Reconstruction of pulmonary artery with porcine small intestinal submucosa in a lamb surgical model: Viability and growth potential

Lorenzo Boni, MD,[a] Fariba Chalajour, MD,[a] Takashi Sasaki, MD,[a] Radhika Lal Snyder, BA,[a] Walter D. Boyd, MD,[b] R. Kirk Riemer, PhD,[a] and V. Mohan Reddy, MD[a]

Objectives: This study investigated the time-dependent remodeling and growth potential of porcine small intestine submucosa as a biomaterial for the reconstruction of pulmonary arteries in a lamb model.

Methods: Left pulmonary arteries were partially replaced with small intestine submucosal biomaterial in 6 lambs. Two animals each were humanely killed at 1, 3, and 6 months. Computed tomographic angiography, macroscopic examination of the implanted patch, and microscopic analysis of tissue explants were performed.

Results: All animals survived without complications. Patency and arborization of the pulmonary arteries were detected 6 months after implantation. There was no macroscopic narrowing or aneurysm formation in the patch area. The luminal appearance of the patch was similar to the intimal layer of the adjacent native pulmonary artery. Scanning electron microscopy showed that the luminal surface of the patch was covered by confluent cells. Immunohistochemical examination confirmed endothelialization of the luminal side of the patch in all of the explanted patches. The presence of smooth muscle cells in the medial layer was confirmed at all time points; however, expression of elastin, growth of the muscular layer, and complete degradation of patch material were detectable only after 6 months. The presence of c-Kit–positive cells suggests migration of multipotent cells into the patch, which may play a role in remodeling the small intestine submucosal biomaterial.

Conclusions: Our data confirmed that remodeling and growth potential of the small intestine submucosal biomaterial are time dependent. Additional experiments are required to investigate the stability of the patch material over a longer period. (J Thorac Cardiovasc Surg 2012;144:963-9)

⟲ Supplemental material is available online.

The reconstruction of defective or hypoplastic pulmonary artery (PA) is a common surgical procedure performed in pediatric patients with congenital cardiovascular disease. Augmentation of the PA is often implemented during the first year of life and requires synthetic or biologic patch materials. Although the outcomes of surgically repaired hypoplastic PAs have improved,[1] frequent reoperations are unavoidable because commercially available graft materials cannot grow with the child's growing vessels. In addition to having growth potential, ideal vascular patches should be readily available, easy to handle, and resistant to degeneration or infection.[2,3] Recently, the application of extracellular matrix (ECM) has been gaining more attention as a possible biomaterial for cardiovascular repair.[4] ECM biomaterials are mammalian decellularized tissues with preserved ECM components that may be harvested from various tissue sources.[5,6] Small intestinal submucosa (SIS) is an ECM biomaterial that is widely used in clinical and animal studies.[7-13] The biologic and mechanical characteristics of SIS biomaterials depend on the source of tissue, the age of the animal, and the preparation process.[14,15] It has been shown that the alteration of biologic and mechanical properties of the implanted SIS patch biomaterials depends on the new in vivo microenvironment.[16,17] The structural integrity of SIS biomaterial is critical to the stability of implantation; however, activation of the host healing process is closely dependent on the degradation of ECM SIS.[5] A balance between the rate of ECM degradation and host remodeling is therefore critical for a successful surgical outcome.

Application of SIS biomaterial for the reconstruction of congenital vascular defects has shown promising results with regard to the patency of repaired vessels in pediatric patients[8]; however, remodeling and growth potential of the SIS patch remain the major concern. The goal of this study was to investigate time-dependent remodeling and

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Abbreviations and Acronyms

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ECM = extracellular matrix
PA   = pulmonary artery
SIS   = small intestinal submucosa
SMA = smooth muscle actin
vWF = von Willebrand factor
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growth potential of porcine SIS biomaterial for the reconstruction of PA in a lamb surgical model.

MATERIALS AND METHODS
Animals
Six Suffolk lambs, weighing approximately 10 to 20 kg, were used for this study (provided by Center for Laboratory Animal Science, Davis, Calif). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html).

Surgical Procedures
Animals fasted for 24 to 48 hours before surgery. All surgical procedures were performed with aseptic technique. Anesthesia was induced either with intravenously administered ketamine hydrochloride (INN ketamine) and diazepam or with isoflurane by mask. After intubation, the animals were mechanically ventilated and anesthesia was maintained with inhalation of 1% to 2.5% isoflurane. The animals were placed in the right lateral decubitus position, their wool was clipped, and a sterile preparation of the area was accomplished. A left lateral thoracotomy was performed. The pericardium was opened anteriorly to the phrenic nerve, and the left PA was isolated. Heparin was administered intravenously at 300 U/kg. Vascular clamps were used to clamp the left main PA temporarily. Partial resection of the vessel was made, and the SIS patch (5 × 10 mm) was placed with 6-0 polypropylene running sutures. After de-airing, clamps were removed. Bupivacaine hydrochloride (INN bupivacaine; 0.25%-0.5%) was injected into the area around thoracotomy for postoperative analgesia before closure of the chest. The chest was closed in the standard fashion, with placement of a temporary chest tube that was removed a few minutes later, just before the animal extubation on the operating table. Aspirin (325 mg) was given orally once a day for 7 days postoperatively to prevent acute thrombosis on the surface of implanted materials.

After recovery, the animals were housed in free-walking pens until elective killing. The patch materials were harvested at 1-, 3-, and 6-month intervals after implantation. Two lambs from each group were made to fast for 24 hours. Each animal was then intubated and anesthetized with isoflurane and mechanically ventilated. The chest was reopened, and macroscopic investigation was done to assess the size and shape of the vessels. Left PA pressures proximal and distal to the patch were recorded by direct puncture. Finally, the animals were humanely killed with intravenous pentobarbital or intravenous saturated potassium chloride, and the vessels were harvested for histological study.

Computed Tomographic Angiography
Patency of the graft was assessed in all animals by macroscopic examination and direct pressure measurement at the moment of the explants. Furthermore, 2 animals underwent computed tomographic angiographic scan of the lungs at 6 months after implantation to assess patency and arborization pattern of the branch PAs before death.

Scanning Electron Microscopy
Small longitudinal sections of the explanted grafts were fixed for scanning electron microscopy (model S-3400N VP-SEM; Hitachi, Tokyo,

Japan). Scanning electron microscopy was performed to show the appearance of the luminal surface of the patched area.

Histologic and Immunohistochemical Studies
The explanted SIS vascular patch, along with a piece of native PA, was dissected and fixed in 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ). The tissues were embedded in paraffin and, 5-μm tissue sections were prepared for Russell-Movat pentachrome staining (American Master Tech Scientific, Inc, Lodi, Calif). Immunohistologic analyses were performed for the endothelial cell marker von Willebrand factor (vWF; Dako Cytomation, Glostrup, Denmark), smooth muscle actin (SMA; Dako Cytomation), elastin (Abcam Inc, Cambridge, Mass), and the stem cell marker CD117 (c-Kit; Dako Cytomation). The thickness of the tissue explants was measured with image analysis software (LAS AF image analysis; Leica Microsystems Inc, Buffalo Grove, Ill).

RESULTS
Postoperative Course and Gross Examination
All 6 lambs survived surgery and the postoperative period without complications or signs of infection. The mean animal weight increased from 17.5 kg at the first operation to 23.9, 51.7, and 53.4 kg at 1, 3, and 6 months, respectively. At the time of explantation of patch material, the areas around the left PA and patch material presented a normal appearance. Formation of adhesion fibers around the suture lines was found 1 month after the first operation (Figure 1, A), and was reduced 6 months after implantation. The patched area did not show any narrowing or aneurysm formation, nor was there a pressure gradient across the patch. The luminal surface of the explanted patch was shiny and smooth, similar to the luminal surface of adjacent native left PA (Figure 1, B). This was observed at all time points. There was no thrombus formation on the endothelial surface of any explanted graft.

In Vivo Imaging Analysis
The computed tomographic angiographic scan with 3-dimensional reconstruction showed patency of the left PA without any sign of stenosis or aneurysm formation. The left and right PA sizes were normal and comparable with one another, as was the distal arborization after each PA branch (Figure 2).

Scanning Electron Microscopy
The scanning electron microscopy revealed the endoluminal aspect of the patch to be covered by confluent cells with the morphologic characteristics of endothelial cells from the first month after implantation (Figure 3).

Histologic and Immunohistochemical Studies
Our histologic analysis showed recellularization of grafted patch after 1 month (Figure 4, A); however, formation of ECM was limited to proteoglycan (Figure 4, B), in contrast to elastin fibers in native artery (Figure 4, C). A diffuse infiltration of inflammatory cells into the adventitia increased the thickness of the grafted patch (Figure 4, D)