuit. In the descending aorta the tip of the Amplatz catheter and the tip of the 6 F coronary catheter were brought together and held constantly in this "kissing position" covering the nitinol guidewire, to avoid damaging the mitral and aortic valves while pulling the nitinol guidewire from the femoral artery to position its conus in the left atrium.

Retrieval of the device was accomplished by advancing the long sheath and simultaneously pulling the nitinol guidewire and the screwdriver catheter from the venous side, thereby folding the right sided umbrella into the long sheath. Similarly, the left sided umbrella was folded in a reverse fashion into the long sheath by the conus when pulling the nitinol guidewire from the femoral vein.

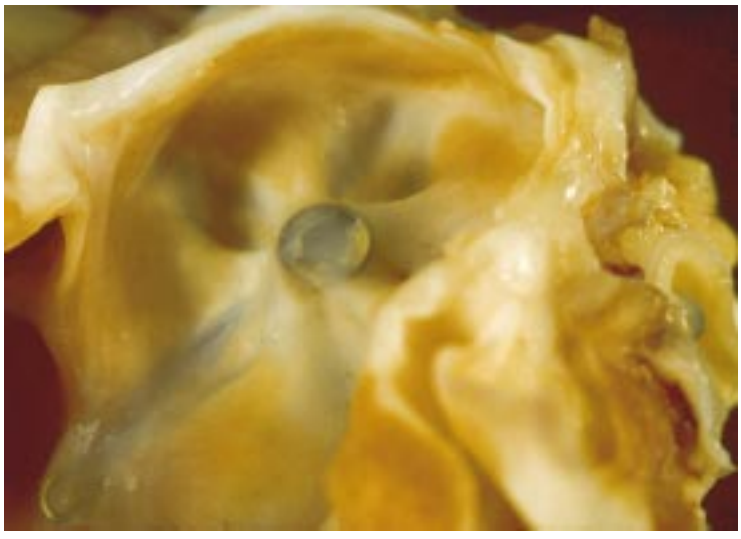

Figure 2 Atrial septum in a pig from group 1 (killed three months after implantation). The device is completely endothelialised and covered by smooth tissue.

SURVIVAL STUDIES

Implantation and follow up

Implantation was performed without complications in eight pigs. All pigs survived follow up. A fever developed in two pigs two months after implantation. Both pigs were treated with ampicillin 1 g and streptocillin 3–4 ml daily for one week: they recovered within three days. Blood cultures were negative, and an explanation for the transient hyperthermia was not found.

Apart from this episode all pigs were fully mobile and had normal appetite. There were no signs of pain, dyspnoea, or abnormal behaviour. Haemolysis was not present in the analysed blood samples.

Electrocardiograms showed sinus rhythm and a normal PQ interval in pigs examined six months after implantation.

Echocardiography

The device was located in the atrial septum in all eight pigs. Therefore, embolisation had not occurred. The device seemed very low in three pigs in group 2, however, interference with adjacent cardiac structures was not detected—that is, integrity of the mitral and tricuspid valve was verified.

MACROSCOPIC EXAMINATION

Global pericardial fibrous adherances were found in one heart. Signs of infection or pericardial effusion were not present in the remaining seven hearts. All hearts were of normal weight, size, and shape. There were no macroscopic signs of embolism or infarction in the spleen, liver, lungs, or kidneys.

Group 1

Macroscopic examination of the hearts showed complete closure of the ASD. The position of each device was correct. All devices were completely covered and embedded in soft tissue (fig 2). The depth of the device locus varied slightly; one device was hardly visible because of the thickness of the covering tissue. Parietal thrombus or fibrinous exudation was not seen, and signs of interference with adjacent structures of the heart or valve damage were not detected. There were no fractures of the metal arms.

Foreign body material corresponding with the intact polyurethane membrane was seen in the myocardial tissue, when cutting through the septum between the nitinol arms.

Group 2

The position of the device was very low in three of the four hearts, as suspected during echocardiographic examination. Additionally, in three of these cases, one or two of the nitinol arms protruded into the atrial chambers and had not been incorporated into the atrial septum. One nitinol arm protruded into the lower right pulmonary vein in one pig, and one nitinol arm protruded into the orifice of the coronary sinus in two pigs. Obstruction of flow, however, was not detected by echocardiography. There were no signs of thrombus

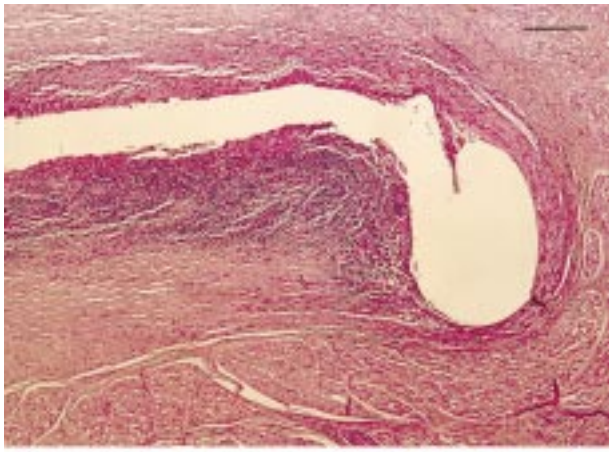

Figure 3 Microphotograph of atrial septal tissue in a pig in group 1 showing a fistula corresponding with the locus of a device nitinol arm. The nitinol arm is surrounded by an acute inflammatory reaction, which decreases with increasing distance from the device. (Bar = 250 μm.)

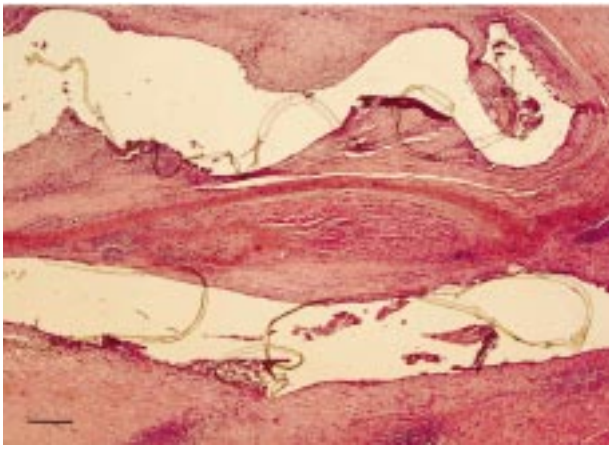

Figure 4 Microphotograph of the atrial septum in a pig in group 2 (killed six months after implantation) showing fistulas corresponding with the loci of two nitinol arms. Fibrosis was present and there was a milder (narrower) inflammatory reaction than in group 1. (Bar = 250 μm.)

formation nor corrosion of the metal surface. The remaining part of the device was completely covered by pale, smooth tissue.

The findings of the last pig in this group were very similar to the findings of all the pigs in group 1. The device position was correct, and the umbrella was completely covered with tissue. Arm fracture had not occurred in any of the cases.

The atrioventricular valves appeared to be intact in all pigs. Intact polyurethane membrane was seen between the nitinol arms.

MICROSCOPIC EXAMINATION

Group 1

Similar characteristics were seen in three of four hearts. Granulation tissue with an acute inflammatory reaction was present around fistulas, corresponding with the locus of a nitinol arm (fig 3). The inflammatory cell reaction diminished with increasing distance from the device and consisted predominantly of histiocytic cells and neutrophils, but a few multinuclear cells were also found.

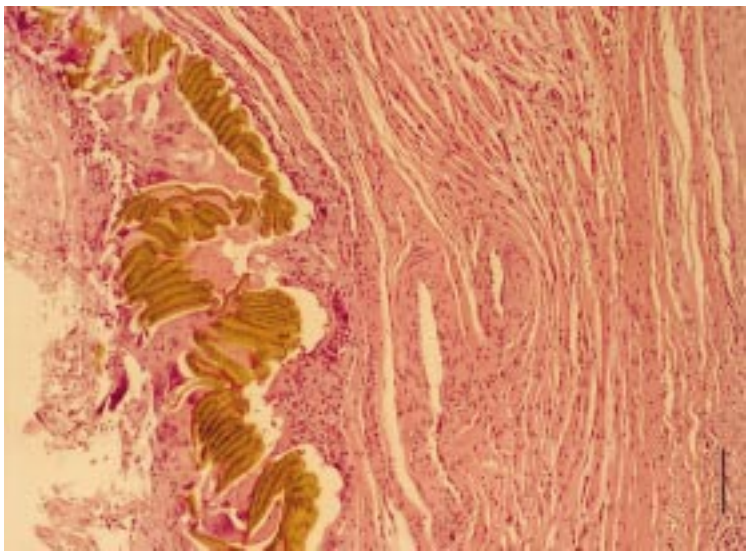

Figure 5 Microphotograph of the atrial septum in a pig in group 2. A few giant cells and very mild fibrosis around the foreign body material (intact polyurethane membrane) are seen. (Bar = 100 μm.)

The inflammatory response was more pronounced in one pig. Fibrin deposits and large numbers of histiocytes, plasma cells, and granulocytes, including eosinophils, were found around the locus of the nitinol arm. Macroscopic signs of pericarditis were present in this pig, as already described, but microorganisms were not seen in Gram stained specimens.

Sections of myocardial tissue adjacent to the polyurethane membrane showed very mild fibrosis in all pigs.

Group 2

The main microscopic finding was fibrosis. The intensity of the inflammatory reaction was greatly diminished compared with that in group 1 (fig 4). All devices were encapsulated in fibrous tissue, and inflammatory cells present were mainly lymphocytes and histiocytes. Tissue adjacent to the polyurethane membrane showed very mild fibrosis and a few giant cells (fig 5). One pig, however, still showed signs of severe inflammation, including eosinophils.

CONTROL STUDY

Epicardial echocardiography and macroscopic examination of the heart in this pig showed closure of the ASD. Minimal fibrosis was detected microscopically but no inflammatory reaction was present in the atrial septum.

Discussion

Transcatheter closure of ASD was first performed more than 20 years ago.[1] For most patients, however, surgery is still the preferred treatment because of complications associated with devices for closure of ASD.[2–6] Common device problems are inaccurate positioning during implantation, separation of the discs, residual shunts, and fracture of the device skeleton. The implantation technique for the ASDOS device seemed theoretically to offer potential advantages by improving the safety and accuracy of implantation, although the feasibility of the method was unknown. Acute pilot studies enabled valuable modification and

refinement of the technique to be achieved. The implantation procedure proved to be feasible in eight chronic animal experimental studies.

The device was considered to be mechanical stabile as fractures and embolisation did not occur during follow up in survival studies. Additionally, ECG and echocardiographic findings, haemolytic variables, and clinical observations concurred with acceptable biocompatibility of the device. Endothelialisation of the device three months after implantation (provided that it was correctly positioned) is in accord with the findings of previously tested devices.[8–10]

The minimal fibrosis detected in the atrial septum of the control pig indicates that the inflammation surrounding the device in the other pigs could not be explained solely on the basis of surgical trauma associated with transseptal puncture and dilatation of the defect. Histological evidence of an acute granulomatous inflammation around the device followed by subsequent development of fibrosis with giant cells corresponding to a typical foreign body reaction in the pigs in groups 1 and 2 was as expected.[11]

There were several potential complications. The pig model was not optimal because rotation is different from that of the human heart and transoesophageal echocardiography for guidance during implantation was not possible. Nevertheless, the disadvantages of the pig model do not exclusively explain the damage to the aortic and mitral valves in two of the pilot studies, and embolisation of one oversized device followed by arm fracture, and the protruding nitinol arms in three surviving pigs.

Arm fracture occurred after embolisation of an oversized device in one pig (table 1). Anatomical position of the device rather than haemodynamic stress is reported to be of greater importance for fracture of the Bard clamshell occluder.[12] In our study, however, the very low position of the device in three pigs in group 2 did not cause arm fracture. The manufacturer of the ASDOS device has found a relation between the angle of the nitinol arms and premature metal fatigue. Therefore, the opening angle of each arm should not exceed more than 180°. The risk of arm fracture must always be considered, particularly, in patients with a thick atrial septum, where the angle between the nitinol arms is bigger. Additionally, implantation of oversized devices should not be performed.

It is not known whether a monoepithelial cell layer covered the protruding nitinol arms in three pigs in group 2 as microscopic examination was not performed, but macroscopic signs of thrombosis or embolism were not seen. Heparin was administered only to pigs during the implantation procedure. Anticoagulation treatment was not given during follow up; however, thromboembolic events were not encountered. This finding implies that the uncovered device and exposed device skeleton are not thrombogenic, but we advocate that the device should not be implanted if the arms of the device are not in line with the atrial septal surface as seen by echocardiography.

Potential complications such as arrhythmias due to fibrosis in the conduction system, or pressure necrosis of the atrial septal tissue between umbrellas were not found.

Significance of the foreign body reaction in all pigs and the continuing acute inflammatory reaction present in two is difficult to evaluate, partly because there are few published studies on this subject. Previous animal studies have often used dogs and sometimes lambs and piglets to investigate biocompatibility.[8–10 13] To our knowledge there is no standard animal model for this purpose. Despite our initial technical problems the pig model seems to be relevant for such investigation, as the porcine cardiovascular system resembles human cardiovascular anatomy more closely than that of dogs and lambs.[14] Additionally, porcine haemostasis variables are characterised by very high activities of most coagulation factors compared with those in humans.[15] Thereby, enhancing the absence of thromboembolic events in this study.

The intensity of the inflammatory reaction around the device was clearly milder in pigs in group 2 than in those in group 1, indicating a decline in foreign body reaction with time.

In conclusion a feasible implantation technique for the ASDOS device has been established in the first experimental in vivo studies. In addition, mechanical stability and clinical impact of the device have been determined. Further studies and critical evaluation of the long term biological effects of implantation are necessary, including comparative pathological investigation with other devices and surgically implanted foreign bodies.

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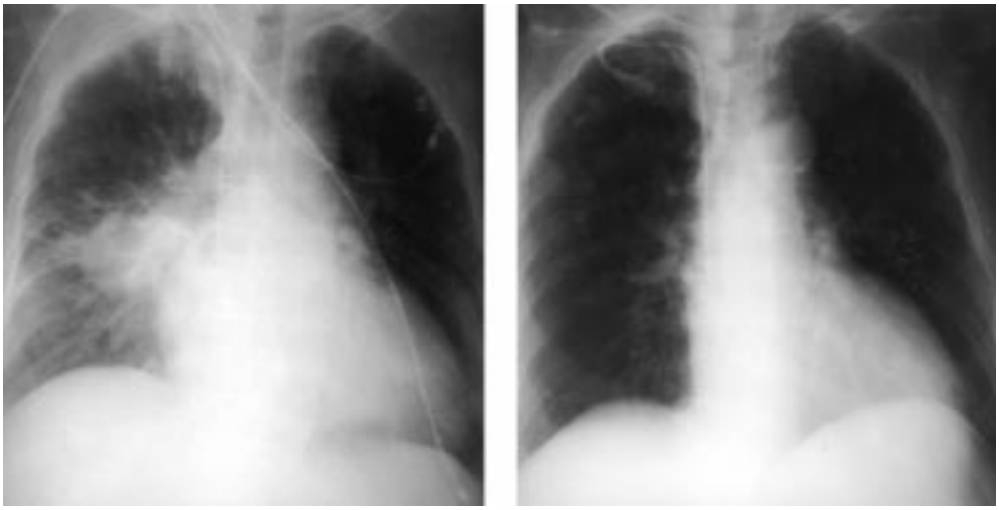
IMAGES IN CARDIOLOGY

Vanishing tumour



A 66 year old woman was admitted to a hospital because of progressive dyspnoea. Chest radiography on admission revealed enlargement of the cardiac silhouette, increased pulmonary vascular marking, and a right hilar tumour-like shadow (left). She was diagnosed with congestive heart failure caused by acute myocardial infarction. The right hilar tumour-like shadow disappeared after treatment for congestive heart failure (right).

MAKOTO KODAMA
YOSHIFUSA AIZAWA

Heart

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