Cardiovascular Pathology 39 (2019) 54–60

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Evaluation of cellular ingrowth within porcine extracellular matrix scaffolding in congenital heart disease surgery☆

Jesse L. Cox [a,*], James M. Hammel [b], Stanley J. Radio [a]

[a] Department of Pathology and Microbiology, University of Nebraska Medical Center, 983135 Nebraska Medical Center, Omaha, NE, 68198-3135
[b] Department of Surgery-Cardiovascular and Thoracic Surgery, University of Nebraska Medical Center, 982315 Nebraska Medical Center, Omaha, NE, 68198-2315

ARTICLE INFO

Article history:
Received 30 August 2018
Received in revised form 7 December 2018
Accepted 11 December 2018

Keywords:
Extracellular matrix
CorMatrix
Congenital heart disease
Great vessel repair

ABSTRACT

The search for an ideal material for cardiac tissue repair has led to utilization of porcine small intestinal submucosa extracellular matrix (CorMatrix). Here, we examine the histologic features of CorMatrix and the associated cellular growth at a variety of time intervals. Tissues with CorMatrix from ten patients (4 male, 6 female) with ages ranging from 2 weeks to 2 years, and implant duration ranging from 1 week to 2 years were included in this study. Samples for analysis were collected at autopsy. Surgical repair sites included great vessel repair (n=9), atrial septum defect (n=1), coronary vessels (n=1), as well as aortic (n=1) and mitral valve (n=2) leaflets. In all specimens, CorMatrix was composed of dense laminated regions of collagen, without appreciable elastin staining. In most grafts, especially those implanted for extended periods of time, tissue with luminal CD31 positivity covered the intimal surface of the CorMatrix graft. This tissue (neo-intima) consisted of spindled myofibroblasts (SMA) and small CD31 positive vessels with occasional mononuclear cells in a matrix composed of collagen, glycosaminoglycans, and rarely elastin, after extended periods of implantation. These features were readily identified in patients as early as 1 month after CorMatrix implantation. The matrix comprising the CorMatrix itself remained largely acellular, despite implantation times up to 2 years, with degradation of the graft material. We provide a framework for histologic expectations when evaluating explanted CorMatrix grafts. In this regard, the CorMatrix matrix is likely to remain acellular without significant elastin deposition, whereas the intimal and adventitial surfaces become coated by proliferating cells in a novel matrix of collagen and glycosaminoglycans.

1. Introduction

Surgical repair of cardiovascular tissues and defects oftentimes requires the use of additional tissues to properly reconstruct structures and vessels. While *in vivo* materials may often be used, certain surgical procedures or repeated surgeries may exhaust the available *in vivo* tissues necessary for proper reconstruction. As an alternative to native tissues, decellularized extracellular matrix (CorMatrix) derived from porcine small intestinal submucosa has been developed and successfully deployed for over 10 years within adult patients [1–3]. For example, CorMatrix, used as a pericardial sac patch, was associated with a twofold reduction in incidence of atrial fibrillation following open-heart surgery in adults [4]. Additionally, CorMatrix has been employed for repair of valvular disease within adult patients [5].

Repair of congenital heart defects, which often includes multiple procedures within the first year of life, requires the use of graft material that can accommodate the growth and development of cardiac structures. Clinically, CorMatrix has been demonstrated by multiple investigators to be a satisfactory material for this purpose, providing a material that remains flexible, resistant to tearing and calcification, and allows for the expansion of cardiovascular structures as the patient ages [6]. With increasing use of CorMatrix in pediatric populations [7,8], reports have been published examining the histologic features of CorMatrix following implantation. These reports present evidence that the bioscaffold material is associated with a chronic inflammatory response, including foreign-body giant cell reaction [9–11]. In contrast to animal models, these investigators did not see tissue integration or recellularization of patch material. Within murine models, extracellular graft material is capable of being resorbed nearly completely after 180 days following implantation [12].

Here, we present the histologic features of CorMatrix used in the reconstruction of great vessels at increasing time intervals, following repair of congenital heart defects, in tissues taken from autopsy specimens. We also provide examples of CorMatrix used to repair different sites within the cardiovascular system, within the same patient, exemplifying the site-specific response to the implanted material. Overall,

☆ Conflicts of interest and source of funding: The authors have no financial interests or significant relationships with commercial entities pertaining to this manuscript. Funding for this project was provided by the Department of Pathology and Microbiology, University of Nebraska Medical Center. The authors of this manuscript have no financial conflicts of interest to declare.

* Corresponding author.
E-mail addresses: jcox@unmc.edu (J.L. Cox), jmhammel@unmc.edu (J.M. Hammel), sradio@unmc.edu (S.J. Radio).

https://doi.org/10.1016/j.carpath.2018.12.003
1054-8807/

Table 1
Patient demographics

Patient ID	Gender	Age	Implant age/location					Clinical history
			Great vessels	Atrial septum	Aortic valve	Mitral valve	Coronary artery	
A	Female	1 Week	1 Week					Pulmonary valve/artery hypoplasia
B	Female	2 weeks	2 weeks	2 weeks				Truncus arteriosus
C	Male	5 weeks	3 weeks					Aortic stenosis and hypoplastic aortic arch
D	Male	3 months	3 months					Atrioventricular septal defect
E	Female	4 months	4 months			1 Month		VSD, aortic stenosis and arch hypoplasia, dysplastic mitral/tricuspid valves, PDA, atrial septal defect
F	Female	5 months	5 weeks					Heterotaxy syndrome with transposition of the great vessels
G	Female	15 months	3 months	6 months				Hypoplastic left heart syndrome with aortic and mitral stenosis
H	Female	15 months	15 months					Atrioventricular septal defect and coarctation of the aorta
I	Male	6 Years			3 months		3 months	Congenital heart disease including aortic valve stenosis
J	Male	26 months	26 months			5 months		Hypoplastic left heart syndrome

our observations support that CorMatrix used to repair great vessels remains acellular and it serves as a scaffold onto which new tissue forms. Together, these data provide a framework for interpretation of CorMatrix histology in a pediatric population.

2. Materials and methods

The demographics of patients examined in this study are summarized in Table 1. Briefly, patients (n=10) included in this study were selected based upon their history of congenital heart defect, as well as repair of defects using CorMatrix (CorMatrix, Roswell, GA). Patient's ages ranged from 1 week to 6 years. Samples were collected during autopsy examination. Sites of CorMatrix implantation were intact and without defect. As indicated in Table 1, the majority of samples collected were from great vessels (aorta and pulmonary artery) augmented with CorMatrix. Implant duration was calculated based upon the time interval from the surgical procedure in which CorMatrix was used until the patient's date of death.

Sections taken were fixed in 10% formalin, embedded in paraffin, and used to prepare hematoxylin and eosin (H&E) and special stains (e.g., Movat pentachrome, Masson trichrome, Verhoeff van Gieson elastin [VVG]) by the histology laboratory at The Nebraska Medical Center. Immunoperoxidase stains were conducted according to standard laboratory protocols. Photomicrographs were captured using an iScan Coreo Au Scanner and iScan Coreo 3.4.0 software (Ventana Medical Systems, Tucson, AZ, USA).

3. Results

3.1. CorMatrix histology

Histologic examination of CorMatrix implanted adjacent to native great vessel in vivo demonstrates a regular pattern of overall anatomy and host response (Fig. 1). After approximately 5 weeks of implantation, the layers of the aorta (tunica intima, tunica media and tunica adventitia) are readily identified on H&E stained sections. At the junction between the native tissue and the CorMatrix, foreign debris and calcification can be seen, consistent with the suture line used to join the two structures. Moving on to the CorMatrix, endothelial (highlighted by CD31) and smooth muscle cells (highlighted by vimentin and smooth muscle actin) are seen spreading onto the intimal surface of the graft material. Similarly, cells and tissues associated with the adventitia can be seen migrating onto the outer surface of the CorMatrix. Special stains for elastin fibers (VVG, Movat) highlight the tunica media of the native vessel. Interestingly, the CorMatrix is void of immunoreactive elastin fibers, following recent (e.g., <12 months) implantation of CorMatrix. A variably intense, but generally mild inflammatory reaction and mononuclear cell infiltration is seen at the interface between the CorMatrix and the native vessel. In particular, a CD68 positive monocyte infiltrate can be seen at the sutured junction of native vessel and CorMatrix. Examination of an additional example of CorMatrix implanted for 5 months reveals similar CD68 findings, and immunoperoxidase staining for CD3 reveals a mild infiltration of T-cells (data not shown). Marked inflammatory infiltration of the issues surrounding the CorMatrix material was not observed in any of the great vessel/CorMatrix anastomoses examined.

3.2. Histologic changes in CorMatrix over time

Great vessel/CorMatrix anastomoses implanted for various lengths of time were examined histologically and compared at low and high power (Figs. 2A, 3, Supplemental Fig. 1). Specifically, sections of aorta, repaired with CorMatrix were sectioned and stained with routine H&E, as well as with VVG-elastin, Movat pentachrome, Masson trichrome, and/or CD31 stains to highlight elastin/collagen fibers and endothelial cells, respectively. Endothelial cells are seen on to the surface of the CorMatrix graft after only 1 week of implantation, and with increasing duration (3 months), small CD31 capillaries are seen in the tissue developing on the luminal surface of the CorMatrix material (Fig. 3A). Accordingly, quantitation of intimal thickness measured after increasing duration of implantation demonstrate a progressive thickening of the intimal layer (Fig. 2B). The majority of cases examined lack elastin fiber staining in the tissue covering the luminal aspect of CorMatrix graft; however, at the longest time points examined (e.g., more than 2 years), a limited amount of fine elastin fibers are identified in the tissue lining the intimal surface of the CorMatrix graft (Fig. 3B). Additionally, the CorMatrix begins to degrade to where little of the original structure of the graft can be seen, and only focal areas of acellular collagen can be identified (Figs. 2A and 3B).

3.3. Variation of CorMatrix tissue response depending on anatomic location

Many additional locations within the cardiovascular system have proven amenable to repair with CorMatrix. We examined samples taken from aortic and mitral valves, atrial septal defects and coronary arteries (Fig. 4). Similar to the histologic features observed in patches of the great vessels, intimal cells adjacent to the graft material migrate on to the surface of the CorMatrix, and the CorMatrix is largely devoid of elastin staining, though deposition of elastin fibers is seen in the tissue overlaying the CorMatrix graft after 5 months of implantation. Of interest, graft material implanted in place of valve leaflets and as septal defect patches demonstrate mild chronic inflammation, which was usually increased as compared to the grafts located in the great vessels. Interestingly, the patient depicted with the coronary artery graft also had a concurrent repair of the aortic valve, which did not show significant inflammatory change, suggesting the response to the CorMatrix was site specific.

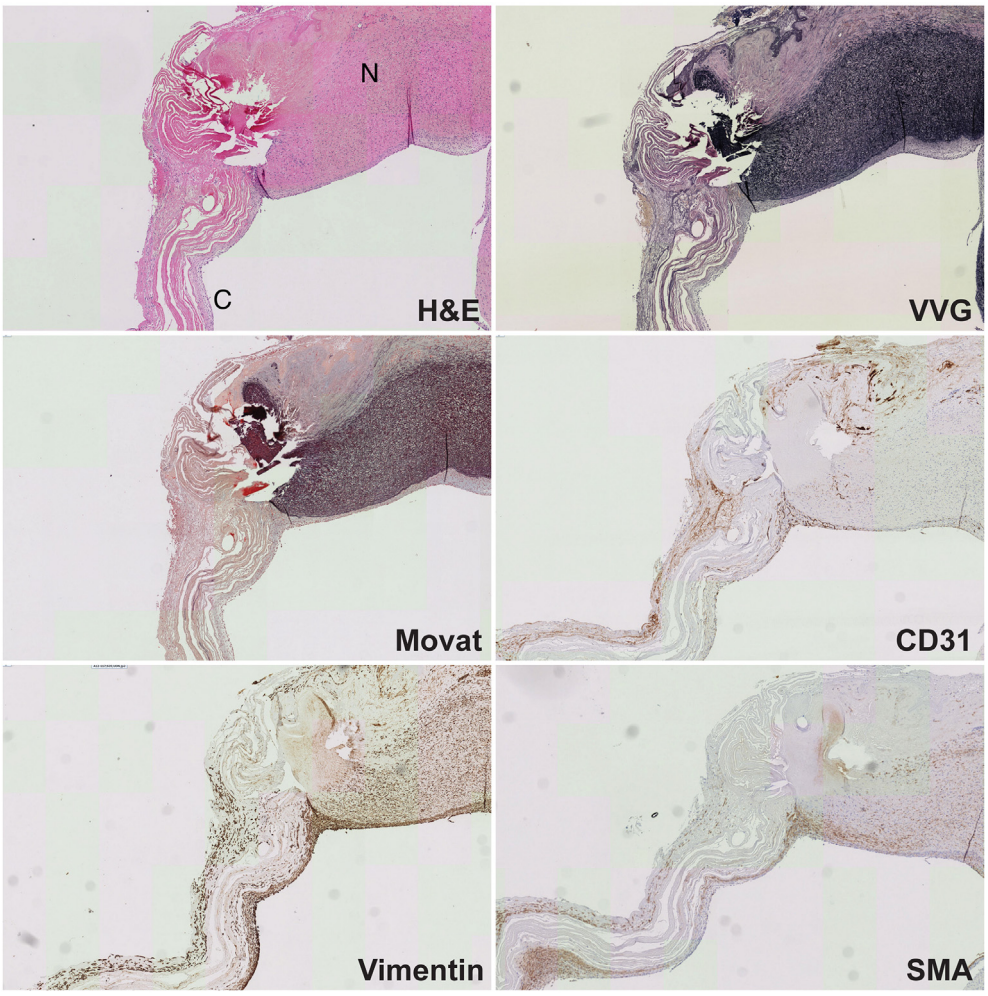


Fig. 1. Anatomy of aorta and ECM anastomosis. Pictured are photomicrographs of aorta and ECM graft junction from Patient C (graft age: 3 weeks). Stains and immunoperoxidase stains are indicated in the bottom right of each photograph. CorMatrix material is denoted with 'C' and native tissue is denoted with 'N'.

4. Discussion

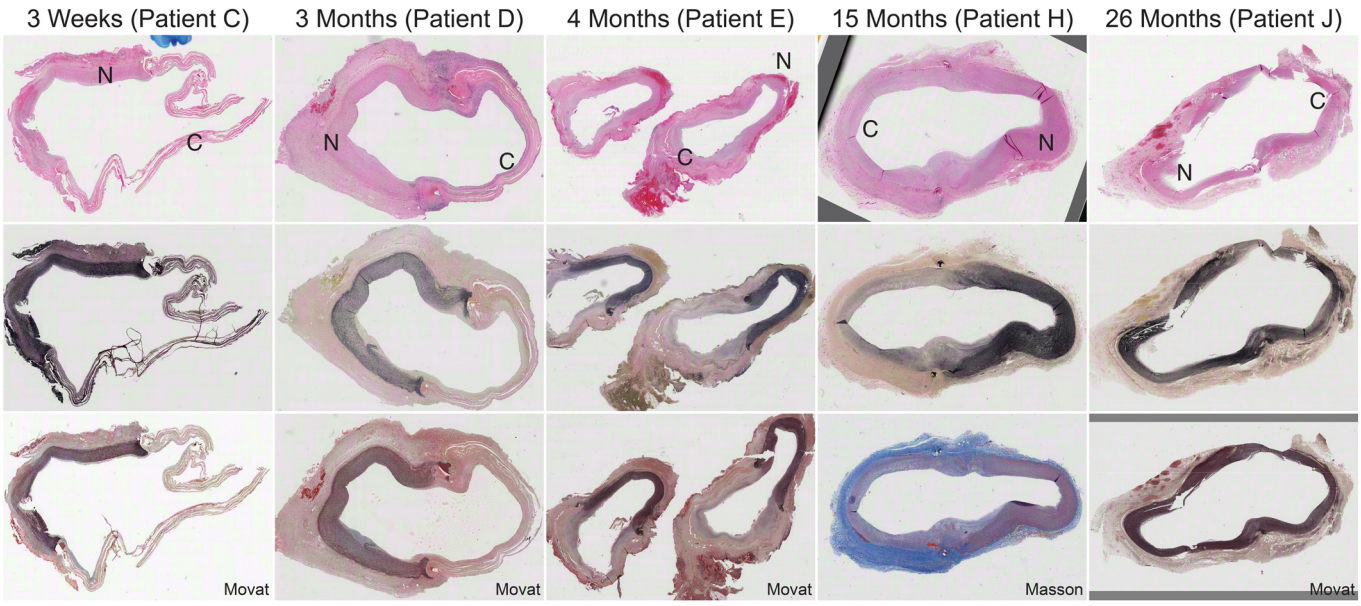
CorMatrix has proven to be a useful tool during the repair of cardiovascular structures in both pediatric [7,8,13,14] and adult populations [4,15,16]. As the use of this material becomes more widespread in the population, and as CorMatrix is employed in ever more diverse anatomic locations, understanding the characteristic histologic features to then identify potential abnormalities will become an important practice going forward.

Currently in our and affiliated institutions, CorMatrix is most commonly employed during the repair of great vessel abnormalities in a pediatric population. Within this group, CorMatrix serves as a scaffolding for new tissues to grow and develop. These layers then progressively thicken as the graft ages, similar to vascular graft healing seen with other graft materials, which are termed 'neo-intima,' in the review by L.M. Buja and F.J. Schoen [17]. In one of the cases presented here, after 2 years of implantation, elastin fibers are seen in the tissue developing on the intimal surface of the graft. This development corresponds with a progressive, though delayed, dissolution of the CorMatrix. Overall, the CorMatrix appears to accommodate the requirement of continued anatomic development over time, such that the grafted vessel may continue to expand as metabolic demands increase downstream of the graft. Interestingly, CorMatrix appears to undergo a similar progression in most other cardiovascular sites examined, including atrial septum and aortic and pulmonary valve leaflets. However, our data indicate that all anatomic sites may not be as equally receptive to CorMatrix implantation.

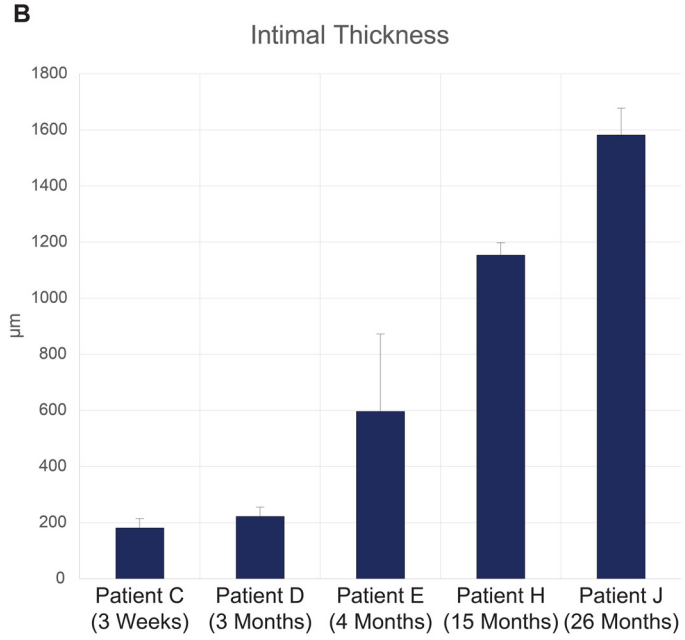
Our observations suggest that the host inflammatory response to CorMatrix is limited in autopsy cases with well-functioning great vessel grafts. However, CorMatrix implantation in certain anatomic locations may be associated with marked tissue inflammation [9,10], especially in cases where redo procedures have been performed [16]. With regard to this study, CorMatrix grafted to coronary artery in our study led to profound inflammatory response in the surrounding tissues; remarkably, the same patient had CorMatrix used for aortic valve repair, which did not demonstrate the same degree of tissue inflammation. This discrepancy may be due to multiple factors. One possibility is increased surgical site trauma at the coronary bed, as compared to the aortic valve, which led to an overall picture of increased inflammatory response. Another is the use of CorMatrix in a site with an already pro-inflammatory milieu, such as the coronary vasculature. Others have demonstrated that implanted CorMatrix within reconstructed

Fig. 2. Tunica intima thickness in relationship to increasing implant duration. (A) Low-power photomicrographs of great vessel and ECM junction are pictured. Duration of in vivo implantation is listed above each column of photomicrographs, along with the corresponding patient (see Table 1). CorMatrix material is denoted with 'C' and native tissue is denoted with 'N'. H&E and VVG-elastin stains are pictured in the top and middle rows, respectively. Movat or Masson stained sections (as indicated) are included in the bottom row. Corresponding high-power images are available in Supplemental Fig. 1. (B) Intimal thickness following graft placement for increasing periods of time are displayed in the graph. The intimal layer (edge of endothelial surface to interior edge of CorMatrix) was measured from the above photomicrographs in multiple locations (n=4); independent measurements were averaged, and standard deviation is represented by the error bars.

A



B



C

	Intimal Thickness (µm, n=4)	Standard Deviation
Patient C (3 weeks)	180.75	33.08
Patient D (3 Months)	221.75	33.18
Patient E (4 Months)	596.5	275.80
Patient H (15 Months)	1153.25	44.21
Patient J (26 Months)	1581.75	95.21

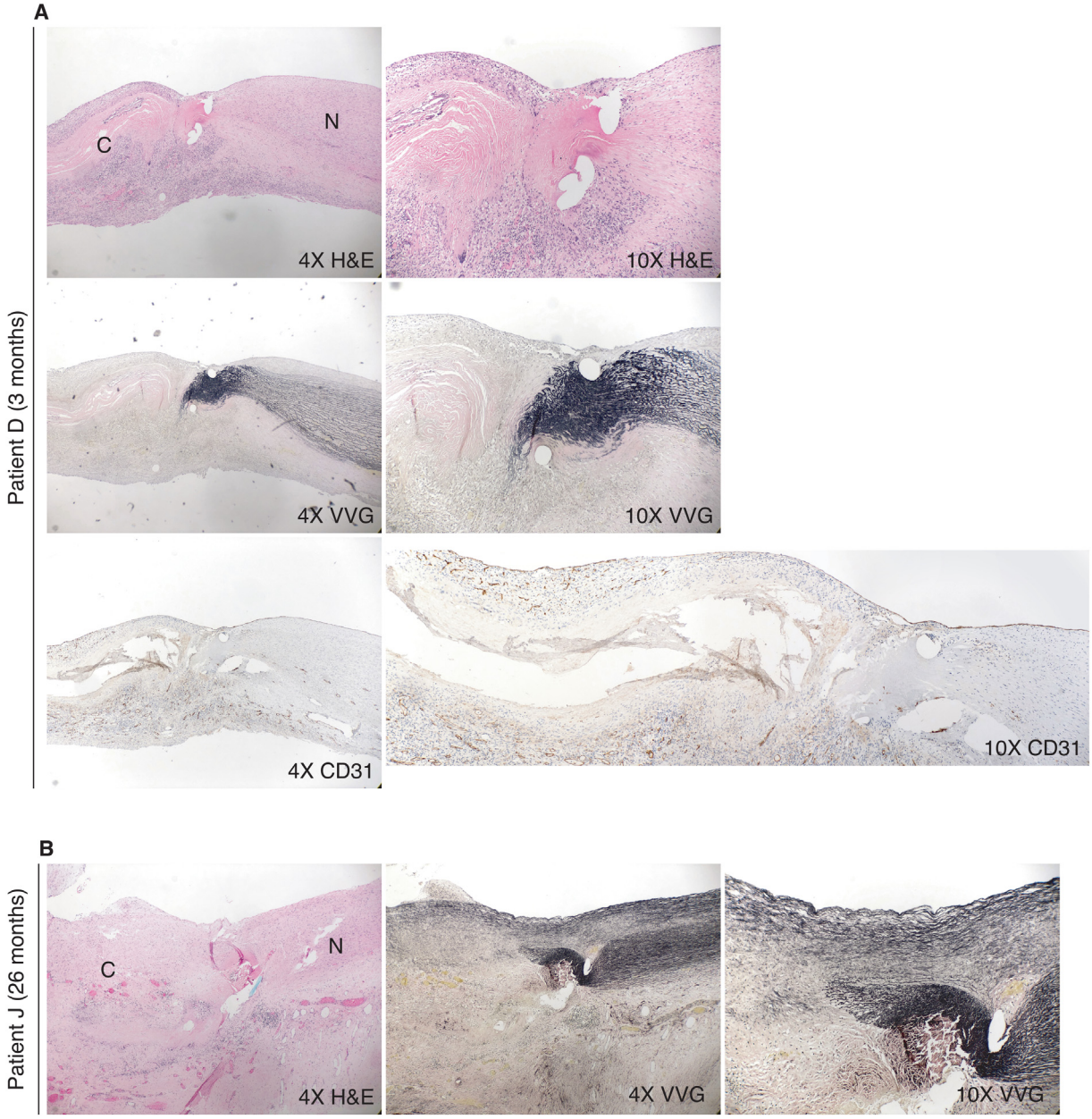


Fig. 3. Changes in luminal tissues covering CorMatrix over time. (A) Photomicrographs of H&E, VVG-elastin and CD31 stained sections of CorMatrix implanted for 3 months (Patient D). (B) Photomicrographs of CorMatrix/native tissue anastomosis implanted for 26 months stained by H&E and VVG-elastin (Patient J). CorMatrix material is denoted with 'C' and native tissue is denoted with 'N'.

valve leaflets is capable of inducing a significant inflammatory response [14]. The variation in inflammatory response to CorMatrix may be secondary, in part, to variable immunogenic response to the porcine epitopes within the xenograft material. The context in which CorMatrix material is histologically examined may also influence expectations for appropriate degree of inflammation.

The pattern of tissue migration and proliferation, with eventual dissolution of the CorMatrix and establishment of an elastin permeated tissue layer on the intimal surface of the CorMatrix graft is similar to findings reported by others in animal studies [6,18]. In seminal studies examining the use of CorMatrix to reconstruct pig pulmonary valve leaflets, histologic examination demonstrated that the CorMatrix supported the recapitulation of the native valve structures and eventual dissolution of CorMatrix within approximately 4 months of implantation [19]. In sheep, reconstruction of tricuspid valve leaflets demonstrated infiltration of host cells onto the atrialis of the CorMatrix bioprosthesis, with inflammatory infiltrate near the site of suture [6]. Additionally, the authors reported that elastin deposition was restricted to the cells which had migrated onto the CorMatrix, and elastin was not found within the CorMatrix itself after 12-months of implantation. Unlike what was seen in pig, significant deterioration of CorMatrix was not readily identified within sheep during the time points examined.

In vivo response to CorMatrix in human cardiac valve leaflets parallel the findings seen in sheep. In this regard, Zaidi and colleagues demonstrated that 'neointima' had formed on the surface of the CorMatrix, along with marked inflammation, with CorMatrix persisting at its nominal thickness after up to 261 days [14]. Examination of CorMatrix after longer implant duration suggests that CorMatrix can be degraded in non-porcine hosts. In addition to our findings presented above, pericardial repair within an adult patient, which remained in vivo for 5 years, demonstrated engulfment of the CorMatrix by native fibroblasts and mesothelial cells, and degradation of the CorMatrix in to rare foci of acellular collagen [1]. CorMatrix grafts may also undergo significant degradation depending upon the site of implantation. A report in

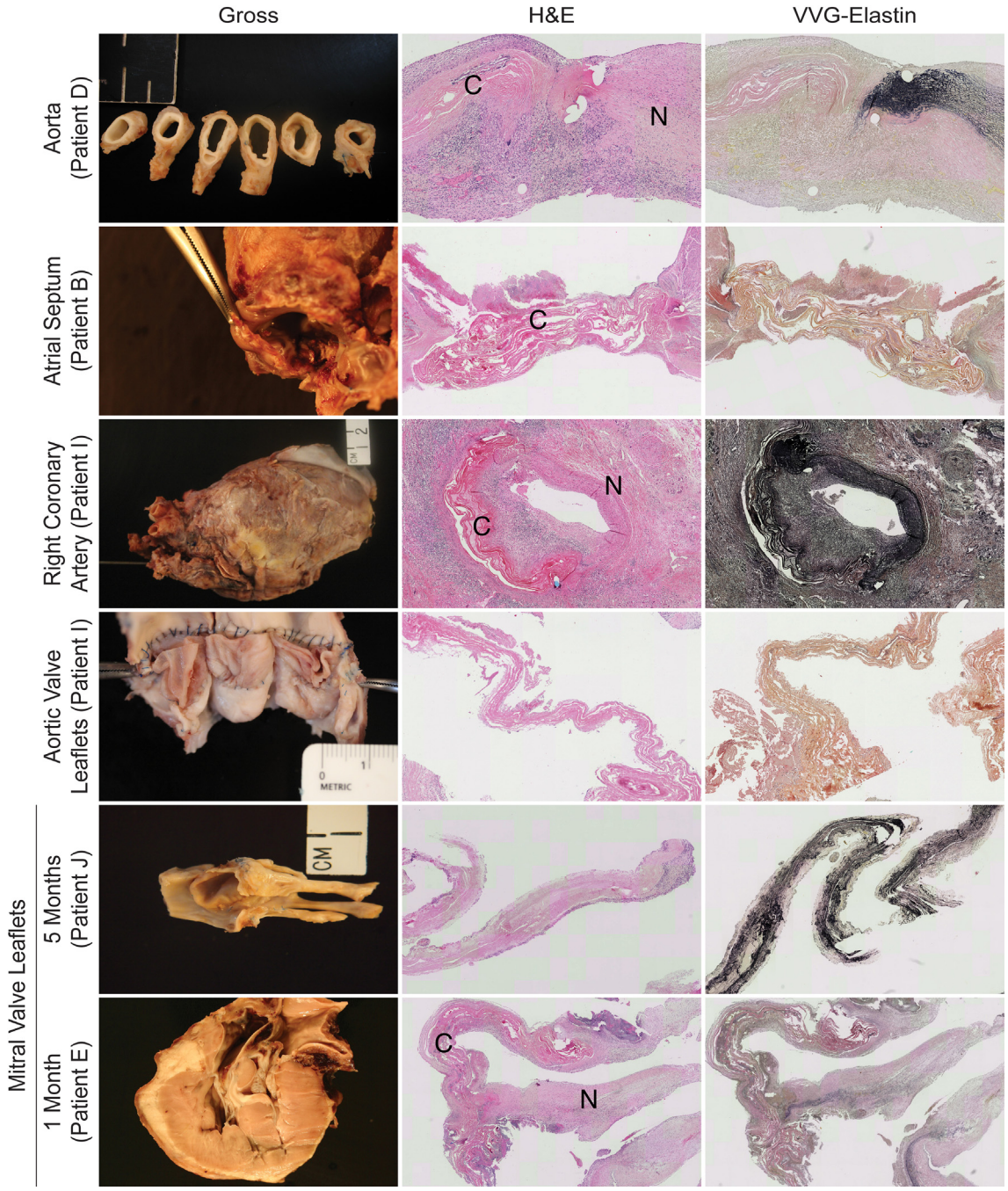


Fig. 4. Histology of CorMatrix implanted at different anatomic locations. Included are gross photographs and corresponding photomicrographs of CorMatrix material implanted into the indicated anatomic locations. CorMatrix material is denoted with 'C' and native tissue is denoted with 'N'. Refer to Table 1 for implant duration.

which CorMatrix was used to repair a unicuspid aortic valve described marked calcification and fibrosis necessitating a redo operation [20]. Thus, it appears that there is variability in the histologic appearance of CorMatrix implanted for longer periods of time.

5. Conclusion

Overall, the in vivo response to CorMatrix appears to follow a regular pattern of anatomic evolution, with variable inflammatory infiltrate seen depending upon anatomic location. Inflammatory response is a key attribute to identify, as profound inflammation in a grafted location without significant trauma may indicate a hypersensitivity reaction and preclude the future use of CorMatrix for that patient. Together, our findings and the observations by others suggest that CorMatrix may provide an adaptable material for the in growth of native tissues and reconstruction of cardiovascular structures.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carpath.2018.12.003.

References

[1] Stelly M, Stelly TC. Histology of CorMatrix bioscaffold 5 years after pericardial closure. Ann Thorac Surg 2013;96:e127–9. https://doi.org/10.1016/j.athoracsur.2013.06.114.
[2] Slachman FN. Constructive remodeling of CorMatrix extracellular matrix after aortic root repair in a 90-year-old woman. Ann Thorac Surg 2014;97:e129–31. https://doi.org/10.1016/j.athoracsur.2013.10.103.
[3] Wallen J, Rao V. Extensive tricuspid valve repair after endocarditis using CorMatrix extracellular matrix. Ann Thorac Surg 2014;97:1048–50. https://doi.org/10.1016/j.athoracsur.2013.05.117.

[4] Boyd WD, Johnson WE, Sultan PK, Deering TF, Matheny RG. Pericardial reconstruction using an extracellular matrix implant correlates with reduced risk of postoperative atrial fibrillation in coronary artery bypass surgery patients. Heart Surg Forum 2010;13:E311–6. https://doi.org/10.1532/HSF98.20091184.
[5] Gerdisch MW, Shea RJ, Barron MD. Clinical experience with CorMatrix extracellular matrix in the surgical treatment of mitral valve disease. J Thorac Cardiovasc Surg 2014;148:1370–8. https://doi.org/10.1016/j.jtcvs.2013.10.055.
[6] Fallon AM, Goodchild TT, Cox JL, Matheny RG. In vivo remodeling potential of a novel bioprosthetic tricuspid valve in an ovine model. J Thorac Cardiovasc Surg 2014;148:333–340.e1. https://doi.org/10.1016/j.jtcvs.2013.10.048.
[7] Witt RG, Raff G, Van Gundy J, Rodgers-Ohlau M, Si M-S. Short-term experience of porcine small intestinal submucosa patches in paediatric cardiovascular surgery. Eur J Cardiothorac Surg 2013;44:72–6. https://doi.org/10.1093/ejcts/ezs638.
[8] Scholl FG, Boucek MM, Chan K-C, 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;1:132–6. https://doi.org/10.1177/2150135110362092.
[9] Woo JS, Fishbein MC, Reemtsen B. Histologic examination of decellularized porcine intestinal submucosa extracellular matrix (CorMatrix) in pediatric congenital heart surgery. Cardiovasc Pathol 2016;25:12–7. https://doi.org/10.1016/j.carpath.2015.08.007.
[10] Hofmann M, Schmiady MO, Burkhardt BE, Dave HH, Hübler M, Kretschmar O, et al. Congenital aortic valve repair using CorMatrix®: a histologic evaluation. Xenotransplantation 2017;24:e12341. https://doi.org/10.1111/xen.12341.
[11] Bustamante-Munguira J, Serrano R, Figuerola-Tejerina A, Aguilar-Blanco EM, Calzada M Is. CorMatrix really a recellularization scaffold as a pericardial substitute in cardiac surgery? J Cardiovasc Surg (Torino) 2018;59:142–3. https://doi.org/10.23736/S0021-9509.16.09763-9.
[12] Padalino MA, Castellani C, Dedja A, Fedrigo M, Vida VL, Thiene G, et al. Extracellular matrix graft for vascular reconstructive surgery: evidence of autologous regeneration of the neoaorta in a murine model. Eur J Cardiothorac Surg 2012;42:e128–35. https://doi.org/10.1093/ejcts/ezs462.
[13] 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;13:569–72. https://doi.org/10.1510/icvts.2011.280016.
[14] Zaidi AH, Nathan M, Emani S, Baird C, del Nido PJ, Gauvreau K, 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. https://doi.org/10.1016/j.jtcvs.2014.02.081 [2216–4–2225.e1].
[15] Badylak S, Kokini K, Tullius B, Simmons-Byrd A, Morff R. Morphologic study of small intestinal submucosa as a body wall repair device. J Surg Res 2002;103:190–202. https://doi.org/10.1006/jsre.2001.6349.
[16] Rosario-Quinones F, Magid MS, Yau J, Pawale A, Nguyen K. Tissue reaction to porcine intestinal submucosa (CorMatrix) implants in pediatric cardiac patients: a single-center experience. Ann Thorac Surg 2015;99:1373–7. https://doi.org/10.1016/j.athoracsur.2014.11.064.
[17] Butany J, Buja LM. Cardiovascular pathology. Academic Press; 2015.
[18] Fallon A, Goodchild T, Wang R, Matheny RG. Remodeling of extracellular matrix patch used for carotid artery repair. J Surg Res 2012;175:e25–34. https://doi.org/10.1016/j.jss.2011.11.001.
[19] Matheny RG, Hutchison ML, Dryden PE, Hiles MD, Shaar CJ. Porcine small intestine submucosa as a pulmonary valve leaflet substitute. J Heart Valve Dis 2000;9:769–74 [discussion774–5.
[20] Mosala Nezhad Z, Baldin P, Poncelet A, Khoury El G. Calcific degeneration of CorMatrix 4 years after Bicuspidization of Unicuspid aortic valve. Ann Thorac Surg 2017;104:e431–3. https://doi.org/10.1016/j.athoracsur.2017.07.040.