Transcatheter placement of a low-profile biodegradable pulmonary valve made of small intestinal submucosa: A long-term study in a swine model

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An additional figure is available online.

Objective: We sought to investigate a placement of a percutaneous low-profile prosthetic valve constructed of small intestinal submucosa in the pulmonary position in a swine model.

Methods: Twelve female farm pigs were stented at the native pulmonary valve to induce pulmonary insufficiency. Once right ventricular dilation occurred, the small intestinal submucosa valve was implanted. The pigs were followed up with transthoracic echocardiographic Doppler scanning. One animal died of heart failure before valve replacement. Animals were euthanized at 1 day, 1 month, 3 months, 6 months, and 12 months after valve implantation.

Results: The small intestinal submucosa pulmonary valve showed effective reversal of pulmonary regurgitation. There were no misplacements during deployment. There were no embolizations. One-year echocardiographic follow-up showed minimal regurgitation and no stenosis for a valve/vessel ratio of 0.78 or greater. Histologic examination demonstrated intensive remodeling of the small intestinal submucosal valve. Within 1 month, the surface was covered by endothelium, and fibroblasts invaded the interior. Over the following months, the small intestinal submucosal valve remodeled without apparent graft rejection.

Conclusion: The small intestinal submucosa valve has the potential for graft longevity without the need for anticoagulation or immunosuppression. Histologic remodeling of the valve tissue provides a replacement capable of resembling a native valve that can be placed percutaneously with low-profile delivery systems.

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Surgical replacement of cardiac valves remains the cornerstone therapy for end-stage valvular disease and substantially improves its natural history.[1] There are 2 types of valve prostheses: mechanical and biologic. The mechanical valves are associated with substantial risks of thromboembolism and hemolysis; the biologic valves tend to become dysfunctional in a relatively short time because of tissue deterioration.[2-5] Furthermore, the current clinically used tissue valves are nonviable; they lack the potential to remodel, and therefore their durability is limited. Porcine small intestinal submucosa (SIS) is an acellular matrix prepared from the jejunum and is used as a xenograft material because it induces variable degrees of tissue-specific remodeling in the organ or tissue into which it is placed.[6-12] It is mostly composed of type I collagen, although it has some type III and type IV collagen in addition to other extracellular matrix molecules, such as fibronectin, hyaluronic acid, chondroitin sulfate A and B, heparin, and heparin sulfate, and some growth factors, such as basic fibroblast growth factor, transforming growth factor β, and vascular endothelial growth factor. Recently, Matheny and colleagues[13] used single-layer porcine SIS as a substitute for mature pulmonary

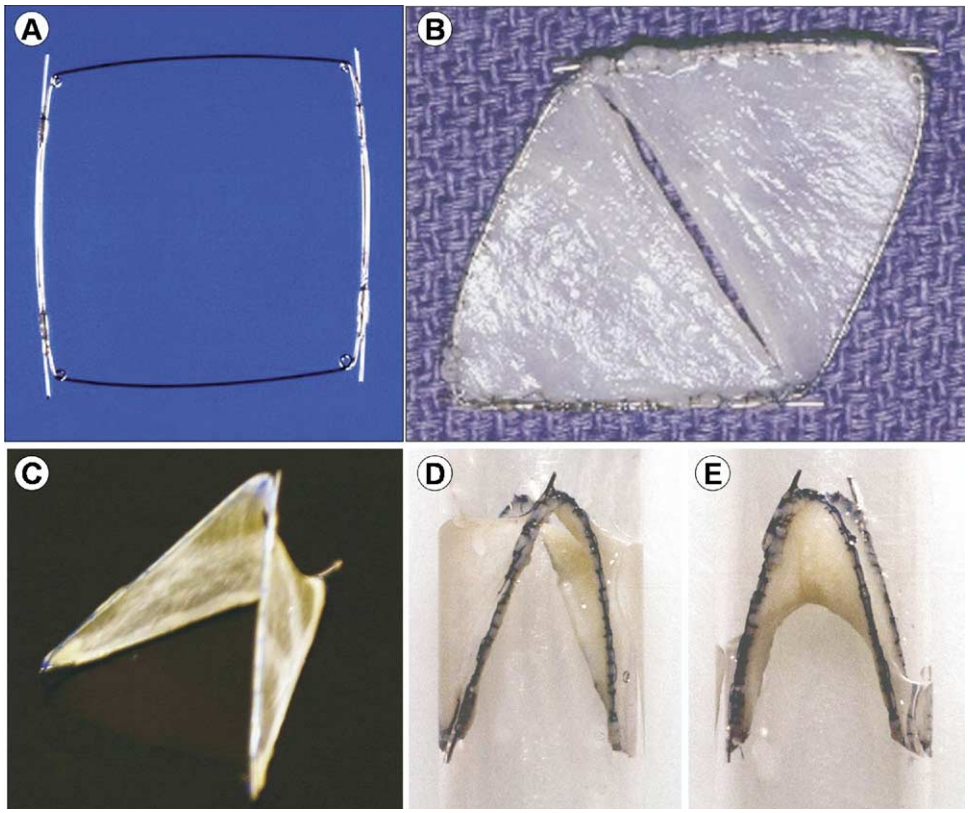

Figure 1. A, Self-expanding square stent. B, SIS material attached to stent. C, SIS valve. D and E, SIS valve tube model during extracorporeal cycling studies at systole (D) and diastole (E).

valve leaflets in a swine model. We hypothesized that porcine SIS tissue mounted on a self-expandable stent might result in remodeled SIS, with similar properties and behavior as native valvular tissue, when implanted near a native cardiac valve.

Therefore, we investigated the percutaneous placement of an SIS valve in 12 farm pigs with induced right ventricular failure. The rationale for this approach was to document the behavior of such an SIS-based valve by echocardiography, postmortem extracorporeal cycling studies, and histopathology, enabling further investigation toward a new therapeutic valve based on SIS material in the future.

Methods

This investigation was approved by the University of Illinois at Chicago's Animal Care Committee and was in compliance with the 1996 "Guide for the Care and Use of Laboratory Animals" and the Animal Welfare Act.

Self-Expandable Valve

The pulmonary valve prostheses were constructed of square stents with 4 barbs (Cook Inc, Bloomington, Ind) and a hydrated sheet of SIS (Cook Biotech, Lafayette, Ind). In this study square stents were made of stainless-steel wire (0.01905 mm in diameter), as previously described.[7] The square stent is a simply constructed, self-expanding device that has 4 corners bent into spring-like coils to reduce stress and metal fatigue. The wire ends are extended 1 mm over the stent frame, forming barbs on opposing stent corners to provide anchors for the stent during placement. Two more anchoring barbs are added by attaching a wire to the contralateral side of the stent frame. Two triangular pieces of SIS were sutured with 7-0 Prolene monofilament (Ethicon, Inc, Somerville, NJ) to the stent frame to form the valve. The layer of SIS sutured to the metal frame had a flap about 2 mm wide, extending beyond the frame (Figure 1).[7,14] All valves were 17 mm in diameter. After construction, the valves were lyophilized and gas sterilized. The lyophilized SIS sheet was 100 to 120 μm thick. The valves were then folded and front loaded into an 8F Teflon sheath 80 cm long (Cook Inc) and delivered coaxially.[7,14] Extracorporeal cycling studies were conducted by the manufacturer in advance of this study.

Preparation of Animals With Right Ventricular Failure

Twelve female farm pigs weighing 8 to 18 kg received sedation with 0.05 mg/kg intramuscular atropine, 4.4 mg/kg intravenous tetrazol, and 2.2 mg/kg xylazine. The animals were endotracheally

intubated and mechanically ventilated, and the gas anesthesia was maintained at stage III, planes 1 to 2, with 2% to 3% isoflurane (Iso-thesia; Burns Veterinary Supply, Rockville Center, NY) in oxygen. The animals then underwent cardiac catheterization for stenting of the native pulmonary valve. Digital fluoroscopic images were obtained and stored with an OEC-9600 C-arm cardiac mobile system (GE Medical Systems, Waukesha, Wis). The native pulmonary valve, the distal part of the right ventricular outflow tract, and the proximal third of the main pulmonary artery (PA) were covered with 2 to 3 metallic stents (Cordis Corp, Miami Lakes, Fla). The stents were mounted on a 14.0 mm × 3.0 cm, 7.0 mm × 2.0 mm BIB-balloon (NuMed, Hopkinton, NY) and deployed under simultaneous digital fluoroscopy and transthoracic echocardiographic (TTE) guidance. Moderate-to-severe pulmonary regurgitation was documented by means of color flow Doppler scanning in all animals. The animals were returned to their community cages for a 2- to 4-week period until significant right ventricular dilatation was documented with TTE.

Percutaneous Valve Implantation

The animals underwent percutaneous access of the right internal jugular vein after achievement of general anesthesia. By using a multipurpose catheter, the stented native pulmonary valve was crossed, and an extrastiff Amplatz wire (AGA Medical Corp, Golden Valley, Md) was advanced to one of the distal PA branches. The multipurpose catheter was exchanged for the 8F delivery system, which was advanced over the guide wire into the main PA just distal to the stented valve. The valve was deployed under fluoroscopic and TTE guidance. After deployment, the valve orifice was not crossed to prevent any dislodgement, and the guide wire was pulled out. The intracardiac echocardiographic probe (AcuNav; Acuson, Mountain View, Calif) was advanced to the mid–superior vena cava to best visualize the newly implanted valve. The pigs received 100 U/kg heparin at the beginning of the procedure, and it was not reversed at the end of the procedure.

Serial Follow-up

All animals were started on 325 mg/d oral aspirin, but no anticoagulants were used. The pigs underwent weekly TTE under sedation for the first 4 weeks after valve implantation. Echocardiograms were obtained with an Acuson Sequoia (Acuson) and a 5-MHz transducer, and the images were digitally stored, as well as videotape recorded. Four weeks after implantation, serial TTEs were obtained every 2 weeks until animal death. The animals were euthanized in the following order: 1 animal at both 24 hours and at 1 month and 3 animals at each of the 3-month, 6-month, and 1-year end points. Euthanasia was performed after achievement of general anesthesia with 100 mg/kg sodium pentobarbital. Before death, all animals received 50,000 U of heparin intravenously to prevent any postmortem clot formation and underwent diagnostic cardiac catheterization, angiography, and intracardiac echocardiography.

Histology and Flow Evaluation

Immediately after death, the distal right ventricular outflow tract–PA bifurcation was carefully dissected and removed. The vessel was rinsed in saline and placed in a container with 0.9% sterile iced saline with gentamicin. The container was then placed on ice and sent to the histology laboratory. Before fixation and histologic processing, video footage of valve function was acquired. Unfixed valves were placed in tubing of appropriate size to hold the valve in place. Blood flow was simulated with an electric pump circulating 0.9% saline solution through the tubing containing the valve. An endoscope was placed close enough to the valve to capture video images of its function. Afterward, the valves were placed in 10% neutral-buffered formalin and allowed to fix for 24 to 48 hours. After fixation, the vessels were dehydrated and embedded in methylmethacrylate plastic. After polymerization, the samples were cut on a plane perpendicular to the orifice of the valve. Longitudinal thick sections (approximately 50 μm) were cut with a rotary saw microtome and stained with hematoxylin and eosin. For immunohistochemical examination, sections of tissue were deparaffinized and rehydrated. Antigen retrieval was accomplished by an antigen retrieval solution (Dako Cytomation, Carpinteria, Calif). Endogenous peroxidase was blocked for 15 minutes with 3% hydrogen peroxide. Nonspecific immunoglobulin binding was blocked by incubation of slides for 10 minutes with a protein-blocking agent (Dako Cytomation) before application of the primary antibodies (all Dako Cytomation). Vimentin immunostain was used to document mesenchymal differentiation, desmin and smooth muscle actin were used for muscle cell differentiation, and von Willebrand factor VIII and CD31 were used for angioformative differentiation. A labeled streptavidin-biotin-peroxidase complex system (Dako Cytomation) was used to visualize all immune reactions. The immunoreaction was visualized with 3,3′-diaminobenzidine substrate (Dako Cytomation). Sections were counterstained with Mayer hematoxylin. Positive immunohistochemical controls included lymph nodes and arteries from normal swine to which the appropriate antisera were added. For negative controls, the primary antibodies were replaced with homologous nonimmune sera.[15] An independent board-certified pathologist experienced in the evaluation of chronically implanted vascular stents performed qualitative histopathologic evaluation of the stented vessels. Slides were evaluated for remodeling of the valve material and to assess potential adverse effects, with particular attention to the interface between the implant and the vessel wall, as well as signs of graft rejection.

Results

All 12 animals had their pulmonary valves stented successfully. One animal died of heart failure before valve implantation. The SIS valve was percutaneously implanted in 11 pigs (median weight, 23.6 kg; range, 17.5-34.4 kg). In 10 animals the valves were implanted just distal to the distal stent within the main PA. In 1 animal expected to survive for 1 year, the valve was implanted at the origin of the right PA to minimize the mismatching effects of somatic growth between the valve and the vessel wall. There were no major clinical complications from any of the procedures, and none were observed during the follow-up period (ie, valve thrombosis and infective endocarditis).

One Day

Echocardiographic results. The presence of moderate tricuspid regurgitation (TR) was observed after stent im-

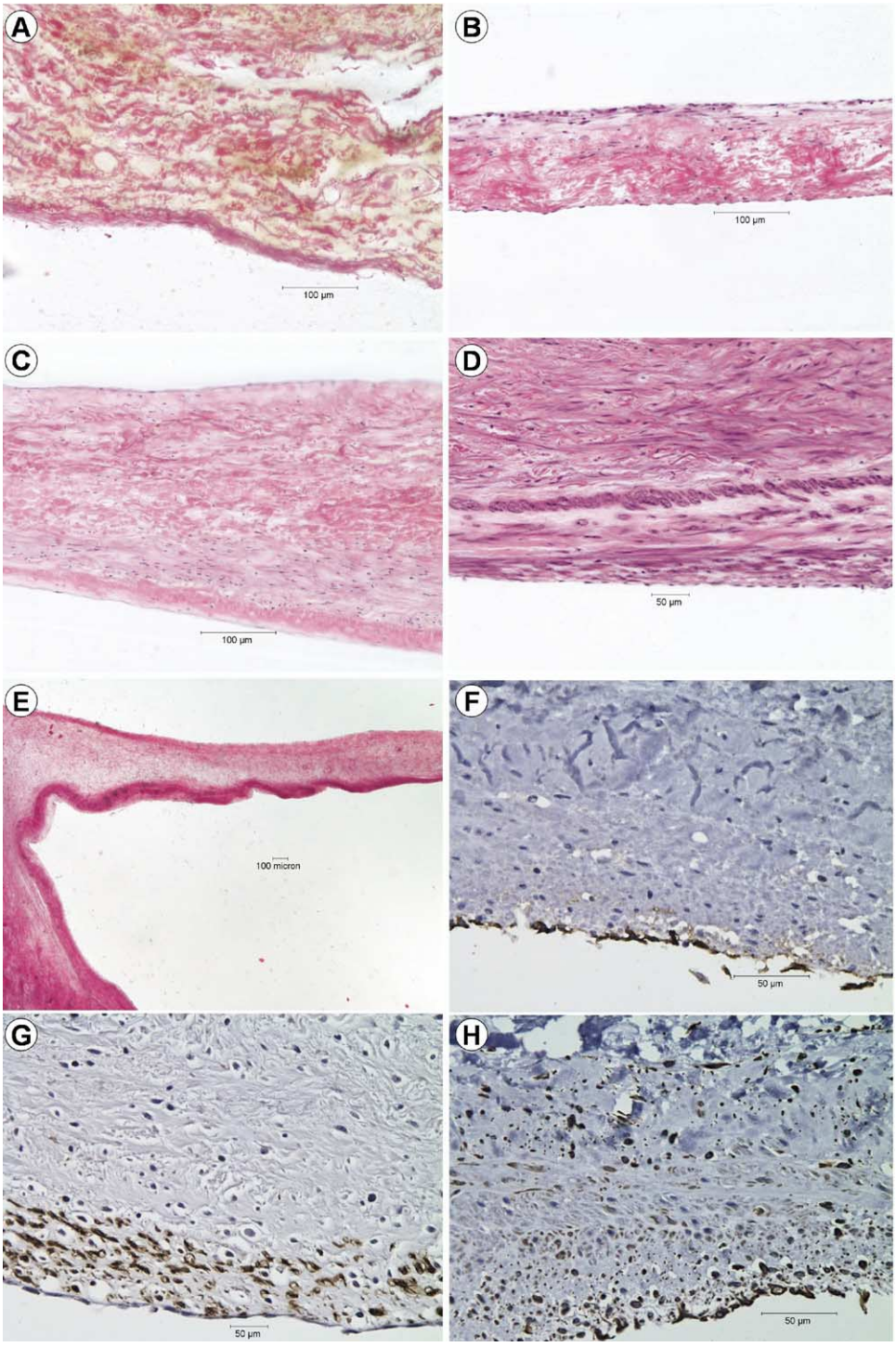

Figure 2. A, SIS valve 1 day after implantation, hematoxylin and eosin staining. Acellular SIS collagen fibers were separated, resulting in marked leaflet thickening by serum, blood, minimal fibrin, and a few platelets. B, Three months after implantation. Early remodeling shows thin neointima coating the valvular surface. C, Six months after implantation. Ongoing remodeling demonstrates a continuous epithelial coating and host connective tissue inside the valve interior. D, Twelve months after implantation. Advanced remodeling showing differentiated epithelial lining; fibroblasts and smooth muscle cells are shown in the interior. E, Native pulmonary leaflet, hematoxylin and eosin staining. F-H, SIS valve 12 months after implantation, antibody staining: F, subendothelial von Willebrand factor VIII; G, interstitial smooth muscle cells; H, connective tissue vimentin.

plantation in all animals, with an average velocity of 2.45 m/s. After valve implantation, the TR improved to trace, and the TR velocity decreased to 1.40 m/s. There was a moderate degree of perivalvular leak, but there was no valvular regurgitation. Perivalvular leak was observed in all implants.

Gross anatomy. The valve appeared well anchored to the PA. The implant was attached to the vessel wall by the hooks; however, there was no tissue attachment between the leaflets and the arterial wall.

Histopathology. The valve leaflets were 17 mm in length, and the diameter of the main PA was 13 mm, resulting in a valve/PA ratio of 1.3. There were no infiltrating host cells. The SIS collagen fibers were separated, resulting in marked edema of the leaflets by means of infiltration of serum, blood cells, minimal fibrin, and a few platelets. There were no signs of graft rejection (Figure 2).

One Month

Echocardiographic results. A trace of TR was still present, and the TR average velocity was 1.08 m/s. There was a trivial central jet of pulmonary insufficiency determined by means of color flow Doppler scanning, and the perivalvular leak was still present in 3 of the 10 animals, although it was significantly decreased.

Gross anatomy. The video recording of the prosthetic valve within the PA in a pump simulator revealed very pliable leaflets with complete coaptation. The base of the leaflets was firmly adhered to the vessel wall. The valve/PA ratio was 1.02.

Histopathology. The leaflets were infiltrated with host fibroblasts and capillaries, but the SIS was still visible. A thin neointima covered large portions of the leaflet. Endothelium partially covered most of the leaflet, some areas only sporadically. There were few lymphocytes and plasma cells, which were not consistent with graft rejection in number and appearance.

Three Months

Echocardiographic results. There was no change in the amount of TR, and the mean velocity was 1.36 m/s. There was still a trivial central jet pulmonary regurgitation determined by color flow Doppler scanning that had not changed from the 1-month follow-up. There were no perivalvular leaks in any of the 9 animals at 3 months.

Gross anatomy. The valve/PA ratio was 0.88. The valve was well anchored to the PA. The prosthetic valve was well incorporated and firmly adhered to the vessel wall. There was a minimal progression of leaflet stiffening noted by in vitro video recording, with an apparent increase in leaflet thickness but with complete coaptation.

Histopathology. The valves were firmly adhered to the vessel wall, and the leaflets were slightly shorter (mean, 15.6 mm). The base of the leaflets had a thin coating of neointima (usually 20 μm thick), which extended to the remainder of the leaflet as a neointimal coating 1 to 2 cells thick. In the middle the leaflets were approximately 200 μm thick and contained mostly dense collagen fibers, with few capillaries and few infiltrating host cells (Figure 2). Endothelium was present but occasionally sporadic. There were no signs of graft rejection.

Six Months

Echocardiographic results. There was essentially unchanged TR, with a mean velocity of 1.49 m/s. There was no change in the amount of pulmonary regurgitation in any of the animals, and there were no perivalvular leaks.

Gross anatomy. The valve/PA ratio was 0.78. All the valves appeared well anchored to the PA. There was further increase in stiffening and thickness of the leaflets, but they still had complete coaptation.

Histopathology. The leaflets were slightly shorter (mean, 14.4 mm) and thicker at the base and in the middle of the leaflet. Neointima was usually 50 to 150 μm thick at the base and tapered toward the tip of the leaflet. In the middle the leaflets were usually 200 to 400 μm thick (one up to 1200 μm thick) and fully infiltrated with spindle-shaped host cells, capillaries, and arterioles and covered with neointima 10 to 20 μm thick and endothelium (Figure 2). There were no signs of graft rejection.

One Year

Echocardiographic results. There was an increase in the amount of TR caused by dilatation of the right ventricle that progressed from the 10- and 11-month follow-ups in the 2 animals that had valves implanted in the main PA. By the 12th month, both animals had wide-open pulmonary regurgitation, and in both animals the valve stent was noted to be broken and separated on one side from the PA wall. The animal that had the valve implanted in the right PA had trivial pulmonary regurgitation through the valve, and there was no gradient across the valve. All 3 animals had significant pericardial and pleural effusion.

Gross anatomy. At autopsy, the 2 animals with implanted valves in the main PA had a dilated right ventricle and right atrium, with large pericardial and pleural effusion. The valve/PA ratio was 0.48. In one animal a fragment of the stent was noted protruding through the main PA wall. The prosthetic valves implanted in the main PA were both fractured and separated on one side from the PA wall. The valve leaflets were torn on one side, and there was a significant mismatch between the valve stent frame diameter (17 mm) and the size of the main PA (34.8 mm) at the time of death. The animal that had the valve implanted at the origin of the right PA had no fractures of the stent, and the

valve was well incorporated within the artery, with no tears noted.

Histopathology. The valve leaflets ranged from 200 to 1200 μm thick and measured 9 mm. In the middle the leaflets were fully infiltrated with fibroblasts, capillaries, arterioles, and a few scattered lymphocytes and covered with neointima 10 to 20 μm thick and endothelium (Figure 2). A few thick, dense collagen fibers were present in the core of the leaflet. Immunohistochemically, the core of the leaflet was phenotypically fibroblastic cells intermixed with dense collagen and capillaries. A layer of spindle-shaped cells that were phenotypically similar to smooth muscle cells surrounded the core (Figure 2). Inflammation was mostly absent. By comparison, native pulmonary leaflets (Figure 2) were approximately 5 mm long and ranged from 1 mm thick at the base to approximately 100 to 300 μm over most of its length. The valve was composed of dense collagenous connective tissue and elastic fibers forming bands under the endothelium on both surfaces. The central surface of the valve, which was continuous with the endocardium, had slightly thicker connective tissue (approximately 100 μm thick) than the peripheral side ($<$50 μm thick).

Discussion

Graft Endurance

Biologic valve replacement has been applied in cardiac surgery for more than 30 years. Many bioprosthetic valves have shown excellent hemodynamic profiles, and unlike their mechanical counterparts, they do not require permanent anticoagulation.[2-4] However, the endurance of biologic tissue after placement is still a significant problem, with only some improvement over the last decades.[16,17] The relatively short durability of bioprosthetic valves limits their application to patients with either a contraindication to anticoagulation (ie, during pregnancy) or to an elderly age group with a low likelihood for reoperation.[2-4,18] The mechanisms by which biologic valves degrade are considered multifactorial, with involvement of immunologic rejection, mechanical wear out, calcification, and enzymatic digestion, with subsequent loss of the original histologic integrity.[19-21] This work shows that the SIS valve provides substantial improvement in many aspects associated with early tissue remodeling, as well as a lack of significant immunologic rejection, which is frequently assessed by histology,[22] suggesting prolonged graft survival. In concurrence with previous work with SIS material, we found no gross histologic evidence of immunologic rejection.[6-13,23] This is most likely due to the lack of antigens, as well as the histologic remodeling of the SIS material, which leads to the expression of host antigens. The SIS material is predominantly composed of porcine extracellular matrix macromolecules, which are closely related to their human equivalent and have a low immunogenicity.

Currently, the mechanisms of bioprosthetic valve degeneration are an area of great interest. A recent work by Simionescu and associates[19] investigated the influence of bioprosthetic valve preparation during manufacturing and whether this can influence durability in a swine model. They described glutaraldehyde-preserving bioprosthetic valves that failed to prevent the breakdown of glucoseaminoglycanes and elastin-associated microfibrils. These elastin-associated microfibrils have important mechanical functions in addition to protecting elastin from calcification.[24] Recently, several attempts have been undertaken to prevent glutaraldehyde-treated valves from calcifying. Vasudev and coworkers[25] found calcification to be slowed by iron and magnesium supplements. In contrast, the SIS valve does not need pretreatment with glutaraldehyde, thus avoiding early calcification. Furthermore, the induction of early remodeling of the SIS leads to a de novo synthesis of extracellular matrix macromolecules, including elastin-associated microfibrils. A comparable approach avoiding glutaraldehyde preparation has been attempted with the SynerGraft valve presented by O'Brien and colleagues[26] as they introduced an artificially decellularized porcine valve. However, the application to human subjects could not keep up with the expectations derived from the preclinical trials. Bechtel and associates[27] reported reduced immunologic response after SynerGraft pulmonary homograft implantation; however, this did not translate into any clinical advantage. The experience from the SynerGraft valve implied that decellularization was effective in decreasing rejection and avoiding calcification, without any improvement in endurance. We hypothesize that the ability of the SIS material to become remodeled to a certain extent might be the key point in creating a valve replacement more similar to the native valve, resulting in prolonged graft survival. Nevertheless, despite promising histologic remodeling results, the progressive thickening of the SIS valves remains of serious concern for the potential use of this material in human cardiac valve applications. Even though the progressive valve thickening did not impair valvular function at 12 months, as shown in the postmortem extracorporeal cycling studies, moderate reduction of leaflet motility was evident. It will be essential to further investigate how this will translate into studies beyond 12 months of SIS valve implantation to assess whether the thickening will lead to functional impairment.

Adaptation to the Vessel Wall

Given the ability of the SIS tissue to remodel, we believed that the SIS valve would be able to adapt to the expanding vascular system. During the early stages of the experiment, valvular coaptation was complete and paravalvular leak did

not occur, thus indicating that the SIS material adapted well to the vessel wall. However, once the mismatch between the implanted valve and the PA size exceeded a certain tolerance, stent fracturing and significant disruption of the prosthetic valve occurred. In retrospect, these findings might be explained by the rapid somatic growth of the swine and the constraints of the stent frame. The SIS valve used in this study included metal square stents, which cannot expand beyond physical limits. Once the valve stent was implanted in the right PA not exceeding the frame constraints, as documented in the third pig killed at 12 months, the valve was not impaired. Therefore, the vessel adaptation of the valve appears limited by the metallic stent and not by the SIS leaflets. This problem will have to be addressed through either modification or removal of the metal stent, resulting in a device with a flexible diameter in future experiments.

Low-Profile Percutaneous Valve Placement

Percutaneous catheter-based systems for the treatment of valvular heart disease have been studied in animal models for several years.[28] The major advantage of this approach is that it does not require surgical intervention. Nevertheless, surgical procedures still make up the vast majority of valve replacements.[29] Recently, Bonhoeffer and coworkers,[30] using a bovine jugular vein valve mounted within a stent, performed the first human percutaneous implantation of an artificial valve in children with right ventricle–pulmonary prosthetic conduits. In addition, Cribier and colleagues[31] described one case report with a successful percutaneous valve replacement in a 57-year-old man. The current implantation procedures are based on large delivery systems. Bonhoeffer and coworkers[30] used 22F catheters for pulmonary valve replacement, and Cribier and colleagues[31] used 24F catheters for aortic valve placement. In this experiment 8F catheter delivery systems were used. We hypothesize that further improvement making the percutaneous placement procedure even less invasive might substantiate the potential of this upcoming technology.

Conclusion

The SIS valve has the potential for combined graft longevity without the need for anticoagulation or immunosuppression. Histologic remodeling of the valve tissue provides a replacement comparable with the native valve. Furthermore, the SIS valve can be placed percutaneously by using a low-profile catheter delivery system.

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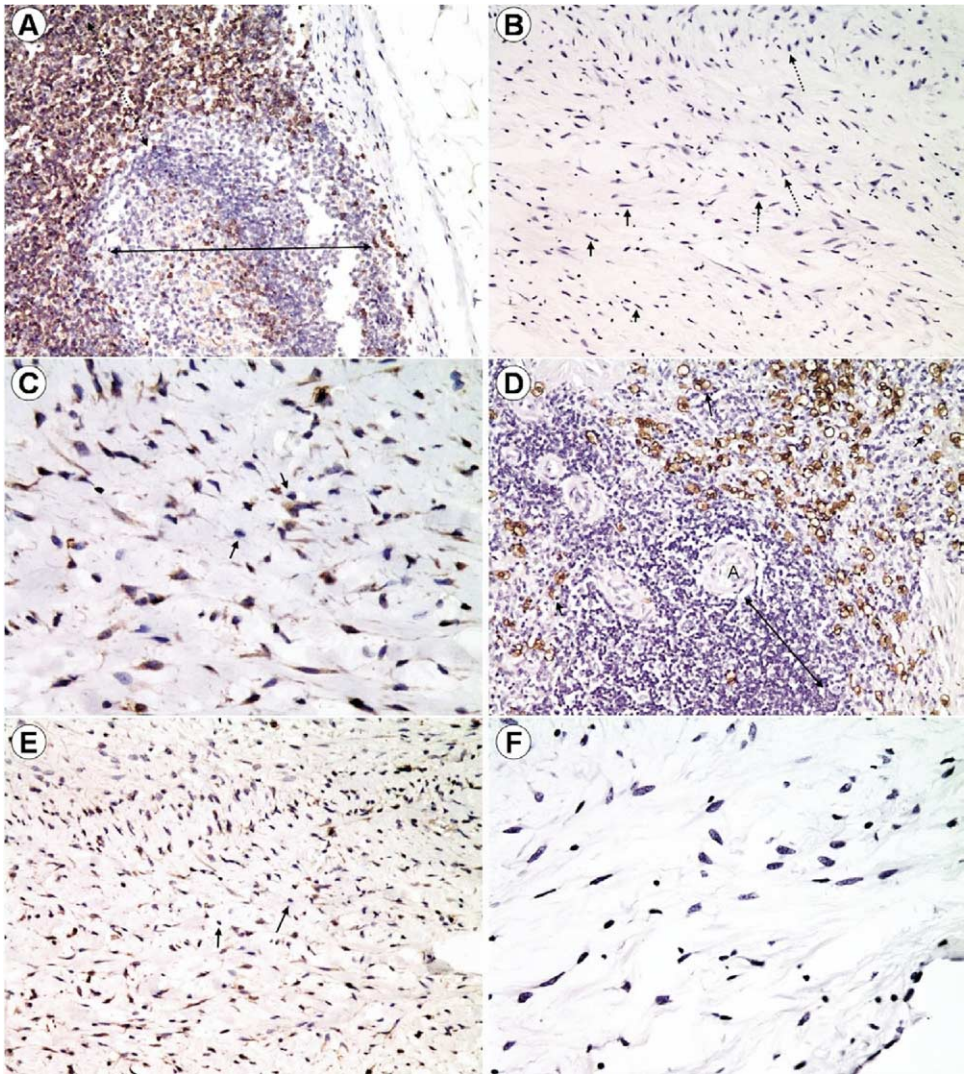

Figure E1. A-C, CD3 antibody staining: A, CD3 control, 200x. *Solid arrow* outlines lymphoid follicle. *Dotted arrow* outlines T-cell rich region that stains strongly with CD3 antibody. B, CD3 on SIS leaflet at 6 months, 200x. Stellate and spindle-shaped host stromal cells have low-grade staining (considered nonspecific). Cells with morphology of lymphocytes *(arrows)* did not stain. C, CD3 on SIS leaflet at 6 months, 400x. Stellate and spindle-shaped host stromal cells have low-grade staining (considered nonspecific). Cells with morphology of lymphocytes *(arrows)* did not stain. D-F, MAC 387 (myeloid) antibody staining: D, MAC 387 control, 200x. *A*, Arteriole; *double arrow*, lymphoid sheath without staining. Large dark-staining cells *(singlehead arrow)* are positive macrophages. E, MAC 387 on SIS leaflet at 6 months, 200x. Stellate *(dotted arrow)* and spindle-shaped *(solid arrows)* host stromal cells have no staining. Round-shaped cells that could represent macrophages were very rare and did not stain. F, MAC 387 on SIS leaflet at 6 months, 400x. Stellate and spindle-shaped host stromal cells have no staining. Round-shaped cells that could represent macrophages were rare and did not stain.