A novel restorative pulmonary valved conduit in a chronic sheep model: Mid-term hemodynamic function and histologic assessment

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ABSTRACT

Objective: To evaluate the safety and the short-term function of a novel pulmonary valved conduit (Xeltis Pulmonary Valved Conduit; XPV) up to 12 months in a sheep model.

Methods: XPV and Hancock bioprosthetic valved conduits (H, used as control) were implanted in adult sheep in the pulmonary artery position. Animals were killed at 2 months (n = 6 XPV), 6 months (n = 6 XPV and n = 3 H), and 12 months (n = 6 XPV) and examined histologically. During follow-up, function of the device as well as diameter of both XPV and H were assessed by transthoracic echocardiography.

Results: Of 18 animals that received an XPV, 15 survived until they were killed; 3 animals that received H survived the planned observational interval. XPV showed mild neointimal thickening and degradation beginning at 2 months with an ongoing process until 12 months. Only 1 of the 18 animals with XPV had significant calcification at 6 months. Pathologic specimen did not show any significant narrowing of the conduit whereas neointimal thickness showed a peak at 6 months. Inflammatory process reached a maximum at 6 months and the degradation process at 12 months. Gel permeation chromatography analysis showed molecular weight loss beginning at 2 months with a peak at 12 months for the conduit with slower absorption for the leaflets. The wall of the H conduits showed more neointimal thickening, narrowing, and calcification compared with XPV, but the leaflets demonstrated minimal changes.

Conclusions: Both conduits demonstrated an acceptable safety and functionality. Significant calcification was rarely observed in the XPV, whereas the H developed more neointimal thickness with calcification of the porcine aortic root portion of the wall. (J Thorac Cardiovasc Surg 2018;155:2591-601)

Histopathology of Xeltis XPV conduit gradually replaced over time with newly formed tissue.

Central Message

We show acceptable safety and functionality of a novel restorative pulmonary valved conduit in the sheep model. Significant calcification was rare with absence of conduit stenosis.

Perspective

The Xeltis Pulmonary Valved Conduit (XPV) is based on a bioabsorbable polymer matrix designed to allow the body to repair itself by healthy tissue from the recipient. XPV has shown encouraging results in the sheep model at 2, 6, and 12 months and could be an improvement over the current conduits available for children with complex congenital heart disease.

See Editorial Commentary page 2602.

Congenital malformations of the right ventricular outflow tract are present in 15% to 20% of all congenital heart defects operated on worldwide.^{1,2} Artificial materials as well

as homografts and xenografts are frequently used to repair complex congenital malformations.^{3,4} Although these implants are usually easy to handle, they may be associated with mid- to long-term complications such as rejection, excessive narrowing leading to stenosis, thromboembolic events, and degeneration with or without calcification.⁵⁻⁷ Unfortunately, current prosthetic materials are

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Abbreviations and Acronyms

ETR	= endogenous tissue restoration
H	= Hancock device
UPy	= 2-ureido-4[1H]-pyrimidinone
XPV	= Xeltis Pulmonary Valved Conduit

unable to grow to accommodate the biology of young recipients, which results in multiple operations to replace the degenerated or outgrown conduits.^{8,9}

To overcome these limitations, significant efforts have been applied to alternative approaches: a tissue-engineering approach using cell culture and the incorporation of bioactive factors into a synthetic absorbable implant.¹⁰⁻¹² More recently, a new technology was proposed based on a bioabsorbable polymer matrix designed to enable endogenous tissue restoration (ETR) without the use of stem cells or animal-derived products.¹³ The prosthetic material allows patient's own cells to infiltrate and trigger a cascade of physiological events leading to ETR, with gradual replacement by native tissue.

This paper summarizes preclinical data to evaluate safety and performance in a sheep model of the Xeltis Pulmonary Valve Conduit (XPV; Xeltis, Eindhoven, The Netherlands), which is a novel bioabsorbable polymer-based medical device designed to enable ETR.

MATERIALS AND METHODS

The XPV used in this study had a length of 5 cm and an inner diameter of 21 mm. The XPV is a sterile, flexible, straight, highly porous conduit with 3 leaflets inside, processed by electrospinning. The XPV is composed of fully synthetic bioabsorbable supramolecular polymers, which are formed by arrays of directed, noncovalent interactions between the building blocks.¹⁴ The blocks are based on the supramolecular 2-ureido-4[1H]-pyrimidinone (UPy), which provides tunable mechanical properties and absorption.¹⁵ The conduit wall consists of a poly-caprolactone-based UPy polymer that gives a robust structure. For the leaflets, a poly-carbonate-based UPy is used to provide flexibility for valve motions. One of the most intriguing innovations of the XPV is its ability to absorb and be replaced by stable and solid autologous tissue. In vitro models have been used to assess the absorption mechanisms and speed of the used materials. This showed that both materials mainly absorb by oxidative oxygen species and less by enzymatic degradation. In vivo, both pathways play a role at the same time and might even influence each other.¹⁶

The control used for this study was the 22-mm Hancock porcine valved conduit (H, model HC150-22; Medtronic, Minneapolis, Minn), which consists of a porcine aortic root within a Dacron conduit (DuPont, Wilmington, Del).

Preclinical Study Design

The study design and animal usage were approved by the Test Facility's Ethical Committee for compliance with regulations and the Helsinki protocol. Furthermore, animal welfare was in compliance with the Directive 2010/63/EU. The study included 21 animals (adult Swifter sheep, age 2-4 years, weight

VIDEO 1. Surgical approach was through a left anterolateral thoracotomy via the third intercostal space. Normothermic cardiopulmonary bypass was instituted and the procedure performed on beating heart. After transection of the native pulmonary artery, the graft was implanted orthotopically, just above the native pulmonary valve, after the leaflets were excised. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(17\)32892-1/fulltext](http://www.jtcvsonline.org/article/S0022-5223(17)32892-1/fulltext).

60-90 kg). Planned sacrifice was after 2, 6, and 12 months (n = 6 each) in animals that received XPV and 6 months for those 3 animals that received H.

Surgical Technique

Surgical approach was through a left anterolateral thoracotomy via the third intercostal space. Normothermic cardiopulmonary bypass was instituted and the procedure performed on beating heart. After transection of the native pulmonary artery, the graft was implanted orthotopically, just above the native pulmonary valve, after the leaflets were excised (Video 1).

Imaging Follow-up

Transthoracic echocardiography was performed at monthly intervals to assess the hemodynamic performance of the conduits. Regurgitation was graded according to the classification by Brown and colleagues¹⁷ (Table E1). Pressure gradients were derived from the flow velocities. The diameter of the conduits was assessed distally to the valve during systole.

Pathologic Evaluation

Pathologic evaluation included gross examination and careful dissection of the neopulmonary trunk to expose the pulmonary valve. Histology was performed after 2 and 6 months in 1 representative longitudinal section including 1 of the valve leaflets, the sinus, and the adjacent conduit wall. In the 6 animals killed at 12 months, the 3 valve leaflets were cut longitudinally and sections of each leaflet including the adjacent wall of the conduit were assessed for histology. Both the proximal and distal anastomoses were examined microscopically. Tissues were dehydrated and embedded in paraffin. Sections were cut at 4 to 6 microns and stained with hematoxylin-eosin and Movat Pentachrome. Von Kossa stain was performed for calcium.

The leaflets and conduits were examined for thrombotic deposits, neointimal growth including collagen deposition, inflammation, architectural changes (matrix absorption), and calcification. Each parameter was semi-quantitatively graded from one section closest to the center of the leaflet with the most pathologic change (Tables E2-E5). Neointimal thickness was graded on the basis of the original thickness of the leaflet/conduit.

Measurements were made at the thickest portion of the neointimal growth (Table E3). Inflammation score was based on the degree and distribution of the infiltration on the leaflet and conduit surfaces by inflammatory cells. Inflammatory scores were based on the scale shown in Tables E6 and E7.

Molecular Weight (Mw)

Mw was assessed as a measure for absorption of the XPV scaffold materials. Therefore, parts of the explants were assessed for both conduit and leaflet material. Newly formed tissue was removed by a 4.6% sodium hypochlorite treatment for 10 (undiluted) and 20 minutes (diluted 1:5 with water) for the leaflet and conduit material, respectively. Samples were washed twice in water, and Mw of the remaining scaffold materials was measured.¹⁶

Statistical Analysis

Variables with non-normal distribution were expressed as median and interquartile range. Normality of distribution was tested with the Shapiro–Wilk test. Continuous variables with normal distribution were expressed as mean ± SD. Comparisons of variables with nonparametric distribution were performed by the Kruskal–Wallis test. A value of $P < .05$ was considered statistically significant. JMP software (version 9.0, Cary, NC) was used for statistical analyses.

RESULTS

Implantation and Follow-up

All implantations were successfully performed without surgical complications. One animal was lost immediately

after surgery due to arrhythmia. One animal died 11 days after surgery: the cause of death was due to a small hole in the XPV: inappropriate clamping during surgery was suspected to be the cause of the rupture. Finally, 1 sheep was killed after 5 months due to XPV endocarditis. The early deaths are believed to be attributable to the model and its limitations and therefore do not relate to safety of the implanted XPV; however, excessive clamping of the device during implantation should be avoided. Our advice for future clinical studies is to avoid clamping the conduit altogether. All other sheep were successfully followed until the planned explant date, resulting in 5 animals each at 2, 6, and 12 months for the XPV conduit and 3 at 6 months for the H.

Transthoracic Echocardiography

Peak gradients, regurgitation levels, and diameters for XPV and H conduits are reported in Figure 1. The average peak gradients and regurgitation levels were lower for the H, which is expected, as the XPV material is stiffer compared with biological tissue during the first months. However, both conduits show acceptable ranges. Pressure gradients for the XPV showed a decreasing trend from 6 months onwards, whereas gradients for the H increased with time. No significant increase in regurgitation was observed during follow-up. The XPV diameter increased

FIGURE 1. A, Diagram explaining the construction of the XPV and gross external appearance of the conduit as well as superior view of the leaflet. B-D, Mean and 95% confidence intervals for diameter (B), peak gradient (C), and regurgitation (D) of the XPV as measured by echocardiography every 4 weeks until sacrifice. The HC is included as well. HC, Hancock conduit

FIGURE 2. Gross images of the Xeltis Pulmonary Valved Conduit at 2, 6, and 12 months. The 2-month implant shows an intact valve with mild intimal thickening at the base of the leaflets. At 6 months, there is focal disruption of the leaflet (*arrows*) close to the free margins of the leaflets, and focal thickening was observed in one leaflet (*arrowheads*). At 12 months, shrinkage of the leaflets appears and rolling of the free margins of 2 of the 3 leaflet (*arrowheads*) with an open orifice (*asterisk*). Also, one of the commissures shows thickening and fusion (*arrow*). The HD conduit shows a competent valve with mild neointimal thickening (*arrowheads*) of the sinus and focal yellow deposits (*arrows*). Note markedly thickened intima over the Dacron conduit (*arrowheads*). HD, Hancock valve.

after 6 months from 2 to 2.5 cm at 7 months but remained stable thereafter ([Figure 1](#)).

Molecular Weight

Mw is shown with time in [Figure E1](#). This demonstrates that absorption is initiated in both the conduit and the leaflets but it was more advanced for the conduit, as the conduit material showed a lower Mw after 6 and 12 months.

Gross and Histopathologic Observation at 2, 6, and 12 Months

XPV 2 months. Grossly, at 2 months, XPV showed intact leaflets ([Figure 2](#)), without any thrombus deposition/calcification and mild thickening at the base of the leaflet. Both

proximal and distal anastomoses appeared intact and were covered with tissue. Histology of the leaflets showed minimal absorption of the pulmonary valve leaflets ([Figure 3](#)). Very small microthrombi were observed on 2 leaflets in 2 separate animals. There was a smooth layer of neointimal tissue found mainly at the base of the leaflets. The site of leaflet to conduit attachment showed infiltration by SMCs/macrophages and proteinaceous deposits. The adventitial surface of the conduit showed presence of few macrophages, giant cells, and lymphocytes with fibroblasts and collagen deposition, accompanied by small arteries, arterioles, and capillaries. There were adhesions between the conduit and surrounding structures. Leaflet matrix absorption was rare and was greater in the conduit than in the

FIGURE 3. Representative histologic images of the XPV at 2 months (A-D) and 6 months (E-H). A-D, 2 months: Note mild neointimal coverage near the base with extension into the distal conduit consisting of smooth muscle cells in a proteoglycan/collagen matrix mostly at the base of the sinus (arrow) with few macrophages, giant cells, and lymphocytes. There is mild degradation mostly at the hinge point (C). High power of the black box (D) shows the valve leaflet infiltration by proteinaceous material (pink) with early cellular migration. The free margin of the leaflets remained uncovered with absence of neointimal tissue. The intimal to adventitial layers of the conduit are shown in (A) and (B) highlight the abluminal surface of the conduit with infiltration by macrophages, lymphocytes, spindle cells, and red cells. E-H, 6 months: Note significantly more pronounced neointimal growth in the sinus with extension into the leaflet. E and F show the conduit, which is infiltrated by macrophages, lymphocytes, and giant cells (green box) from the conduit. Note detachment of the leaflet material from the underlying conduit. G shows the base of the leaflet with fibrin/proteinaceous material with the leaflet matrix and inflammation at the edges. H shows an area of discontinuity of the leaflet at the hinge point with intense inflammatory infiltrate consisting of macrophages with interspersed giant cell and few lymphocytes. The free margin of the leaflets remained uncovered.

leaflets, but no calcification was observed in any of the leaflets nor in the wall of the conduit.

XPV 6 months. Grossly, at 6 months, XPV showed focal disruption in some leaflets close to the free margins of these leaflets (Figure 2). There was partial coverage by neointimal tissue at the base of the sinus with extension into the basal half of the leaflet. Maximal thickness of the neointimal tissue was observed at the base of the leaflets, with tapering toward the free margin that remained uncovered. However, the polymer was infiltrated by proteinaceous material with fibrin deposition. In some cases, encasement by

neointimal tissue at the base caused infolding of the leaflets, resulting in shortening of the leaflet length. The conduits and anastomosis were intact with more thickening than at 2 months' observation (Figure 3). Absorption of the leaflet was typically observed at hinge point from inflammatory infiltrate, whereas the conduit showed greater absorption mainly at the base of the leaflet and was accompanied by profuse inflammatory infiltrates with macrophage, giant cell, and lymphocyte infiltration. Calcification was observed at the tip of a leaflet in 1 animal only, and no calcification was observed in any of the conduits (Figure 4).

FIGURE 4. Representative histologic images of the XPV at 12 months are shown in sections 1 to 3. The base of the leaflets shows significant neointimal thickening with distal tapering and presence of small fibrin thrombus at the tip (A). B is the base of the leaflet. Note greater neointimal formation over the leaflets most marked on the pulmonary surface. The degree of inflammation is variable, with the greatest amount seen at the hinge point similar to 6 months (C and D). Note D showing profuse giant cell reaction with macrophages and lymphocytes and smooth muscle cells admixed. Section 3 shows mild infolding, whereas 2 shows severe infolding that is most marked near the commissures. Scaffold matrix absorption remain primarily at the base and is associated with areas of complete disruption of leaflet. Conduits are completely covered by neointima consisting of smooth muscle cells and fibroblasts intermingled with collagen and minimal elastic fiber deposition without any calcification and fibrosis (E). The tip of the leaflet is also covered by neointima (F).

XPV 12 months. Gross examination of the 12-month XPV showed complete coverage of the leaflets by neointima without any thrombus deposition. Tears and fragmentation of the polymer leaflets were observed (Figure 2). All conduits were intact and showed more extensive adventitial than intimal thickening.

Histology of the base of the leaflets showed significant neointimal thickening (Figure 5); however, 4 leaflets from 2 animals showed presence of small fibrin deposits at the tip. Overall, the inflammation on the leaflets was mild and only focally observed except at the hinge point. Leaflet infolding with retraction that resulted in shortening of the leaflets was observed in 6 leaflets from 3 animals with surrounding neointimal growth and inflammation. Leaflet matrix absorption occurred primarily at the base in the other

leaflets associated with areas of complete disruption of the leaflets. The XPV conduit wall was completely covered by neointima without any calcification. The conduit scaffold material showed more advanced absorption compared with the leaflet scaffold (Figure 6). Both proximal and distal anastomosis were intact, with tissue growth consisting of mature neointima and collagenous tissue in regions with degraded conduit.

The semiquantitative histologic data are shown in Figure 7 and Tables E8 and E9: the XPV leaflet absorption as well as inflammation increase with time, but these changes are less important in the leaflets than in the conduit itself. Leaflet thickness was maximal at 12 months; the biggest difference seen was between 2 and 6 months ($P = .005$). Neointimal thickness was diffuse over the

FIGURE 5. Bar graphs showing the semiquantitative histologic responses to the leaflets and conduits. A, Leaflet characteristics. B, Conduit characteristics.

conduit but the maximal thickness appeared to be most pronounced in the sinus (Figure 7).

Hancock at 6 months. Gross examination of the 6-month H (Figure 2) showed intact leaflets without any thrombus. All leaflets were pliable without any calcification or thickening; however, moderate thickening was observed at the base of the sinus and the conduit. The aorta of the porcine aortic valve showed presence of calcification in all H conduits (Figure 4), with moderate neointimal thickening, which was greater in H than in XPV. Histologic images of leaflets (Figure 5) showed focal fibrin deposition with rare inflammatory cell presence without calcification. Leaflets close to the base were covered by neointimal tissue. Moderate-to-severe calcification was observed in the porcine aortic root portion of the wall of the H conduit. The inner and outer surfaces of the Dacron conduit showed moderate presence of giant cells, macrophages, lymphocytes, fibroblasts, and collagen deposition. Thickness of the valve leaflets was significantly less pronounced in H as compared with XPV at 6 months ($P = .02$) (Figure 7).

DISCUSSION

Although clinical studies will be needed for confirmation, this animal study is an important step in demonstrating the safety and efficacy of a novel restorative polymer-based pulmonary valved conduit. The Hancock porcine valved conduit was used as a control. Both the XPV and the H showed acceptable performance assessed by echocardiography throughout the observational interval. Interestingly, a decreasing trend in peak gradient was observed from 6 months onwards in the XPV: this finding corresponds to the absorption of the polymeric conduit that loses its mechanical integrity after 6 months in vivo. We hypothesize that increased compliance of the conduit wall results in a decrease in gradient for XPV at 6 months. Progressively the relative stiff polymer is replaced by more pliable autogenous tissue.

The sheep model with similar anatomy and physiology to the human is an established animal model for long-term assessment of tissue valves. Previous reports on juvenile sheep models also showed severe calcification of the

FIGURE 6. Of the 18 XPV, 2 valves showed some degree of leaflet calcification from animals killed at 6 and 12 months. Six months' image shows significant calcification of the leaflet accompanied by fibrin deposition. Twelve months' histologic examination of the leaflets shows mild focal calcification near the base of the leaflet.

Hancock conduit (aortic root portion). In addition, cusp calcification and extensive fibrous sheathing of the cusp was observed.^{18,19} Our findings in the adult model confirm extensive neointima formation on the conduit, but we did not yet see the cusps being affected by calcification or fibrous sheathing. We hypothesize that this relates to difference in age between the models. The reaction against foreign tissue (porcine aortic valve) results in severe immunologic reaction and stenosis in children as well.^{18,19} Biocompatibility of the XPV materials used in this study was assessed and did not show any signs of toxicological or carcinogenic effects on their environment (data not shown). In addition, distal organs of the animals in this study were fully assessed and showed no signs of chronic toxicity.

Another interesting finding of the study is that intimal coverage of the XPV conduit started at 2 months, peaked at 6 months, and decreased thereafter without any

calcification. The neointimal coverage started at 2 months from the base of the leaflets with progressive extension toward the free margin and reached the maximum at 12 months. Leaflet calcification was detected in 1 of 5 valves at 6 months and in 1 of 5 at 12 months, thus showing no progression in calcification. The relatively low amount of calcification in the XPV, especially in the sheep model known for heavy calcification, is a promising finding (Figure 7).

The XPV is composed of 2 different synthetic bioabsorbable supramolecular polymers based on the UPy motif. Histologic and molecular weight analysis confirmed that the poly-carbonate-based UPy used in the leaflets is absorbing slower compared with the poly-caprolactone-based UPy in the conduit wall. This is an important design feature, as histology revealed that ETR processes are similar but delayed in the leaflets compared with the wall. This means that a prolonged support is required for the

FIGURE 7. Representative histologic images of the Hancock conduit at 6 months are shown in images 1 and 2. Note mild neointimal coverage of the leaflet with rare surface inflammatory cell (mostly macrophages) (arrow) without calcification (A). B, Shows the native porcine valve with mild platelet/fibrin thrombus on both the pulmonary and ventricular surfaces and few acute inflammatory cells. There is transmural calcification of the porcine aortic wall (image 2, C) with overlying neointimal growth. Von Kossa stain shows the area of calcification of the aortic wall over the Dacron conduit. The adventitial surfaces of the Dacron conduit showed presence of moderate number of macrophages, giant cells, and lymphocytes with surrounding fibroblasts and collagen deposition (D). Image 2 shows marked neointimal thickening starting at the base with mild calcification of the cardiac muscle (E) (arrow) with superimposed neointimal thickening. The region in F at the base of the sinus shows organizing thrombus (F).

implant material. Similar molecular weight values of the leaflets after 6 and 12 months were observed, whereas histology scores showed increased absorption after 12 months. This could have been caused by rinsing of small material particles during the tissue removal step, thereby ending up with a similar molecular weight after 6 and 12 months. Extensive work using in vitro degradation models has also shown that the leaflet material absorbs slower compared with the conduit material. Here, both oxidative and enzymatic solutions were used to assess the degradation pathways of the materials. Although this provides more insight into the degradation mechanism, a real correlation with in vivo degradation speed cannot be determined using these models as in vivo both the oxidative and enzymatic

degradation mechanisms play a role at the same time and even might affect each other. Histologic analysis of the leaflets showed infiltration by proteinaceous material with gradual increasing inflammation up to 12 months. The absorption of the leaflet material mostly begins at 6 months; it starts at the hinge point and continues to 12 months. Although there is progressive absorption, the process is incomplete at 12 months (mean degradation score was 2.0 ± 0.6), with retraction and curling of the free margin. Inflammation score of the XPV was numerically greater compared with H (Figure 7). The inflammation of the XPV leaflets and conduit occurs through infiltration by macrophage, giant cells, and lymphocytic infiltrate accompanied by SMCs and proteoglycan/collagen matrix. The

degree and extent of the inflammation is much more pronounced in the conduit than in the leaflets. The more severe inflammation associated with XPV is related to polymer absorption and decreases when matrix absorption nears completion.

Several groups have been working on bioabsorbable materials to be inserted as pulmonary valved conduits in children and infants.^{20,21} Sugiura and colleagues²¹ evaluated the feasibility of an arterial bioabsorbable vascular graft, which has a porous sponge-type scaffold, as a small-diameter arterial conduit and compared the difference in cellular infiltration, followed by tissue remodeling for different pore sizes. The grafts were constructed by a 50:50 poly (1-lactic-co-ε-caprolactone) copolymer scaffold reinforced by a poly (1-lactic acid) nanofiber. Histologic assessment demonstrated favorable cell infiltration into scaffolds, neointimal formation with endothelialization, smooth muscle cell proliferation, and elastin deposition. There were no rupture nor aneurysmal formation despite the fact that this implant was inserted into the arterial circulation.

The group of Shinoka developed a tissue engineered vascular graft with the ability for growth and remodeling and have already used in humans.²⁰ Autologous bone marrow was obtained and the mononuclear cell component was collected. Mononuclear cells were seeded onto a biodegradable scaffold composed of polyglycolic acid and epsilon-caprolactone/L-lactide and implanted as extracardiac cavopulmonary conduits in patients with single-ventricle physiology. The results were very gratifying: there was no evidence of aneurysm formation, graft rupture, graft infection, or ectopic calcification, but stenosis was observed. Etiology of this problem should be investigated.

Other groups have tried to fashion a valved conduit using porcine small intestinal submucosa extracellular matrix (CorMatrix; CorMatrix Cardiovascular, Rosewell, Ga) and examined biodegradation, subsequent replacement by autologous native tissue, and durability. They were able to show that CorMatrix is biodegradable; however, it failed to remodel in a structural and anatomical fashion in the arterial environment.²²

Some limitations must be summarized. First, biological responses to the implants may be different in the humans than in the sheep model. Therefore, generalizing the current experimental findings into clinical outcomes in humans may not be valid. Second, we used an adult sheep model (2–4 years old), with less calcification to be expected. Finally, the duration of this study was too short to show the complete leaflet absorption in GPC analysis. Long-term evaluation studies to confirm the long-term functionality of the XPV and complete absorption of the leaflets are currently underway.

In summary, we believe that the XPV shows encouraging results in the sheep model up to 12 months and may be a significant improvement over the current conduits available on the market for children with congenital heart disease who

need pulmonary valve/artery replacement. Nevertheless, clinical experience is needed to confirm the safety and performance of this bioabsorbable valved conduit.

Conflict of Interest Statement

Cox, Svanidze, and Brugmans are employees of and hold shares/options in Xeltis. Dr Virmani and Prof Bennink are paid advisors to Xeltis. All other authors have nothing to disclose with regard to commercial support.

References

1. Walters HL III, Mavroudis C, Tchervenkov CI, Jacobs JP, Lacour-Gayet F, Jacobs ML. Congenital Heart Surgery Nomenclature and Database Project: double outlet right ventricle. *Ann Thorac Surg.* 2000;69:S249-63.
2. Costello JM, Pasquali SK, Jacobs JP, He X, Hill KD, Cooper DS, et al. Gestational age at birth and outcomes after neonatal cardiac surgery: an analysis of the Society of Thoracic Surgeons Congenital Heart Surgery Database. *Circulation.* 2014;129:2511-7.
3. McElhinney DB, Reddy VM, Hanley FL. Homografts in congenital heart disease: current applications and future directions. *Isr J Med Sci.* 1996;32:880-5.
4. Bobylev D, Breymann T, Boethig D, Haverich A, Ono M. Semilunar valve replacement with decellularized homograft after Damus-Kaye-Stansel anastomosis and fontan procedure. *Ann Thorac Surg.* 2014;97:1792-5.
5. Lis GJ, Rokita E, Podolec P, Pfitzner R, Dziatkowiak A, Cichocki T. Mineralization and organic phase modifications as contributory factors of accelerated degeneration in homograft aortic valves. *J Heart Valve Dis.* 2003;12:741-51.
6. Joudinaud TM, Baron F, Raffoul R, Pagis B, Vergnat M, Parisot C, et al. Redo aortic root surgery for failure of an aortic homograft is a major technical challenge. *Eur J Cardiothorac Surg.* 2008;33:989-94.
7. Grubitzsch H, Laule M, Stangl K, Lembcke A, Sander M, Christ T, et al. Surgical-transcatheter approach for endocarditis of a calcified aortic homograft. *J Heart Valve Dis.* 2013;22:751-3.
8. DiBardino DJ, Jacobs JP. Current readings: long-term management of patients undergoing successful pediatric cardiac surgery. *Semin Thorac Cardiovasc Surg.* 2014;26:132-44.
9. Aroca A, Polo L, Pérez-Farinós N, González AE, Bret M, Aguilar E, et al. Risk factors for surgery of congenital heart disease in adults: twenty-two years of experience. Who should operate them? [in Spanish]. *Arch Cardiol Mex.* 2014;84:262-72.
10. Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, et al. Functional living trileaflet heart valves grown in vitro. *Circulation.* 2000;102:III44-9.
11. Mol A, Rutten MC, Driessen NJ, Bouten CV, Zünd G, Baaijens FP, et al. Autologous human tissue-engineered heart valves: prospects for systemic application. *Circulation.* 2006;114:1152-8.
12. Talacua H, Smits AI, Muylaert DE, van Rijswijk JW, Vink A, Verhaar MC, et al. In situ tissue engineering of functional small-diameter blood vessels by host circulating cells only. *Tissue Eng Part A.* 2015;21:2583-94.
13. Bockeria L, Svanidze O, Kim A, Shatalov K, Makarenko V, Cox M, et al. Total cavo-pulmonary connection with a new bioabsorbable vascular graft: first clinical experience. *J Thorac Cardiovasc Surg.* 2017;153:1542-50.
14. Lehn JM. From supramolecular chemistry towards constitutional dynamic chemistry and adaptive chemistry. *Chem Soc Rev.* 2007;36:151-60.
15. Sijbesma RP, Beijer FH, Brunsveld L, Folmer BJ, Hirschberg JH, Lange RF, et al. Reversible polymers formed from self-complementary monomers using quadruple hydrogen bonding. *Science.* 1997;278:1601-4.
16. Brugmans MC, Sontjens SH, Cox MA, Nandakumar A, Bosman AW, Mes T, et al. Hydrolytic and oxidative degradation of electrospun supramolecular biomaterials: in vitro degradation pathways. *Acta Biomater.* 2015;27:21-31.
17. Brown JW, Ruzmetov M, Rodefeld MD, Vijay P, Darragh RK. Valved bovine jugular vein conduits for right ventricular outflow tract reconstruction in children: an attractive alternative to pulmonary homograft. *Ann Thorac Surg.* 2006;82:909-16.
18. Herijgers P, Ozaki S, Verbeken E, Van Lommel A, Meuris B, Lesaffre E, et al. Valved jugular vein segments for right ventricular outflow tract reconstruction in young sheep. *J Thorac Cardiovasc Surg.* 2002;124:798-805.

19. Herijgers P, Ozaki S, Verbeken E, Van Lommel A, Ràcz R, Zietkiewicz M, et al. Calcification characteristics of porcine stentless valves in juvenile sheep. *Eur J Cardiothorac Surg*. 1999;15:134-42.
20. Hibino N, McGillicuddy E, Matsumura G, Ichihara Y, Naito Y, Breuer C, et al. Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg*. 2010;139:431-6. 436.e1-2.
21. Sugiura T, Tara S, Nakayama H, Kurobe H, Yi T, Lee YU, et al. Novel bioresorbable vascular graft with sponge-type scaffold as a small-diameter arterial graft. *Ann Thorac Surg*. 2016;102:720-7.
22. Mosala Nezhad Z, Poncelet A, de Kerchove L, Fervaille C, Banse X, Bollen X, et al. CorMatrix valved conduit in a porcine model: long-term remodelling and biomechanical characterization. *Interact Cardiovasc Thorac Surg*. 2017;24:90-8.

Key Words: pulmonary valve, endogenous tissue restoration, animal study, restorative synthetic implants

FIGURE E1. Molecular weight of both the leaflet and conduit materials. Molecular weights of the conduit and leaflet materials decreased with time. The leaflet material absorbs slower compared with the conduit material.

TABLE E1. Regurgitation grades

Grade	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Regurgitation severity	No	No to minimal	Minimal	Minimal to mild	Mild	Mild to moderate	Moderate	Moderate to severe	Severe

TABLE E2. Description of leaflet/conduit semiquantitative histology scores

Attribute	Score	Description of assigned score
Leaflet/conduit matrix deposition	0	No collagen deposition
	1	Collagen deposition <10% leaflet/conduit length
	2	Collagen deposition 10% to <25% leaflet/conduit length
	3	Collagen deposition 25% to <50% leaflet/conduit length
	4	Collagen deposition 50% to <75% leaflet/conduit length
	5	Collagen deposition ≥75% leaflet/conduit length
Calcification	0	No calcification
	1	<1% overall leaflet/conduit area involvement
	2	1%-5% overall leaflet/conduit area involvement
	3	5%-10% overall leaflet/conduit area involvement
	4	>10% overall leaflet/conduit area involvement
Surface fibrin/thrombus score	0	No surface fibrin/thrombus deposition
	1	Minimal fibrin/thrombus deposition involving <10% of the leaflet/conduit surface
	2	Mild fibrin/thrombus deposition involving 10%-25% of the leaflet/conduit surface
	3	Moderate fibrin/thrombus deposition involving >25% to 50% of the leaflet/conduit surface
	4	Heavy fibrin/thrombus deposition involving >50% of the leaflet/conduit surface

TABLE E3. Neointimal growth of leaflet/conduit

Neointimal growth	Score	Description of assigned weight
Thickness	0	Absent
	1	Less than leaflet/conduit thickness
	2	Greater than leaflet/conduit thickness but less than 2× the thickness
	3	Greater than 2× leaflet/conduit thickness but less than 4× the thickness
	4	Greater than 4× the thickness
Length	1	<1/4 the length of the leaflet/conduit
	2	1/4 to <1/2 the length of the leaflet/conduit
	3	1/2 to <3/4 the length of the leaflet/conduit
	4	≥3/4 the length of the leaflet/conduit

TABLE E4. Severity of neointimal growth of leaflet/conduit

Severity of neointimal growth	<1/4 the length of the leaflet/conduit	1/4 to <1/2 the length of the leaflet/conduit	1/2 to <3/4 the length of the leaflet/conduit	≥3/4 the length of the leaflet/conduit
Absent	0	0	0	0
Less than leaflet/conduit thickness	0	1	2	3
Greater than leaflet/conduit thickness, but less than 2× the thickness	1	2	3	4
Greater than 2× leaflet/conduit thickness, but less than 4× the thickness	2	3	4	5
Greater than 4× the thickness	3	4	5	5

Neointimal growth of leaflet was assessed based on the thickness relative to the leaflet (0 = absent, 1 = less than leaflet thickness, 2 = equal to leaflet thickness, 3 = more than leaflet thickness, 4 = >2× leaflet thickness) and the length (1 = <1/4 the length of the leaflet, 2 = 1/4 to <1/2, 3 = 1/2 to <3/4, 4 = ≥3/4) (Table E3).

TABLE E5. Matrix absorption of leaflets/conduit

Attribute	Score	Description of assigned score
Matrix absorption score	0	No absorption of matrix
	1	<10% leaflet/conduit length shows matrix absorption
	2	10% to <25% leaflet/conduit length shows matrix absorption
	3	25% to <50% leaflet/conduit length shows matrix absorption
	4	50% to <75% leaflet/conduit length shows matrix absorption
	5	>75% leaflet/conduit length shows matrix absorption

TABLE E6. Inflammation of leaflet and conduit

Inflammation characteristics	Score	Description of assigned weight
Degree of inflammation	0	Absent
	1	Rare inflammatory cells infiltration
	2	Mild infiltrate and is not the predominant component of the associated tissue
	3	Infiltrates up to 1/2 of the leaflet
	4	Infiltrates >1/2 of the leaflet
Distribution of inflammation	1	Focal
	2	Multifocal
	3	Diffuse

TABLE E7. Severity of inflammation of leaflets and conduit

Inflammation grading	Focal	Multifocal	Diffuse
Absent	0	0	0
Rare inflammatory cells infiltration	0	1	2
Mild infiltrate and is not the predominant component	1	2	3
Infiltrates up to 1/2 of the leaflet	2	3	4
Infiltrates >1/2 of the leaflet	3	4	4

TABLE E8. Semiquantitative histologic responses to the leaflets

Leaflet response	Leaflet thickness	Leaflet collagen replacement	Leaflet calcification	Leaflet surface fibrin/thrombus score	Leaflet matrix absorption	Inflammation severity score leaflet
2 mo, n = 5	2.0 ± 1.9	0.4 ± 0.5	0.0 ± 0.0	0.6 ± 0.5	1.0 ± 0.0	1.4 ± 0.5
6 mo, n = 5	4.4 ± 0.5	2.0 ± 0.7	0.6 ± 1.3	0.6 ± 0.9	1.4 ± 0.5	2.0 ± 1.0
12 mo, n = 5	4.9 ± 0.1	3.9 ± 0.2	0.0 ± 0.1	0.2 ± 0.3	2.6 ± 0.5	2.6 ± 0.4
Hancock 6 mo, n = 3	2.0 ± 1.0	1.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.0		0.3 ± 0.6

TABLE E9. Semiquantitative histologic responses to the conduits

Conduit response	Conduit thickness	Conduit collagen replacement	Conduit calcification	Conduit surface fibrin/thrombus score	Conduit matrix absorption	Inflammation severity score conduit
2 mo, n = 5	3.2 ± 0.4	2.6 ± 0.9	0.0 ± 0.0	0.4 ± 0.9	2.2 ± 0.4	2.0 ± 0.0
6 mo, n = 5	4.2 ± 0.4	4.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.9	3.4 ± 0.9
12 mo, n = 5	3.9 ± 0.4	4.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.1	3.7 ± 0.5	3.3 ± 0.3
Hancock 6 mo, n = 3	4.7 ± 0.6	3.3 ± 0.6	3.7 ± 0.6	1.7 ± 0.6		2.7 ± 0.6