Experimental Aortic Valve Cusp Extension with CorMatrix in a Porcine Model

Zahra Mosala Nezhad[1] Alain Poncelet[1] Caroline Fervaille[2] Laurent de Kerchove[1] Pierre Gianello[3]

[1] Department of Cardiovascular and Thoracic Surgery, Université Catholique de Louvain, Cliniques Universitaire Saint Luc, Brussels, Belgium
[2] Laboratory of Anatomy Pathology, Université Catholique de Louvain, Mont-Godinne Hospital-CHU, Mont-Godinne, Belgium
[3] Division of Experimental Surgery and Transplantation (CHEX), Institute of Experimental and Clinical Research (IREC), Université Catholque de Louvain, Brussels, Belgium

Address for correspondence Zahra Mosala Nezhad, MD, MSc, Department of Cardiovascular and Thoracic Surgery, Université Catholique de Louvain, Cliniques Universitaire Saint Luc, Avenue Hippocrate 10, Brussels 1200, Belgium (e-mail: zahra.mosalanezhad@gmail.com).

Thorac Cardiovasc Surg 2017;65:206–210.

Abstract

Background We tested the feasibility of using porcine small intestinal submucosal extracellular matrix (CorMatrix) for aortic valve (AV) repair in porcine model examining its resorption and remodeling potential.

Methods The non-coronary cusp was replaced with CorMatrix in four animals for 120 days. Valve function was assessed by echocardiography. Explants were examined by histology, immunohistochemistry, and collagen assessment.

Results CorMatrix was almost totally replaced with tissue resembling the native cusp with a partial two-layer architecture. However, function was lost due to thickening and calcification.

Conclusions Tested in high-pressure AV position in a pig model, CorMatrix degrades and remodels, but also loses function.

Keywords
- aortic valve
- CorMatrix
- porcine small intestinal submucosal extracellular matrix

Introduction

The long-term outcomes of tissue substitutes used in aortic valve (AV) repair are variable and sometimes disappointing due to patch thickening, fibrosis, and calcification.[1,2] Technically, any patch material (of ~1 mm thickness) can be used for AV repair, provided that it aids cusp coaptation and shows longevity with adequate mobility in this high-pressure area. The main limiting factor dictating long-term function of extended aortic cusps seems to be the durability of the material.[1]

Porcine small intestinal submucosal extracellular matrix (SIS-ECM; CorMatrix; CorMatrix Cardiovascular, Roswell, Georgia, United States) provides a promising alternative to pericardial patches for improved longevity of AV repair. CorMatrix is a decellularised, non-crosslinked, and treated material that should, therefore, have low immunogenicity. CorMatrix is designed to be biologically adaptive and promote "constructive" remodeling; that is, allow recipient cell ingrowth, regeneration, and remodeling that grows with the patient.[3]

Since clinical outcomes are related to both the microenvironment (location) and biomaterial used,[4] we sought to test the technical feasibility and biological responses of using CorMatrix for AV repair in a pig model. We hypothesized that CorMatrix would completely resorb by four months and be replaced with functional cusp-like tissues that resist failure.

Materials and Methods

The local ethics committee approved the study protocol and animals were handled according to guidelines for the humane care for laboratory animals established by national and local legislation according to European regulations. Four commercially sourced adult Landrace pigs were evaluated (mean weight, 64.5 ± 12 kg). The study period was 120 days, and one animal was used to serve as a control at day 0.

received
April 27, 2016
accepted after revision
July 4, 2016
published online
September 1, 2016

DOI http://dx.doi.org/10.1055/s-0036-1586756.
ISSN 0171-6425.

Perioperative anesthesia, pain management, and care are discussed elsewhere.[5] A 4-ply sheet of CorMatrix (for cardiac repair) was used for all cusp extensions.

Surgical Technique

After left lateral thoracotomy in the fourth intercostal space, arterial and venous cannulation (femoral artery, left atrium) were established for cardiopulmonary bypass (CPB) with myocardial arrest by cold crystalloid cardioplegia. Perioperative valve sizing was performed using echocardiography. Aortotomy and valve inspection were performed followed by excision of the non-coronary cusp with a residual rim to aid patch replacement with 5.0 prolene (Prolene polypropylene suture, Ethicon US, Somerville, New Jersey, United States). After CPB weaning, cusp coaptation and function were reassessed by echocardiography. After hemostasis, an intercostal drain was placed prior to closure.

Histology

Histological sections were prepared and processed using standard techniques. For polarized light processing, picrosirius red stained sections were digitized using an Axio Scope 40 microscope (Zeiss, Wetzlar, Germany). Images were quantified using FRIDA software (Johns Hopkins University). A specialist cardiac pathologist used a semiquantitative numerical scale for grading (0 = no abnormality; 1 = mild; 2 = moderate; 3 = severe).[5]

Results

Surgical

Surgery was feasible but technically challenging due to the limited exposure to the aortic valve in the small porcine aortic roots as well as to the frequency of ostial cardioplegic injections. Immediate technical outcomes were acceptable (►**Fig. 1A**). All animals showed persistent arrhythmias soon after removal of the aortic cross clamp, requiring vigorous defibrillation and pharmacological support, rendering the operation eventful. Following CPB weaning, echocardiography revealed lesser mobility of the replaced cusp compared with the native leaflets resulting in mild to moderate, mainly commissural regurgitation. At terminal reoperation, at 120 days, we accessed the heart through median sternotomy and observed diffuse rough pericardial adhesions and significantly enlarged hearts. Echocardiography showed hypertrophic heart with moderate aortic regurgitation and immobile replaced noncoronary aortic cusp.

Macroscopic Explant Analysis

At day 0, CorMatrix was transparent and thin (1 mm) and successfully replaced the non-coronary cusp (►**Fig. 1B**). By day 120, replaced cusps were shortened, retracted, and thickened (5 mm) compared with normal cusps, with focal cartilage-like accumulations and calcification at the cusp base

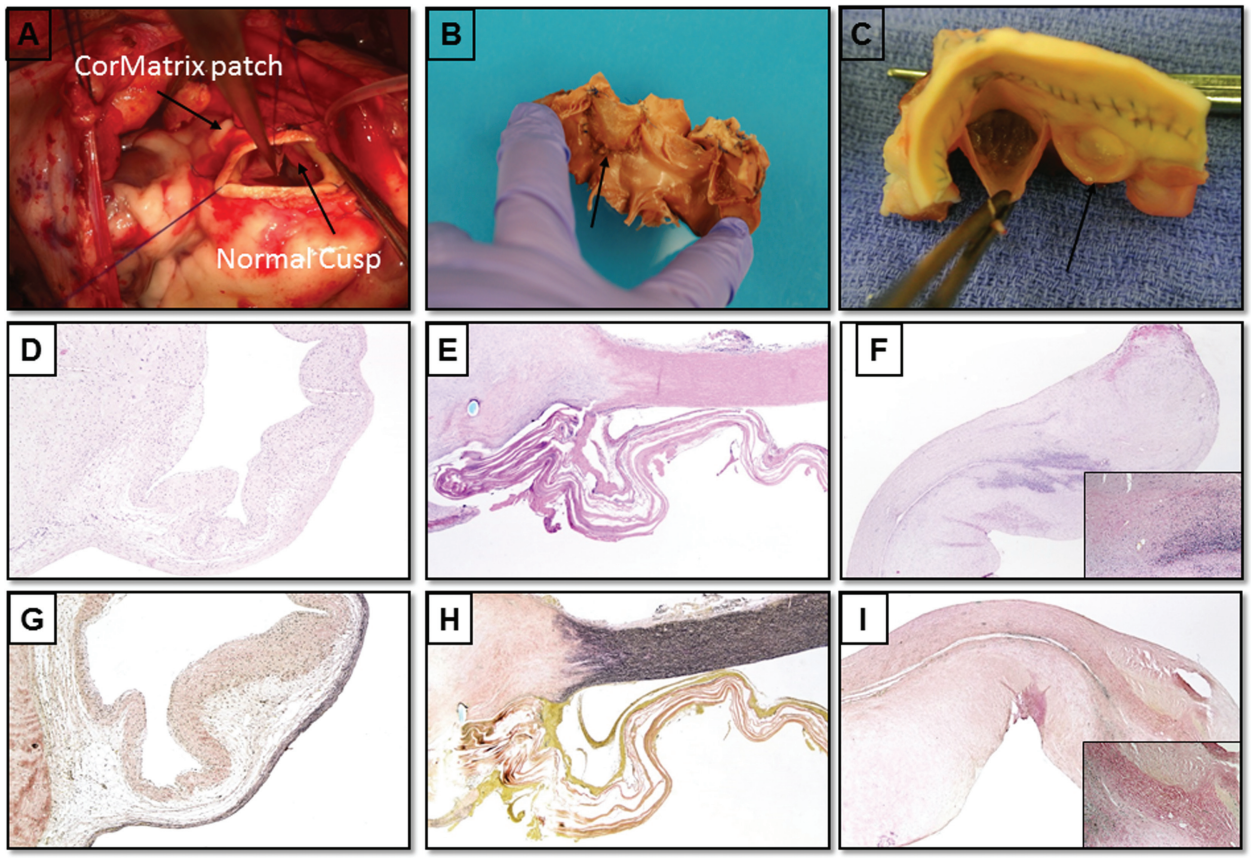

Fig. 1 (**A**) CorMatrix patch in the non-coronary aortic valve cusp position next to a normal cusp (arrows). (**B and C**) CorMatrix (arrow) explanted at day 0 and day 120, respectively. All photomicrographs are at ×100 magnification. (**D**) Hematoxylin and eosin (H&E) and van Gieson (VG) stains showing normal aortic cusp with its three distinct layers. (**E, H**) H&E and VG stains of CorMatrix in the cusp position at day 0 showing collagen bundles. Occasional nuclei are evident. (**F, I**) H&E and VG stains showing remodeled CorMatrix replacing the non-coronary aortic cusp at day 120.

(ventricular side) close to the suture line. There was slight overgrowth of the patch beyond the suture line. The native cusps had grown, but the CorMatrix cusp had not. No tissue defects or cusp calcifications were seen on the remodeled cusp (►**Fig. 1C**).

Light Microscopy Explant Analysis

The CorMatrix cusp harvested on day 0 was intact and covered with a patchy fibrino-leukocytic membrane (►**Figs. 1E** and **H**). At 120 days, the normal aortic valve cusp was a thin sheet of loose connective tissue (spongiosa) and a denser layer (fibrosa) covered by endothelial cells (►**Fig. 1D** and **G**). In comparison, on the CorMatrix cusp, a thin portion of partially fragmented (grade 3) residual patch was identifiable at the center and free edge associated with moderate lymphoplasmacytic inflammation (grade 2) and small, congested capillaries (►**Fig. 1F** and **I**). The tissue surrounding the residual patch near the implantation site at the base of the cusp on the aortic side, consisted of fibrous tissue with chondroid metaplasia and focal calcification (►**Fig. 2A**). The remainder of the cusp had two distinct layers: a fibrous layer on the aortic side and a looser layer on the ventricular side. Focally, endothelial cells could be identified on the surface of the new cusp. There was no evidence of calcification, hemorrhage, or necrosis in any other part of the valve. The implantation base of the CorMatrix (aortic side) cusp showed chondroid metaplasia.

Immunohistochemistry and collagen analysis: Dominant collagen bundles were evident in CorMatrix patch at day 0 (►**Fig. 3A** and **B**), which at four months were replaced by thick type 1 mature collagen with scattered smooth muscles and residual patch in the midsection of the cusp (►**Fig. 3C** and **D**). The fibrous tissue surrounding the CorMatrix patch (120 days) was focally positive for smooth muscle actin (present in myofibroblasts and small capillaries (►**Fig. 2B**).

Discussion

There is no consensus on the best biomaterial for aortic valve repair. The use of patches is limited by mechanical or biological failure and lack of growth. We have previously shown that patch-related factors (size and type) and the location and the extent of patching influences valve repair outcomes.[1] CorMatrix is an acellular collagen scaffold into which host cells migrate and settle to start "constructive" tissue remodeling. Over time, mature collagen is formed on and around the patch that resembles the surrounding structure and the original material is resorbed. This provides the capacity for tissue self-renewal and, therefore, the potential to provide more durable repair with the potential to grow.[3]

Porcine SIS-ECM has been used in a wide variety of applications in animals and patch models, with similarly variable remodeling and functional results reported.[4] In our experience, hydrated CorMatrix patches were easy to use, fashion, and suture and provided good retention without breakage. The CorMatrix was not completely acellular and nuclei were visible by light microscopy; however, this does not indicate the potential for significant immunogenicity or mitotic activity. After four months, CorMatrix was almost completely transformed from a thin collagen-based scaffold into thick and differentiated cusp-like tissue. Residual fragmented CorMatrix was present in the midsection and free edge, suggesting the remodeling pattern likely starts from the base and progresses toward the periphery. The chondroid metaplasia present at the base of the cusp, which in our experience is an unusual finding, had progressed into focal calcification in the same area away from the cusp base. Smooth muscle actin, T lymphocytes, and macrophages were present in the remodeled cusp. Type 1 collagen was dominant and more concentrated at the base of the cusp by four months.

There have been some promising early clinical results on CorMatrix use for intra-cardiac repair[6–8]; however, initial enthusiasm has been tempered by severe inflammatory reactions observed without matrix degradation in some CorMatrix-repaired valves.[4,9,10]

Remodeling is a multifactorial ongoing process. CorMatrix was not tested in such position, thus our experiments and our results are new. We found that the 4-ply sheet was almost completely degraded by 120 days but showed signs of

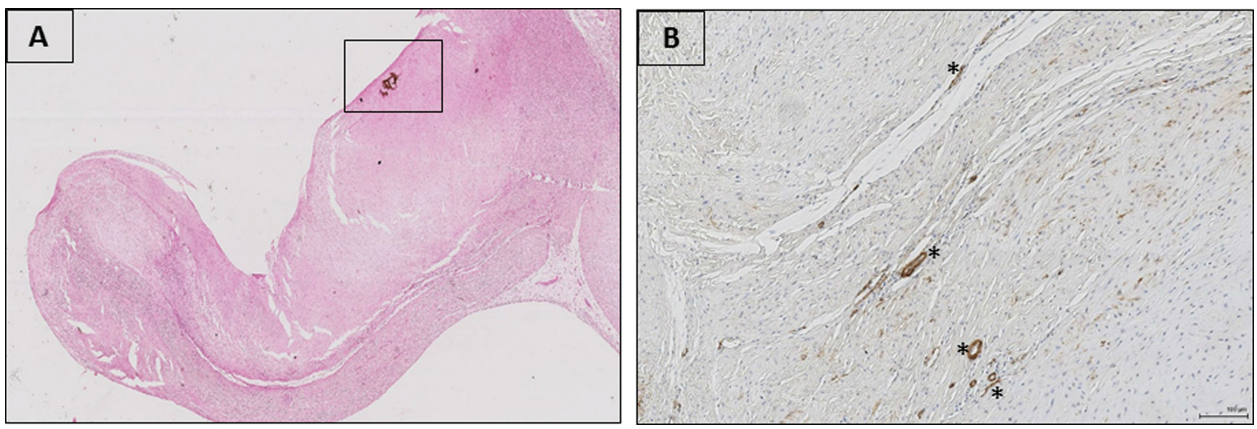

Fig. 2 (**A**) CorMatrix at 120 days stained with von Kossa showing a focal calcification at the aortic side of the cusp concavity (inset, magnified). The area is surrounded by chondroid tissue. (**B**) Immunohistochemistry for α smooth muscle actin (Biogenex, 1/50) showing focal positivity in myofibroblasts and small capillaries.

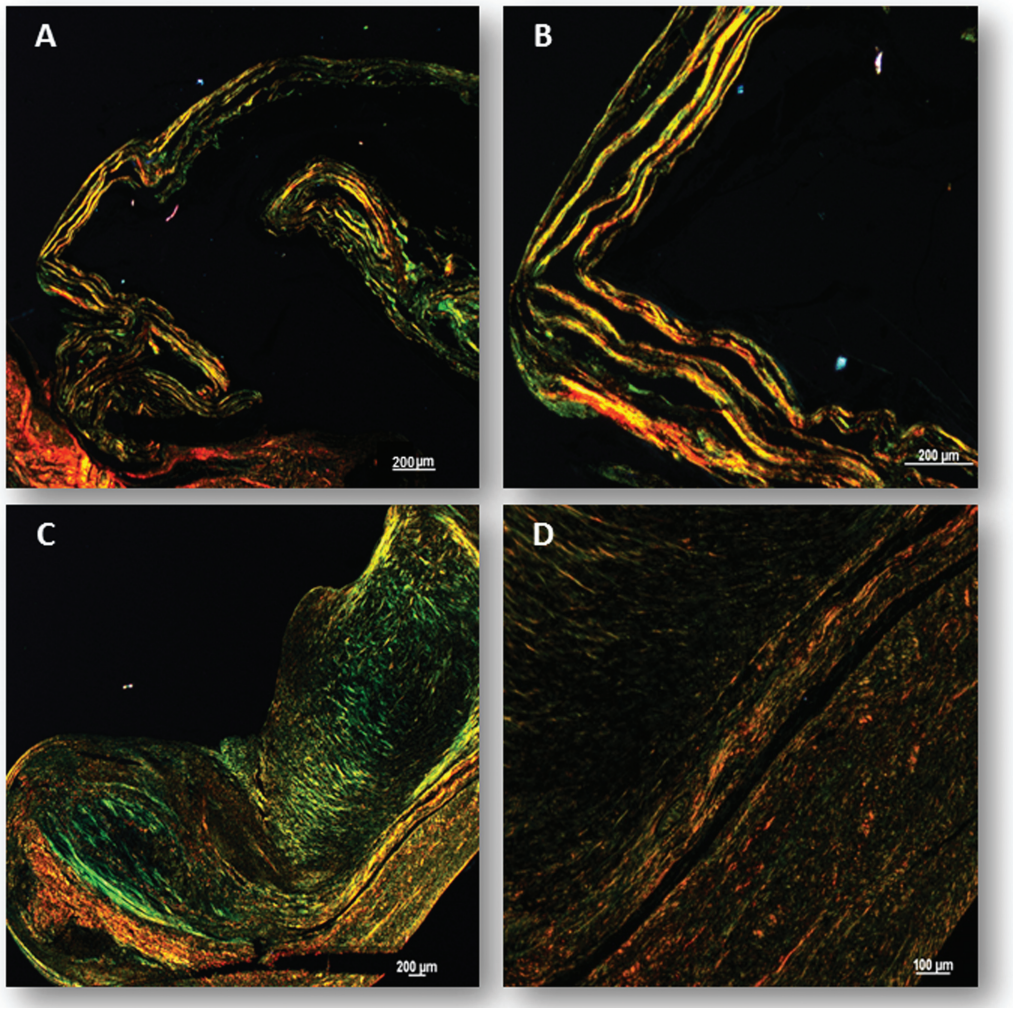

Fig. 3 Polarized light images of CorMatrix at day 0 showing the collagen scaffold (**A, B**). At day 120 (**C, D**), the clear demarcation of the patch into two layers is easily seen. Thin collagen fibers appear green and thicker fibers appear yellow or orange under polarized light, and red indicates mature collagen.

functional failure in the form of thickening, retraction, and rigidity. Possible explanation are; that in addition to high pressure vortices, the presence of a residual commissural regurgitant jet might have influenced the observed remodeling. The floating free edge of CorMatrix and possible entrapment of blood corpuscles in between the microspores of the multilaminated sheet might have altered its microstructure and environment. Finally, as the pig grew fast in size, naturally the heart and aortic valve structures also grew but CorMatrix patch did not. The process of natural, physiologic annular enlargement could have further worsened valve regurgitation and subsequent CorMatrix outcome.

Our preliminary experience with this experimental model of aortic valve repair demonstrates that as an aortic valve-extended cusp, CorMatrix has the potential to remodel and closely resemble the native cusp. However, the observed functional failure and some undesirable remodeling features need to be addressed appropriately before clinical application.

Nevertheless, these experimental results may serve as a framework for future CorMatrix studies in different applications. There are still open questions as to whether the 4-ply CorMatrix sheet is suitable for valve repair, especially when reconstructions are complex or performed in high-pressure environment. Further preclinical studies examining the multifactorial elements of remodeling are required.

Funding
This study was funded by the Laboratory of Experimental Surgery and Transplantation (CHEX-UCL) and the Department of Cardiovascular and Thoracic Surgery, saint luc hospital-UCL.

References

1 Mosala Nezhad Z, de Kerchove L, Hechadi J, et al. Aortic valve repair with patch in non-rheumatic disease: indication, techniques and durability. Eur J Cardiothorac Surg 2014;46(6):997–1005, discussion 1005

2 Myers PO, Tissot C, Christenson JT, Cikirikcioglu M, Aggoun Y, Kalangos A. Aortic valve repair by cusp extension for rheumatic aortic insufficiency in children: Long-term results and impact of extension material. J Thorac Cardiovasc Surg 2010;140(4): 836–844
3 Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater 2009;5(1):1–13
4 Mosala Nezhad Z, Poncelet A, de Kerchove L, Gianello P, Fervaille C, El Khoury G. Small intestinal submucosa extracellular matrix (CorMatrix) in cardiovascular surgery: a systematic review. Interact Cardiovasc Thorac Surg 2016;22(6):839–850
5 Mosala Nezhad Z, Poncelet A, de Kerchove L, et al. CorMatrix valved-conduit in a porcine model: Long-term remodeling and biomechanical characterization. Interactiv Cardiovasc Thorac Surg J 2016;24(1):ivw314
6 Quarti A, Nardone S, Colaneri M, Santoro G, Pozzi M. Preliminary experience in the use of an extracellular matrix to repair congenital heart diseases. Interact Cardiovasc Thorac Surg 2011;13(6): 569–572
7 Scholl FG, Boucek MM, Chan KC, Valdes-Cruz L, Perryman R. Preliminary experience with cardiac reconstruction using decellularized porcine extracellular matrix scaffold: human applications in congenital heart disease. World J Pediatr Congenit Heart Surg 2010;1(1):132–136
8 Witt RG, Raff G, Van Gundy J, Rodgers-Ohlau M, Si MS. Short-term experience of porcine small intestinal submucosa patches in paediatric cardiovascular surgery. Eur J Cardiothorac Surg 2013;44(1):72–76
9 Zaidi AH, Nathan M, Emani S, et al. Preliminary experience with porcine intestinal submucosa (CorMatrix) for valve reconstruction in congenital heart disease: histologic evaluation of explanted valves. J Thorac Cardiovasc Surg 2014;148(5):2216–4, 2225.e1
10 Woo JS, Fishbein MC, Reemtsen B. Histologic examination of decellularized porcine intestinal submucosa extracellular matrix (CorMatrix) in pediatric congenital heart surgery. Cardiovasc Pathol 2016;25(1):12–17