Comparing the Host Reaction to CorMatrix and Different Cardiac Patch Materials Implanted Subcutaneously in Growing Pigs

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Abstract

Background Comparing the structural changes, and local host reactions to CorMatrix (CorMatrix Cardiovascular Inc., Roswell, Georgia, United States) and different biomaterials implanted subcutaneously in growing pig model.

Methods Four pigs harboring implanted patches of CorMatrix, Vascutek porcine pericardium (Vascutek; Scotland, United Kingdom), SJM bovine pericardium (St. Jude Medical, Inc., Minnesota, United States), and Gore-Tex (W. L. Gore & Associates GmbH, Flagstaff, Arizona, United States) were studied for 1, 3, 6, and 12 months. The explants were examined histologically.

Results CorMatrix showed gradual and consistent patch resorption and subsiding inflammatory and fibrosis process. Full scaffold degradation and replacement by mild fibrosis and subcutaneous tissue were seen by 1 year. Xenopericardial patches remained intact, and the initially severe inflammatory and fibrotic reactions reduced gradually to moderate fibrosis and chronic inflammation. Gore-Tex showed foreign body reaction.

Conclusions Patches were biotolerated by pigs. Xenopericardial patches elicited encapsulating fibrosis and no remodeling. CorMatrix resorbs completely and degrades consistently without leaving residues. Lack of encapsulating fibrosis toward CorMatrix allows tissue ingrowth and matrix remodeling.

Keywords
- CorMatrix
- pericardium
- experimental study

Introduction

The development of tissue-engineered acellular extracellular matrices (ECM) has delivered various commercial patch materials that vary in terms of composition, processing, preservation techniques, in vivo reactions, and remodeling potential. The ECM provides structural support and regulates various functions.[1] Acellular xenogeneic ECM materials have been used as patch substitutes for cardiovascular repair.[1]

Here, we compared the in vivo reaction of SIS-ECM (CorMatrix; CorMatrix Cardiovascular Inc., Roswell, Georgia, United States) to other commercial biomaterials in the subcutaneous space of growing pig model, hypothesizing that CorMatrix: (1) is optimally nonimmunogenic by being

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acellular; (2) permits cellular attraction and ingrowth through degradation and new tissue formation without leaving residues; (3) remodels without encapsulation. Other biological materials are expected to initiate fibrotic encapsulation.

Materials and Methods

Patch Materials

Four patches were evaluated: (1) Gore-Tex preclude pericardial membrane (W. L. Gore & Associates GmbH, Flagstaff, Arizona, United States): an e-PTFE (expanded polytetrafluoroethylene) membrane (0.1 mm thick);[2] (2) SJM bovine pericardium (SJM pericardial patch with EnCap technology, St. Jude Medical, Inc., Minnesota, United States): glutaraldehyde-fixed and treated bovine pericardium;[3] (3) Vascutek porcine pericardium (Vascutek; Scotland, United Kingdom): an ultrathin (0.32 mm) flexible glutaraldehyde-fixed and preserved porcine patch; (4) CorMatrix: porcine SIS-ECM designed for cardiac repair.[4]

Surgery

Four miniature pigs (28.8 ± 2.3 kg) were studied for 1, 3, 6, and 12 months, respectively, each harboring the four patches. Perioperative care was discussed by Mosala Nezhad et al,[5] and the local ethics committee approved the study (2010/UCL/MD/036). Patches were prepared according to the manufacturers' instructions, cut to 4 × 4 cm, and implanted separately into subcutical flaps via small, right-sided skin incisions. Patches were fixed at the corners with 4–0 Prolene (Ethicon Inc.) for identification. Animals were euthanized by T61 injection. Materials were explanted in one block, inspected, and divided into two equal parts along the long axis and fixed in formalin for histological processing and analysis. Specimens were coded for blind evaluation by a histopathologist.

Histology and Immunohistochemistry

Controls: Off-the-shelf patches were first examined histologically to characterize "normal" structures.

Explants: Samples were processed using standard techniques.[5] 5 µm thick sections were stained with hematoxylin and eosin, Van Gieson for connective tissue, and von Kossa for mineralization. Immunohistochemistry (IHC) was performed with antibodies targeting macrophages (CD163; LSBio, Seattle, Washington, United States; calprotectin clone MAC387; Abcam, Cambridge, United Kingdom), T lymphocytes (CD3; Thermo Fisher Scientific, Waltham, Massachusetts, United States), α smooth muscle actin (α-SMA; Biogenex, Fremont, California, United States), and proliferation (Ki67; Dako, Glostrup, Denmark). We used a semiquantitative numerical scale for grading (1, mild; 2, moderate; and 3, severe).[5]

Results

Controls

Gore-Tex was amorphous synthetic material (►**Fig. 1A**). Pericardial patches showed three well-defined pericardial layers, with bovine pericardium's collagen bundles larger and coarser than those in the porcine pericardium (►**Fig. 1B**), the latter composed of dense collagenous tissue containing fibroblasts and rare vessels (►**Fig. 1C**). CorMatrix was composed of separated parallel collagen bundles containing a few recognizable nuclei, fibroblasts, and small vessels (►**Fig. 1D**).

Explant Microscopy

The findings are summarized in ►**Figs. 2** and **3** and ►**Table 1**.

Gore-Tex: At 1 month, Gore-Tex was visible as exogenous material surrounded by dense fibrous tissue containing an abundant inflammatory infiltrate (2 +) composed of numerous hemosiderin-laden macrophages and lymphocytes. There was a histiocytic reaction with giant cells around the patch, mostly peripherally. At 3 months, there was an early formation of a fibrotic capsule containing lymphocytes, plasma cells, hemosiderin-laden macrophages, a few neutrophils, and ancient steatonecrosis. At 6 months and up to 1 year, the patch appeared as exogenous material delimited by fibrous tissue with residual mild chronic inflammation and giant cells (►**Fig. 2**).

SJM: At 1 month, bovine pericardium appeared as amorphous eosinophilic material adjacent to dense fibrous tissue containing an inflammatory infiltrate composed of hemosiderin-laden macrophages, lymphocytes, and neutrophils, similar to the Gore-Tex reaction, but less than other patches. Few giant cells were visible surrounding the patch (►**Fig. 2**). Fibrosis was moderate (2 +). At 3 months, the fibrosis remained moderate (2 +) and encapsulated the patch, with persistent moderate inflammation (2 +) with lymphocytes, eosinophils, and a few plasma cells and multinucleate giant cells. At 6 months and 1 year, fibrosis remained reduced, and inflammation persisted with lymphocytes and giant cells, the patch appearing slightly fragmented (►**Fig. 2**).

Vascutek: At 1 month, the patch appeared as eosinophilic material surrounded by fibrous tissue (2 +) containing a severe inflammatory infiltrate (3 +) with giant cells, lymphocytes, eosinophils, and neutrophils (►**Fig. 2**). At 3 months, there was moderate but more organized fibrosis (2 +), moderate perivascular inflammation (2 +) with lymphocytes and hemosiderin-laden macrophages, and a few residual foreign body giant cells around the patch. At 6 months and 1 year, no patch changes, moderate fibrosis, mild inflammation (1 +) with rare giant cells (►**Fig. 2**).

CorMatrix: At 1 month, residual CorMatrix was surrounded by dense fibrous tissue (3 +), and a severe inflammatory infiltrate (3 +) containing lymphocytes, plasma cells, eosinophils, and hemosiderin-laden macrophages (►**Fig. 3**). By 3 months, there was partial patch resorption with a reduction in fibrosis and inflammation (2 +), essentially perivascular. At 6 months, the patch had resorbed making it difficult to identify, and inflammation had further reduced to mild chronic (1 +) with residual perivascular infiltrates containing hemosiderin-laden macrophages and scattered but moderate fibrosis (2 +). At 1 year, the patch was not visible with the area only identifiable by the presence of residual macrophages and fibrosis at the implantation site.

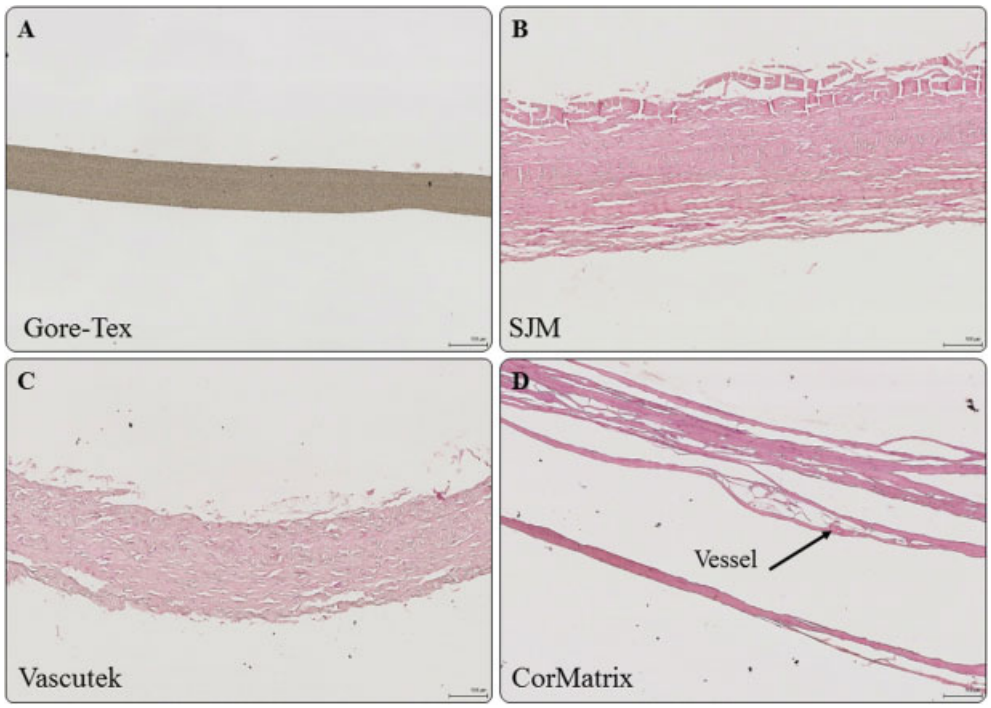

Fig. 1 Patch histology (controls). (**A**) Gore-Tex appearing as gray amorphous tissue. (**B** and **C**) Bovine and porcine pericardium, respectively, both are glutaraldehyde fixed and have three layers: (1) serosa, with a mesothelial cell surface layer and a narrow submesothelial space; (2) fibrosa, containing variously oriented layers of collagen fibrils and small elastic fibers; (3) epipericardial connective tissue layer, containing mainly large coarse bundles of collagen forming part of the pericardiosternal ligament. (**C**) The porcine pericardium is composed of more dense collagenous tissue containing fibroblasts and rare vessels. (**D**) CorMatrix shows lamellar collagenous tissue with fibroblasts and rare vessels with many nuclei seen.

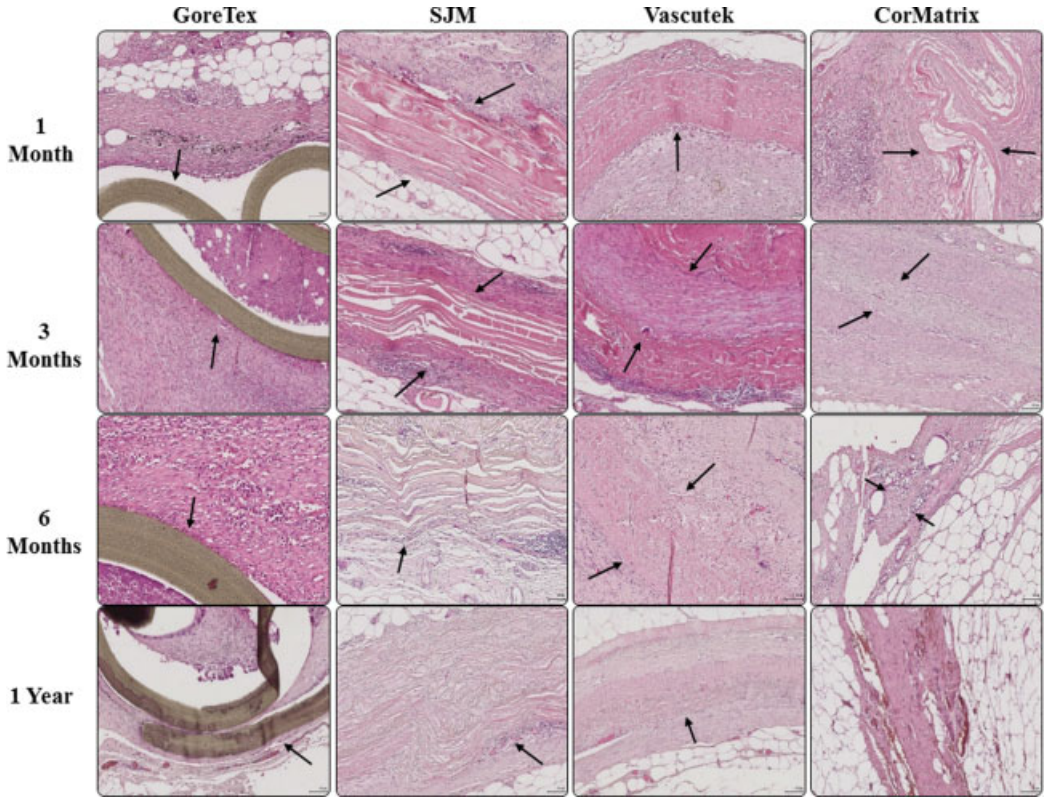

Fig. 2 Photomicrographs of ×10 hematoxylin and eosin-stained sections of all patches throughout the study period. Gore-Tex: Exogenous material delimited by fibrous tissue tending to diminish with time and residual chronic inflammation and giant cells around the patch. SJM: Little fibrosis, persistent lymphocytic infiltrate, patch slightly fragmented at 1 year. Vascutek: Encapsulated with severe fibrosis that reduced after 3 months. CorMatrix: Progressive patch degradation and resorption, no patch found at 1 year, only fat and residual fibrosis.

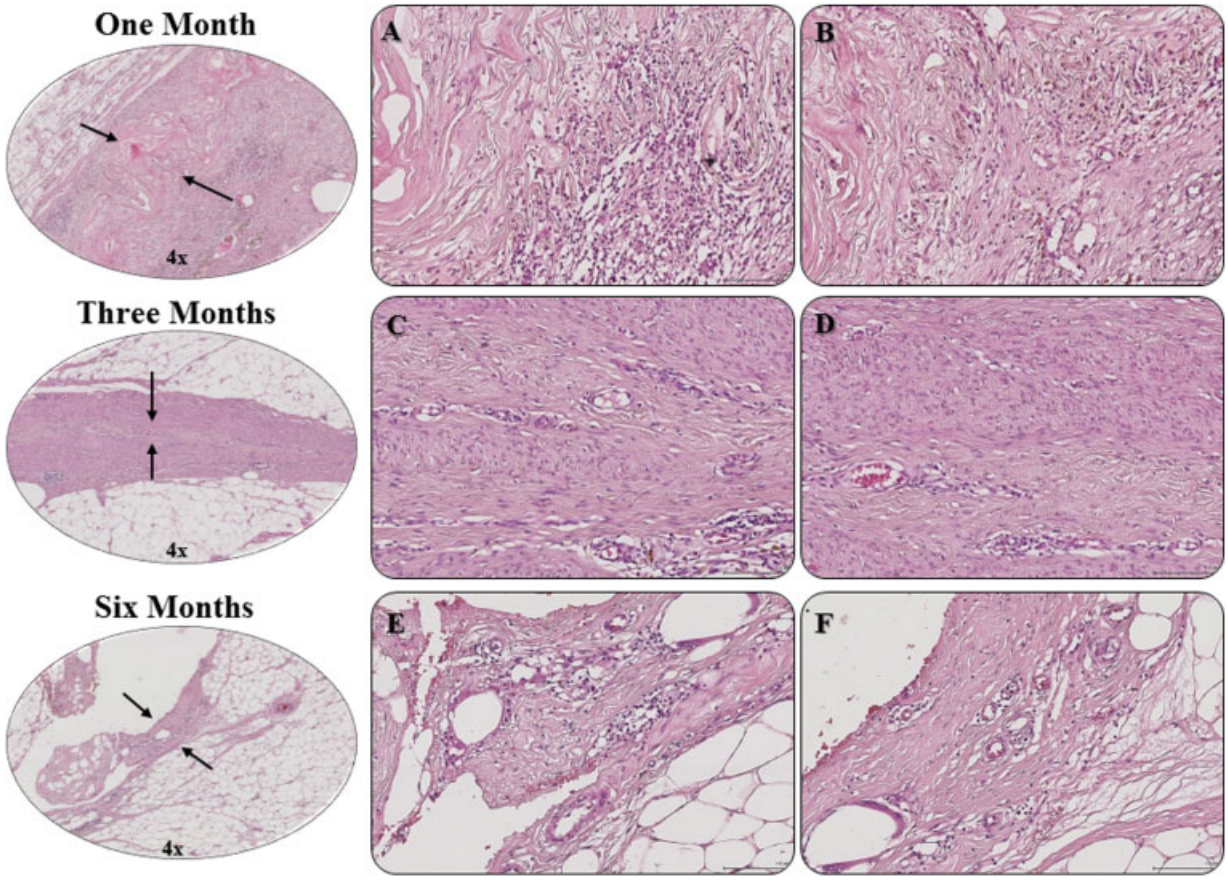

Fig. 3 CorMatrix explants at 1, 3, and 6 months. Hematoxylin and eosin stains at ×4 (circles) and ×20 magnification. At 1 month, the patch is still visible and surrounded by an intense inflammatory reaction. (**A**), (**C**), and (**E**) show predominantly lymphocytes that reduce in number over time but are still present at 6 months. (**B**), (**D**), and (**F**) show predominantly macrophages and neovessel formation that also reduce over time. The CorMatrix patch progressively degrades and resorbs and is replaced by subcutaneous tissue and fibrosis as shown in the left-hand side circles, with the patch area indicated by arrows.

Immunohistochemistry

IHC of tissue sections of CorMatrix at 1 month is shown in ►**Fig. 4**. The T-cell infiltrate (CD3+ cells) at 1 month was more severe than at 3 and 6 months, with a change in distribution from generalized to more intravascular or small peripheral aggregates. There was strong and progressive α-SMA positivity, indicating either focal smooth muscle differentiation or scar tissue formation. Ki67 staining for proliferation

Table 1 Inflammatory changes, fibrosis, and degradation of the different patch materials over time

	1 mo			3 mo			6 mo		
	Inflammatory cells	Fibrosis	Degradation	Inflammatory cells	Fibrosis	Degradation	Inflammatory cells	Fibrosis	Degradation
Gore-Tex	2+ Lymphocytes, giant cells, macrophages	2+	No	2+ Chronic, mainly neutrophils	2+ Encapsulating the patch	No	1+ Chronic	1+	No
SJM	2+ Lymphocytes, macrophages, neutrophils, giant cells	2+	No	1+ Chronic, lymphocytes eosinophils, macrophages	1+ Scar tissue	No	2+ Chronic, lymphocytes, giant cells	1+	Signs of early degradation
Vascutek	3+ Lymphocytes, eosinophils, macrophages, giant cells	3+	No	2+ Chronic. lymphocytes macrophages giant cells	3+ Encapsulating the patch	No	2+ Chronic, giant cells	3+	No
CorMatrix	3 + + Lymphocytes, plasma cells, eosinophils, macrophages, giant cells	3+	Partial	3+ Lymphocytes plasma cells macrophages, no giant cells	2+ No capsule	moderately degraded	3+ Macrophages	3+	Almost completely

Note: For CorMatrix the fibrosis and inflammation were significantly less (1), and it was fully degraded at 1 year.

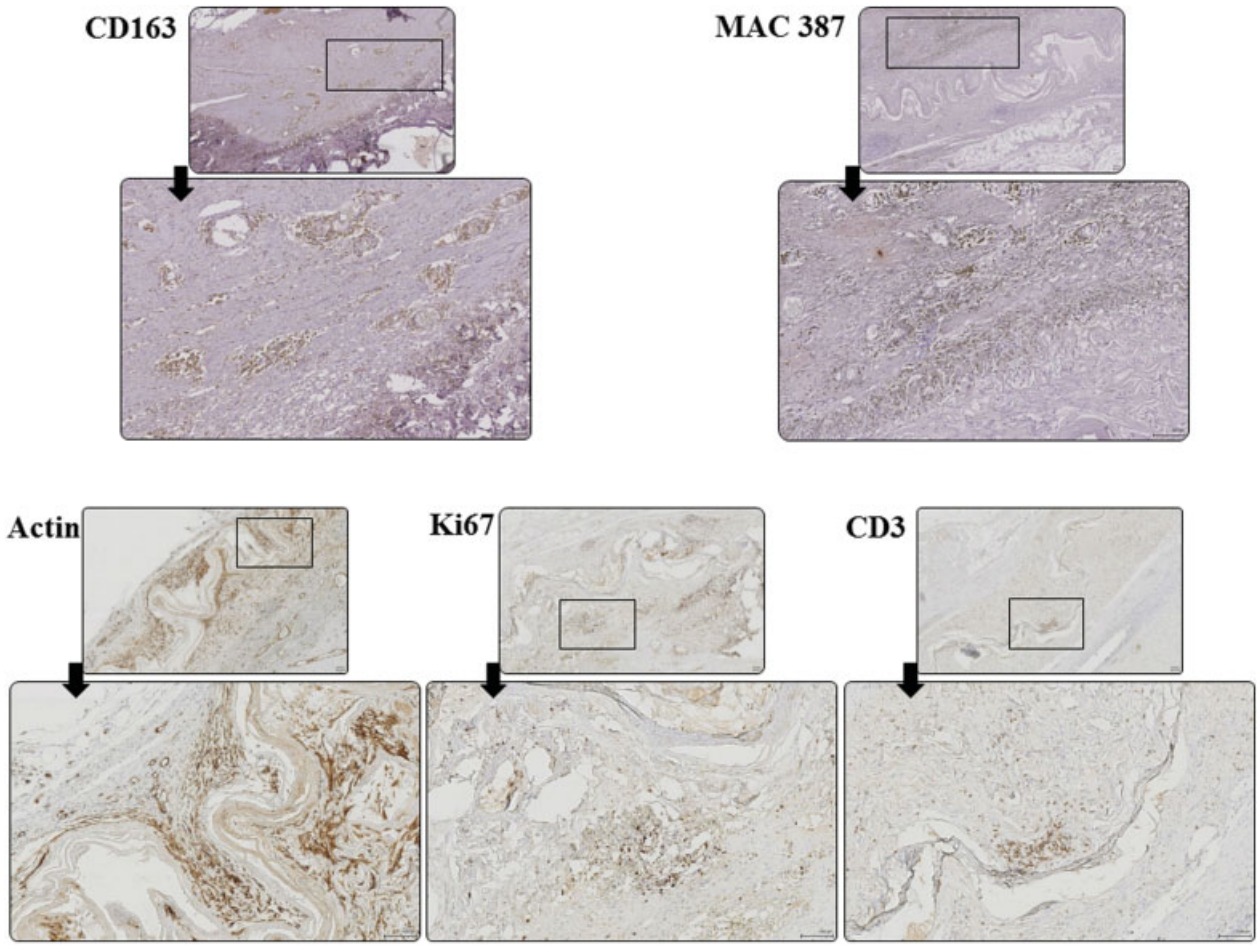

Fig. 4 Immunohistochemistry of CorMatrix explant at 1 month (×4 and ×10 magnification). Antimacrophage antibodies CD163 and MAC 387, SMA, Ki67, and CD3.

indicated high proliferation rate at one month that subsided to be sporadic and mainly intravascular later on. Antibodies targeting macrophages (CD163, calprotectin) were moderately positive with a variable distribution at one month that became mild and sporadic by three to six months.

Discussion

Outcomes from biomaterial use are related to the complex interactions between the scaffold's microenvironment, structure, and host responses. Decellularization and commercial processing influence the in vivo behavior and remodeling outcome. Appropriately configured materials can induce a shift in the healing response from inflammation and scar tissue formation to constructive remodeling and functional tissue restoration.[6] The transition from the initial acute inflammatory response against local injury to complete resolution is an active and highly coordinated process regulated by the immune system.[7] Although the exact inflammatory and healing pathways toward functional remodeling remain unestablished, the process is thought to proceed in phases from initial host response to final mature tissue. (1) An acute inflammatory reaction to the implanted material and release of enzymes that recruit cells, particularly neutrophils,[8] and the release of specific growth factors.[9,10] (2) Progression to chronic inflammation and fibrosis or scar tissue formation, at the end of which the patch would ideally be resorbed and replaced by new (albeit weak) tissue. (3) Remodeling of the matrix and the formed fibrosis with the help of endothelial and mesenchymal progenitor cells[8] into a newly differentiated matrix that matches the surrounding tissues. Vessels mature, leading to the final phase of functional tissue remodeling and ideally new and functional tissue.

We compared the composition of different commercial patches used for valve repair and then evaluated the differences in patch degradation and local host responses to CorMatrix and conventional glutaraldehyde-fixed xenopericardial patches. The key difference between the biomaterials used, apart from their origin, manufacturing, and preparation for use, is the local host response, and the remodeling of CorMatrix. We observed a general pattern of early-onset acute inflammation followed by fibrosis in all materials. CorMatrix showed a much more intense initial inflammatory response. Although there was also a fibrotic reaction to CorMatrix, it replaced parts of the sheet instead of surrounding it. The fibrosis encapsulated the other patches produced more of a foreign body reaction, even with allograft (Vascutek). The SJM patch showed evidence of late tissue degradation (after 6 months). Only CorMatrix showed progressive and complete resorption and degradation with the replacement of the scaffold with subcutaneous tissue and residual fibrosis. It showed consistent and progressive

remodeling starting with acute inflammation followed by fibrosis, neovessel formation, and then full replacement of the matrix with host tissue resembling the surrounding tissues. The resorption happened from the periphery to the center.

Invariably and regardless of the material used, the host responded with varying degrees of acute inflammation and fibrosis. Only CorMatrix, which is acellular and glutaraldehyde-free, was not encapsulated.

CorMatrix showed progressive and complete degradability, and the potential to remodel and resemble the surrounding tissues without leaving residues. All implanted biomaterials elicited a variable host tissue response depending on their type and manufacturing. CorMatrix appears to be as bioadaptable as cross-linked xenopericardium but without encapsulation. This study identifies the lack of fibrotic encapsulation reaction against CorMatrix, which facilitates cell migration and matrix degradation and subsequent new matrix formation.

Disclosure

Patch materials were donated with no influence on the study design or outcome. No commercial affiliation or conflict of interest.

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