Published in final edited form as:

World J Pediatr Congenit Heart Surg. 2016 July ; 7(4): 475–483. doi:10.1177/2150135116651113.

Pulmonary Valve Replacement With Small Intestine Submucosa-Extracellular Matrix in a Porcine Model

Jacob R. Miller, MD¹, Matthew C. Henn, MD¹, Timothy S. Lancaster, MD¹, Christopher P. Lawrance, MD¹, Richard B. Schuessler, PhD¹, Mark Shepard, CCP², Mark Anderson, CCP², Attila Kovacs, MD³, Robert G. Matheny, MD⁴, Pirooz Eghtesady, MD, PhD², Ralph J. Damiano, MD¹, Umar S. Boston, MD⁵

¹Section of Adult Cardiac Surgery, Barnes-Jewish Hospital, Washington University School of Medicine, St Louis, MO, USA

²Section of Pediatric Cardiothoracic Surgery, St. Louis Children's Hospital, Washington University School of Medicine, St Louis, MO, USA

³Section of Cardiology, Barnes-Jewish Hospital, Washington University School of Medicine, St Louis, MO, USA

⁴CorMatrix Cardiovascular, Inc, Roswell, GA, USA

⁵Section of Pediatric Cardiovascular Surgery, LeBonheur Children's Hospital, University of Tennessee Health Science Center, Memphis, TN, USA

Abstract

Background: Prosthetic materials available for pediatric pulmonary valve replacement (PVR) lack growth potential, inevitably leading to a size mismatch. Small intestine submucosa-derived extracellular matrix (SIS-ECM) has been suggested to possess regenerative properties. We aimed to investigate its function and potential to increase in size as a PVR in a piglet.

Methods: An SIS-ECM trileaflet valved conduit was designed. Hanford minipigs, n = 6 (10–34 kg), underwent PVR with an intended survival of six months, with monthly echocardiograms evaluating valve size and function. The conduit was excised for histologic analysis.

Results: Of the six, one was sacrificed at three months for midterm analysis, and one at month 3 due to endocarditis. The remaining four constituted the study cohort. The piglet weight increased by 186% (19.56 ± 10.22 kg to 56.00 ± 7.87 kg). Conduit size increased by 30% (1.42 ± 0.14 cm to 1.84 ± 0.14 cm; $P < .01$). The native right ventricular outflow tract increased by 43% and the native pulmonary artery by 84%, resulting in a peak gradient increase from 10.08 ± 2.47 mm Hg to 36.25 ± 18.80 mm Hg ($P = .03$). Additionally, all valves developed at least moderate regurgitation. Conduit histology showed advanced remodeling with myofibroblast infiltration, neovascularization, and endothelialization. The leaflets remodeled beginning at the base with the

Corresponding Author: Umar S. Boston, Section of Pediatric Cardiothoracic Surgery, Division of Cardiothoracic Surgery, LeBonheur Children's Hospital, 49 N Dunlap St, Memphis, TN 38111, USA. uboston@uthsc.edu.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

leaflet edge being less cellular. In addition to the known endocarditis, bacterial colonies were discovered within a leaflet in another.

Conclusions: The SIS-ECM valved conduit implanted into a piglet demonstrated cellular infiltration with vascular remodeling and an increase in diameter. Conduit stenosis was a result of slower rates of size increase than native tissue. Suboptimal leaflet performance requires design modifications.

Keywords

congenital heart disease; pulmonary valve; pediatric; biomaterials; animal model

Introduction

Congenital cardiac malformations necessitating valve replacement represent a challenge when selecting the correct prosthetic material. Specifically, for patients with tetralogy of Fallot, pulmonary atresia or truncus arteriosus, the pulmonary valve is either replaced or repaired with a transannular patch reconstruction or a valved right ventricle (RV) to pulmonary artery (PA) conduit. Unfortunately, as the child grows, the initial repair will become undersized and require a pulmonary valve replacement (PVR). Thus, reoperations involving the pulmonary valve are one of the most prevalent valve replacements in congenital heart surgery.^{1,2} Unfortunately, the currently available prosthetic valves utilized in pediatric patients degenerate or become undersized and lead to failure in approximately 80% of pediatric PVRs, with the majority requiring reoperation.³ Further, the patient's future growth potential complicates surgical timing, as ideally the new valve will be implanted once the patient is fully grown, however, data have shown that the valve should be replaced prior to the development of RV dysfunction.¹ Earlier valve replacement requires the use of a smaller prosthetic and increases the likelihood of an additional operation becoming necessary.

Given this, the ideal prosthetic valve would remodel and increase in size with the patient, as well as possess good hemodynamic performance, be durable, nonimmunogenic, nonthrombogenic, resist infection, accept suture, and be readily available. Porcine small intestine submucosa-derived extracellular matrix (SIS-ECM), composed of type I collagen, fibronectin, laminin, and glycosaminoglycans, possesses many of these attributes.⁴ It has been shown to remodel,⁵⁻⁹ endothelialize quickly,^{8,9} release growth factors as it degrades,¹⁰⁻¹² resist infection,¹³⁻¹⁶ and release chemoattractants for undifferentiated cells.^{17,18} When used as a superior vena cava interposition graft, it was shown to substantially increase in size.¹⁹ Further, it performed well as a valve in the tricuspid position out to eight months in an ovine model with the potential to increase in diameter similar to native valves.²⁰ For that reason, SIS-ECM has been utilized in adult and pediatric cardiac surgery, often as a patch but as a valve in some circumstances.²¹⁻²⁶ There is little published data on the regenerative remodeling potential of this material as a valved conduit. As such, we aimed to investigate the short-term function and potential of an SIS-ECM valved conduit to increase in size in the pulmonary position of young piglets.

Materials and Methods

A total of six immature female Hanford minipigs with an average weight of 17.97 ± 8.51 kg (range: 10-34 kg) and age 15.17 ± 4.96 weeks (range: 10-24 weeks) underwent successful PVR with an SIS-ECM valved conduit. The porcine model was chosen due to the growth similarities of their cardiovascular system from birth to four months with that of the human in their midteens.²⁷ Two additional adult pigs were utilized as blood donors for cardiopulmonary bypass (CPB) prime. One animal which underwent PVR was sacrificed at three months postoperatively to analyze intermediate tissue effects. The remaining animals were survived for six months. Echocardiograms were performed intraoperatively and postoperatively at two weeks, one month, and then monthly to analyze valve function and size. While killing, the valve was excised for histologic analysis. This study was approved by the Washington University in St. Louis animal studies committee in compliance with the 1996 "Guide for the Care and Use of Laboratory Animals," with priority given to each animal's overall comfort.

The SIS-ECM Valve Production

The SIS-ECM valved conduit was manufactured as previously described.²⁸ Briefly, production occurred in four main steps: (1) mechanical delamination of the mucosa and muscularis, (2) decellularization using paracetic acid to remove any remaining antigenic epitopes, (3) sterilization utilizing ethylene oxide to allow for a lower temperature to prevent protein cross-linking, preserving the three-dimensional structure, and (4) fashioning of a sheet of SIS-ECM into a trileaflet valved conduit with dimensions as previously described.²⁹ The diameter of the valved conduits were produced in two millimeter increments (10-20 mm), with the size chosen intraoperatively to most closely match the size of the diameter of the native pulmonary valve annulus. Initially, the valve was fashioned such that both the conduit and the valve leaflets were two-ply. Subsequently, it was fashioned to have a two-ply conduit with a one-ply leaflet as demonstrated in Figure 1 and previously described.²⁴ Additionally, the leaflets were elongated to allow for prolonged leaflet coaptation, as our initial experience indicated that the conduit may increase in diameter prior to the leaflets increasing in length causing the conduit insufficiency. All valves performed similarly and were therefore analyzed together.

Valve Implantation

The day prior to the PVR, 750-1,000 mL of blood were collected from an adult donor pig via femoral vein, which was stored on a rocker in a cold room overnight. One hour prior to the operation, the piglet was premedicated with solumedrol (10 mg/kg), buprenorphine (0.1 mg/kg), and carprofen (4 mg/kg) via intramuscular (IM) injection. Piglets were induced with IM midazolam (0.5 mg/kg) and ketamine (33 mg/kg), isoflurane (2%-5%) was used for maintenance. Cefazolin (25 mg/kg) was given intravenously (IV). A continuous fentanyl (20 mg/kg) IV infusion was added prior to incision.

A left thoracotomy was performed via the fourth intercostal space. A catheter was placed in the innominate artery for continuous blood pressure monitoring and for serial arterial blood gas measurements. The pericardium was incised. Purse-string 5-0 polypropylene sutures

were placed in the aortic arch and right atrial appendage. Heparin (600 U/kg) was administered, and once the activated clotting time (ACT) was greater than 400 seconds, the aorta and right atrium were cannulated and CPB initiated keeping the piglet at 36°C. While on CPB, the ACT was checked every 30 minutes and heparin was redosed as necessary.

Cardiopulmonary bypass consisted of a Sorin SIII roller pump (Sorin Group USA, Inc, Arvada, Colorado) utilizing a CAPIOX RX05 oxygenator (Terumo Cardiovascular Systems Co, Ann Arbor, Michigan) and an air/oxygen gas mixer (Sechrist Industries, Inc, Anaheim, California). An initial flow rate of 80 to 120 mL/kg/min was obtained and adjusted to maintain a mean arterial pressure of 35 to 50 mm Hg. A Hemocor HPH 400 hemoconcentrator (Medivators, Inc, Minneapolis, Minnesota) provided modified ultrafiltration at the end of CPB.

Once on CPB, the proximal main PA was divided approximately 1 cm from the pulmonary valve annulus. The proximal PA and valve leaflets were then completely excised down to the level of the right ventricle. The SIS-ECM valved conduit was then rehydrated for five minutes in normal saline. The proximal anastomosis was performed with running 5-0 polydioxanone suture (PDS) on the posterior wall and interrupted 5-0 PDS on the anterior wall. The distal anastomosis was performed in a similar fashion with 6-0 PDS (Figure 2). Prior to the last interrupted suture being placed, CPB flow was decreased to de-air the conduit and check for suture line leaks. As CPB was weaned, amiodarone (2.5-5 mg/kg) was administered. Following separation from CPB, protamine (1 mg/100 U heparin) was given and redosed until the ACT was corrected. Prior to closure, an epicardial echocardiogram was performed to confirm valve function.

The piglet was extubated and transported to an isolette with precise oxygen and temperature control for two to four hours. Furosemide (1 mg/kg) was given IV every six hours for four doses with the initial dose just after coming off bypass. The pig was given full access to food and water once out of the isolette. Postoperatively, pain was controlled initially with buprenorphine (0.1 mg/kg IV or IM every six hours) and carprofen (5 mg/kg by mouth every eight hours), which were weaned until no longer necessary. Antiplatelet therapy was initiated on postoperative day 1 with aspirin (5 mg/kg) and continued until killing.

Echocardiogram

For each echocardiogram, induction was achieved with either ketamine (20 mg/kg) or TKX (telazol [4.4 mg/kg], ketamine [2.5 mg/kg], and xylazine [2.2 mg/kg]) and maintenance using isoflurane (2%-5%). All echocardiograms were performed by the same staff cardiologist (A.K.) who has expertise in porcine echocardiograms. Echocardiograms were performed postoperatively at two weeks, one month, and then monthly utilizing a Siemens Acuson Sequoia C512 echocardiography system. Transthoracic echocardiograms (TTEs) were performed at all time points. Transesophageal echocardiograms were also performed at four months and beyond, as TTEs became insufficient for evaluating valve function as the animal increased in size. For consistency, all size measurements were obtained via TTEs and compared to the TTEs obtained at two weeks postoperatively. The conduit was measured in three areas, the proximal anastomosis (proximal), mid-conduit above the valve leaflets (middle), and at the distal anastomosis (distal). Leaflet lengths were unable to be reliably

measured by echocardiogram, therefore, these data were excluded. Additionally, the right ventricular outflow tract (RVOT) just below the proximal anastomosis and PA distal to the conduit were measured and the RV was assessed for size and function.

Histology

After the valve had been in place for six months, the animal was sacrificed and the valve excised and stored in 10% formalin. Specimens were evaluated for cellularity, loss of ECM structure, inflammation, endothelialization, and neovascularization. Analysis was performed by an independent pathologist (Alizée Pathology, LLC, Thurmont, Maryland). Stains performed included hematoxylin and eosin, trichrome, von Willebrand factor, Von Kossa, and Vimentin.

Statistical Analysis

Continuous variables were reported as mean $\pm$ standard deviation and were compared with a Student *t* test. Categorical variables were compared with a Fisher exact test. A univariate logistic regression analysis was performed comparing valve size over time. A *P* value $< .05$ was considered statistically significant.

Results

Pulmonary valve replacement was performed successfully on six piglets. One was sacrificed at three months as planned, one developed endocarditis after being bitten on the tail by another animal and had to be sacrificed early, and the remaining four were sacrificed as planned at six months. These four composed the group for echocardiographic analysis.

Valve Size

Echocardiograms showed a progressive increase in valve size at all three points measured: proximal, middle, and distal. The mean initial proximal measurement was 1.25 ± 0.17 cm and increased to 1.88 ± 0.16 cm, an increase of 50% ($P = .002$). The middle measurement increased from 1.42 ± 0.14 cm to 1.84 ± 0.14 cm, an increase of 30% ($P = .005$; Table 1, Figure 3). The distal measurement increased from 1.31 ± 0.17 cm to 1.93 ± 0.15 cm, an increase of 47% ($P = .002$). Univariate logistic regression analysis confirmed a significant increase in size over time ($P < .001$). Despite this increase in size, each of the four valves developed significant stenosis as evidenced by an increase in mean gradient from 4.95 ± 0.81 mm Hg to 19.50 ± 12.23 mm Hg and peak gradient from 10.08 ± 2.47 mm Hg to 36.25 ± 18.80 mm Hg (Figure 4). The increase in gradient correlated with the more rapid growth of the surrounding native structures than the valved conduit, as the native RVOT increased in size from 1.30 ± 0.15 cm to 1.86 ± 0.10 cm, a 43% increase, and the native PA increased in size from 1.46 ± 0.23 cm to 2.69 ± 0.25 cm, an 84% increase (Figure 5).

Valve Function

The intraoperative epicardial echocardiogram showed good valve position and function for each animal. All valves implanted had trace or trace to mild regurgitation with leaflets that coapted well, usually mid-leaflet due to leaflet redundancy. Although initially the leaflets functioned well, each eventually developed at least moderate regurgitation that progressively

worsened until killing (Table 1). This regurgitation was secondary to poor leaflet coaptation, with leaflets often becoming flail. Despite the development of regurgitation and stenosis, no pigs developed right ventricular hypertrophy, dysfunction, or dilation, likely due to the gradual development and limited duration.

Complications

One animal, 3.5 months post-PVR, developed endocarditis after being bitten on the tail by another animal. Four days later, the animal developed purplish discoloration of her snout and ears. An echocardiogram demonstrated endocarditis. Blood cultures confirmed the bacteria, *Streptococcus dysgalactiae*, a component of the porcine oral flora. Due to the severity of the endocarditis and the resulting valve dysfunction, the animal was sacrificed. Only one other animal developed a postoperative complication, cardiac tamponade on postoperative day 5. Her activity level progressively decreased and she developed discoloration of her snout and ears. Echocardiogram showed tamponade and a pericardial drain was placed. This was left in place and drained every eight hours until the output was minimal, approximately two days. Her aspirin was discontinued for five days during this episode. No additional pigs had any clinical signs or symptoms of compromised heart function.

Killing: Gross Inspection

At explant, there was adhesion formation in all mediastinal structures including the SIS-ECM valved conduit. All conduits showed a significant size mismatch with the main PA and the RVOT. Internally, the conduits incorporated well at both the proximal and distal anastomoses, with the distinction between ECM and native tissue being impossible with the exception of remaining suture lines. On inspection of leaflets, they appeared redundant and some of the leaflets had fused to themselves. All leaflets were freely mobile without any leaflets being fused together or to the conduit. One leaflet did have a firm nodule present that was later confirmed to be a nodule of heterotopic calcification.

Histology

At the three-month period, there was a generalized cellular ingrowth of fibroblasts and complete endothelialization of the conduit with a small amount of neovascularization. The leaflets showed only minimal remodeling occurring at the base progressing outward from their attachment to the conduit (Figure 6). Endothelialization followed a similar pattern involving only the base of the cusp without neovascularization. Inflammation was limited to rare lymphocytes at the leading edge of tissue ingrowth at the base of the leaflets.

By 6 months, the conduit showed extensive advanced remodeling and tissue integration throughout the conduit forming a fibrocellular to fibromuscular wall (Figure 7A). This was also present in the cusps, though incomplete, initiating from the base of the cusp and progressing variable distances (Figure 7B). Endothelialization mimicked cellular remodeling, involving the entire conduit (Figure 8A) and progressing variably along the cusps (Figure 8B). Inflammatory cells were rare and limited to lymphocytes.

There were additional unexpected findings. These included the development of a chondro-osseous nodule, metaplastic bone, at the tip of the free edge of a cusp in one animal. Another

valve, from an animal who had not shown signs of infection (#2), was found to have numerous bacterial colonies. Lastly, there were areas of thinning of the conduit (Figure 9). Although generally the conduit was approximately 2 to 2.5 mm in thickness, the conduit at the infundibulum was thinned in nearly all specimens to 0.5 to 1.0 mm, even in the three-month specimen. However, there was no aneurysm formation or evidence of rupture.

Comment

The Achilles heel of many congenital cardiac operations is the use of valves that lack growth potential. Developing a valve from a material with regenerative properties has the potential for growth and indefinite durability. This is one of the first survival piglet studies evaluating the potential of SIS-ECM as a valved conduit to increase in size when implanted in the pulmonary position, with mixed results. Increase in conduit diameter was clearly demonstrated, confirming the findings of previous studies.^{19,20} However, the increase in conduit dimensions was not commensurate to the rate of growth of the adjacent native tissue or to the pig's somatic growth resulting in significant gradients. Additionally, valve function was poor with the consistent development of regurgitant flow. The histological analysis was promising, revealing a cellular infiltrate with remodeling and endothelialization. There were, however, some unanticipated findings including thinning of the infundibulum and the infiltration of bacterial colonies in two animals, despite previous reports of antibacterial properties of SIS-ECM.

Zafar et al recently showed that an SIS-ECM valve in the tricuspid position functioned well over 8 months in an ovine model. Though our study confirmed some of their findings, including the incorporation with surrounding native tissue, paucity of inflammatory cell infiltrate, lack of calcification, complete endothelialization, and the increase in host cellularity progressing from the annulus to leaflet tip, our data did differ in some important areas.²⁰ Our data indicate that this valve did not function well in the pulmonary position, with the eventual development of regurgitation. The primary factor leading to regurgitation was most likely related to leaflet design. Leaflets were created by simply folding the SIS-ECM material into the lumen of the conduit and, therefore, did not have the anatomy or complex geometry of a true semilunar valve. The mode of failure was often a flailed leaflet resulting from the mechanical stress encountered during each cardiac cycle. Furthermore, the lack of remodeling of the ECM leaflet could have been due to these abnormal mechanical stresses experienced by the leaflets. In addition, since the ECM leaflets were invaginated foldings onto the conduit, there was minimal contact with surrounding tissue leading to potentially slower migration of native stem cells to the ECM leaflets.

The results of our study are consistent with recent studies of SIS-ECM use in humans. Although it has only undergone preliminary investigation in humans, some concerns have been raised.^{21,22,26} Specifically, its use in semilunar valve reconstruction has led to a high failure rate, a significant number of which require reoperation. Our data confirm these findings, demonstrating that the material does not possess the structural integrity to maintain its form without being secured on all sides.

Interestingly, in our small study susceptibility to leaflet infection was evident. This material has previously been shown to resist infection. Accordingly, it has been utilized in human patients with infective endocarditis.³⁰ Though the number of implants in our study was small, and the risk of infection was high (ie, animals with trimmed hooves walking near feces, a tail bite leading to the exposure to oral flora in addition to the turbulent flow associated with both stenosis and regurgitation), the material showed a susceptibility to infection not previously demonstrated. Previous research has shown that infection during the acute postoperative period is unlikely due to the antibacterial properties of the ECM breakdown products.¹³⁻¹⁵ The duration of antibacterial peptide release with actual implantation, however, is unknown. Therefore, these animals likely became colonized after significant remodeling had occurred and the bulk of the breakdown products had been exhausted. In our study, one animal, which was bitten, developed endocarditis three months after valve implantation, after significant remodeling had already occurred. The second animal, harboring an unexpected bacterial infection, likely developed this subclinical infection even later.

As has been previously reported, the SIS-ECM did not calcify in our study, with one exception. There was the notable development of a chondro-osseous nodule. Metaplastic bone formation on a cardiac valve is a known phenomenon, occurring in up to 10% of excised stenotic valves,³¹ typically developing with valvular stenosis and calcification.

Limitations

Our study had several important limitations. Most significantly, our study was small, impacting the statistical analysis. Valve measurements were made using echocardiogram, a modality with known user variability. Additionally, our study lacked a control group. To limit the impact of these limitations, the echocardiograms were performed and read by a single cardiologist with expertise in animal echocardiography; and despite the small sample, the dramatic changes in size allows for confidence in the statistical analysis. Lastly, our study demonstrates the utilization of a porcine-derived SIS-ECM in a porcine model. The lack of a gal epitope in human participants would likely significantly impact the inflammatory response. Therefore, extrapolating the lack of inflammation induced by this valve when used in pigs to its use in humans may not be accurate.

Conclusion

The SIS-ECM valved conduit placed as an allograft in a piglet model did increase in size and demonstrated histologic evidence of remodeling. Valvular dysfunction was significant and could be attributed to flawed design and outmatched growth of the surrounding native tissues. Further studies of SIS-ECM as a bioscaffold and improved valve designs with this material are warranted.

Acknowledgments

The authors would like to acknowledge Diane Toeniskoetter and Naomi Still for their diligent and compassionate animal care.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by CorMatrix Cardiovascular, Inc, Roswell, GA, with the authors maintaining full control of the design of the study, methods used, outcome parameters, analysis of data and production of the written report. Robert Matheny reports consulting fees and equity ownership in CorMatrix. Specifically, CorMatrix Cardiovascular, Inc, Roswell, GA, funded the study itself. NIH funding (T32 HL007776) was utilized to pay the salaries of the research fellows involved in the study, including: Jacob Miller, Matthew Henn, Timothy Lancaster and Christopher Lawrance. The authors have no additional relevant conflicts of interest to disclose.

Abbreviations and Acronyms

ACT	activated clotting time
CPB	cardiopulmonary bypass
IM	intramuscular
IV	intravenously
PA	pulmonary artery
PDS	polydioxanone suture
PVR	pulmonary valve replacement
RV	right ventricle
RVOT	right ventricular outflow tract
SIS-ECM	small intestine submucosa–derived extracellular matrix
TTE	transthoracic echocardiograms

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Figure 1.

Small intestine submucosa–derived extracellular matrix (SIS-ECM) valved conduit construction, as described by Gilbert et al.²⁴ In the first image, L is the length of the patch which becomes the circumference of the valved conduit, W is the width of the patch, C is the length of the sewing cuff, and D is the distance between which the patch is folded to create a two-ply conduit. In the second image, D is one-third of the length and marks both the points of fixation of the two layers and the location where one layer of the conduit is cut to form each leaflet.

Figure 2. Small intestine submucosa–derived extracellular matrix (SIS-ECM) valved conduit after pulmonary valve replacement with a size closely approximating the native pulmonary artery (PA) and right ventricular outflow tract (RVOT).

Figure 3. Mid small intestine submucosa–derived extracellular matrix (SIS-ECM) conduit size, measured in the middle, at each month after implantation.

Figure 4.

Average peak gradient and regurgitation based on month since implant. Regurgitation graphed as none = 0; trace = 1; mild = 2; moderate = 3; severe = 4. When listed as between two values, such as “mild to moderate,” the higher value was used.

Figure 5.

Average conduit dimensions of the small intestine submucosa-derived extracellular matrix (SIS-ECM) valved conduit (ECM conduit) compared with the growth of native pulmonary artery (PA) and right ventricular outflow tract (RVOT) and the piglet weight. The weight increase is also shown.

Figure 6.

Vimentin stain, small intestine submucosa-derived extracellular matrix (SIS-ECM) at three months postimplantation. Single arrows denote the mesenchymal cell remodeling occurring within the conduit and the base of the leaflet. Double arrows span the acellular leaflet.

Figure 7.

Vimentin stain, small intestine submucosa-derived extracellular matrix (SIS-ECM) at six months postimplantation. A, Dense cellular infiltrate was present throughout the conduit denoted by clear arrows. B, The leaflet also showed diffuse cellular infiltrate starting at the leaflet base.

Figure 8.

A, von Willebrand factor stain of the conduit with the clear arrows illustrating complete endothelial coverage with early formation of vaso vasorum denoted by the clear arrowheads. B, von Willebrand factor stain of a leaflet with the clear arrows illustrating complete endothelial coverage with early formation of vaso vasorum denoted by the clear arrowheads.

Figure 9.

A specimen with pronounced conduit thickness variability. The arrow is the native pulmonary artery, the arrow head is a suture, the double arrows are different portions of the conduit with variability in thickness.

Table 1.

Echocardiographic Findings Post Conduit Placement.

Pig	Initial diameter^a	Final diameter^a	Final peak gradient (mm Hg)	Month developed moderate regurgitation
#1	1.24	1.99	35	1
#2 ^b	1.53	1.89	60	6
#3	1.56	1.65	36	4
#4	1.41	1.84	14	4

^aIn centimeters measured at the midpoint of the conduit across the valve.^bFound to have subclinical endocarditis at the time of histologic analysis.