

Evaluation of Explanted CorMatrix Intracardiac Patches in Children With Congenital Heart Disease

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Background. Animal data demonstrate that intracardiac patches of decellularized porcine small intestine submucosa (CorMatrix; CorMatrix Cardiovascular, Inc, Atlanta, GA) become repopulated with native cells, suggesting the possibility of a substrate for regenerative tissue in humans. We report the only prospective series to date of explanted CorMatrix patches placed in infants with congenital heart disease.

Methods. CorMatrix patches were implanted as the hemi-Fontan baffle in patients during their second stage of palliation. The patch material was explanted as part of the standard Fontan operation. Specimens were analyzed with the use of hematoxylin and eosin, Movat pentachrome, and trichrome stains.

Results. Of the 12 implantations, 10 specimens were explanted. Two patients did not undergo Fontan

because of unfavorable hemodynamics. Acellular material, chronic inflammation, fibrosis, and foreign body giant cell reaction was seen in all explanted patches. Also noted in explanted specimens was calcification (n = 2), elastic fibers (n = 2), and eosinophils (n = 2). No explanted CorMatrix material showed evidence of ingrowth of native cells or transformation into cardiac tissue at a median of 21 months after implantation.

Conclusions. Although the CorMatrix remained pliable and most did not exhibit calcification, these intracardiac patches did not show evidence of native heart tissue ingrowth at a median of 21 months in vivo.

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The ability to replace damaged or absent heart structures with the patient's own tissue remains one of the ultimate, but as of yet unachieved, goals of congenital heart surgeons. Regenerated native tissue, whether cultivated outside the body and implanted or developed over time on an implanted biological scaffold, would have many advantages. These advantages include durability and the potential for growth, important in the pediatric population. One such scaffold, porcine small intestinal submucosa extracellular matrix (SIS ECM), has shown promise in regenerating native tissue. The ECM is implanted and by a process of ingrowth of native cells may be theoretically transformed into native tissue. CorMatrix (CorMatrix Cardiovascular, Inc, Atlanta, GA) is a commercially available ECM product that is approved by the Food and Drug Administration for use as a pericardial replacement and as an intracardiac patch.

Animal studies have shown remodeling of CorMatrix patches into myocardial tissue with contractile properties

[1]. However, recent retrospective human studies have had disappointing results [2–4]. Of all available studies to examine explanted CorMatrix patches used for intracardiac use in congenital heart disease, none have shown native tissue ingrowth [2–5]. Prior studies are limited by their retrospective nature, heterogeneity of implant sites, and short times in vivo [2–4]. To our knowledge, the current study represents the first prospective histologic study of CorMatrix for intracardiac use in children with congenital heart disease.

The purpose of this study was to describe the histopathologic process of a prospective series of explanted CorMatrix patches used as a hemi-Fontan baffle in children undergoing routine single-ventricle palliation.

Material and Methods

The University of Michigan Medical School Institutional Review Board approved this study. Informed, written consent was obtained from all patients by their parents or legal representative.

Study Population

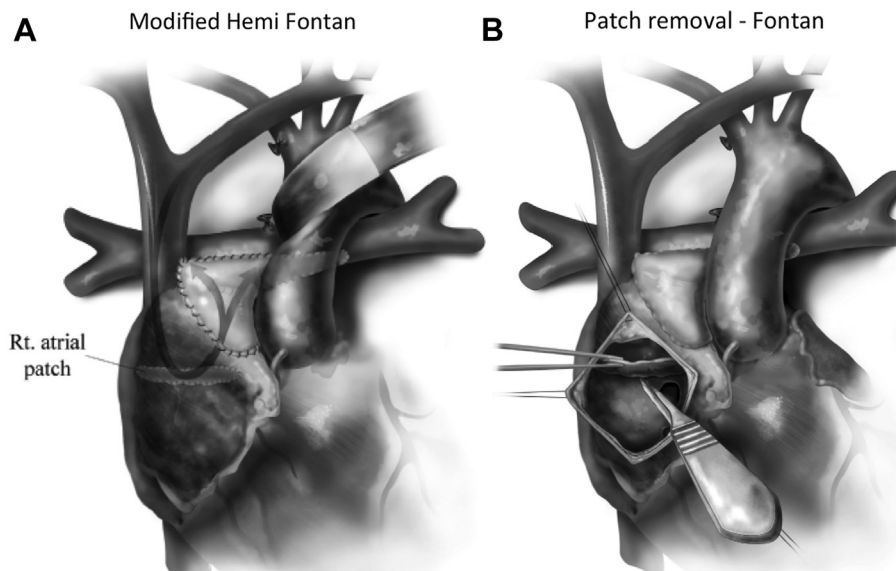
The medical records for consecutive patients scheduled for stage 2 palliation for a single-ventricle lesion between June 1, 2012, and February 1, 2013, at C.S. Mott Children's

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Fig 1. (A) Hemi-Fontan patch baffle location within the right atrium. The patch separates the superior vena caval/pulmonary artery junction from the body of the right atrium. (B) Patch removal at Fontan operation. (Reprinted with permission from Elsevier from Bove EL, Mosca RS. Surgical repair of the hypoplastic left heart syndrome. *Prog Pediatr Cardiol* 1996;5:23–35.)



Hospital were reviewed. Patients with anatomy amenable to hemi-Fontan procedure (HFP) were approached for parental consent. Enrollment was closed after the recruitment of 12 patients.

Operative Details

On the basis of institutional preference, a modified HFP is performed for stage 2 palliation whenever anatomically possible. Our technique is described elsewhere [6]. A variety of patch materials are available for routine use for intracardiac baffle creation, including polytetrafluoroethylene, bovine pericardium, pulmonary homograft, autologous pericardium, and decellularized porcine small intestine submucosa (CorMatrix; CorMatrix Cardiovascular Inc).

In this study, all patients received circular 4-ply CorMatrix patches that measured approximately 20 × 20 mm implanted as the standard hemi-Fontan baffle. The baffles were sutured with polypropylene monofilament in a running fashion completely within the right atrium beneath the superior vena caval-pulmonary artery connection (Figs 1A, 1B). Therefore, the entire circumference of the CorMatrix patch was in contact with the right atrial wall.

After operation, patients received routine postoperative care. In general, the baffles were exposed to low (<20 mm Hg) pressures while in vivo. When clinically appropriate according to institutional criteria, patients underwent completion lateral-tunnel Fontan. As a routine part of the Fontan operation, the previously implanted CorMatrix patches were excised (Fig 1B).

Histologic Examination

Explanted CorMatrix patches were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections 5- μ m thick were stained with hematoxylin and eosin, Masson's trichrome, and Movat pentachrome stains. The histologic examination was evaluated for evidence of native tissue ingrowth, specifically any tissue resembling

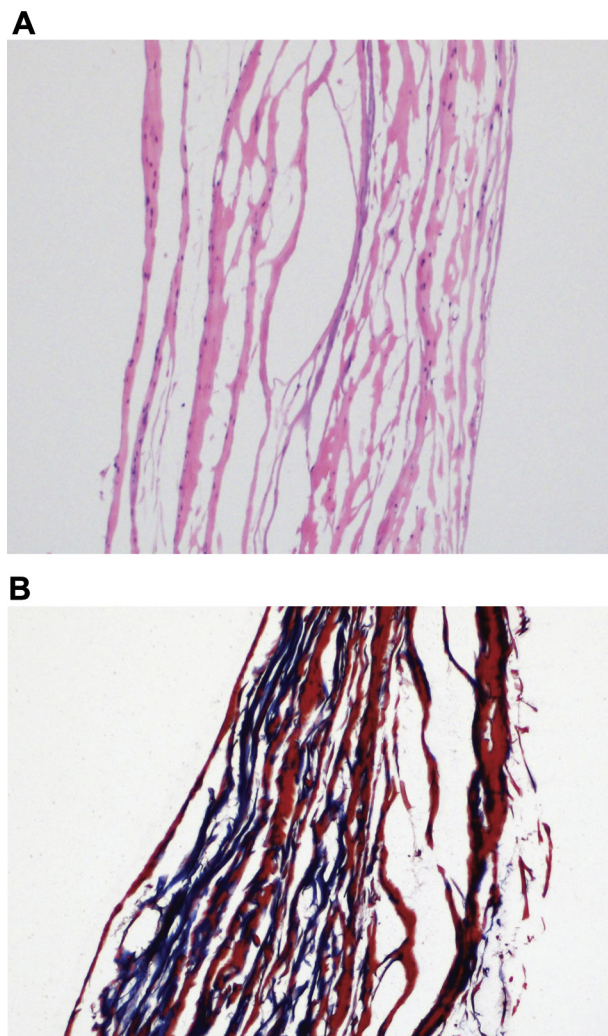


Fig 2. (A) Control; collagen and ghost outlines of nuclei are shown. (Hematoxylin and eosin; $\times 10$.) (B) Control; connective tissue with collagen stained blue. (Trichrome; $\times 10$.)

Table 1. Clinical Characteristics of Study Population (n = 12)

Characteristic	Value*
Male sex	9 (75)
Diagnosis	
HLHS	7 (58)
DILV	1 (8)
DORV, HRV, PS	1 (8)
Unbalanced AVSD, HLV, DORV, heterotaxy, dextrocardia, interrupted IVC	1 (8)
DIRV, DORV	1 (8)
DORV, unbalanced AVSD, HLV	1 (8)
Operation at implant	
HFP	10 (83)
HFP/Kawashima	2 (17)
Operation at explant (n = 10)	
Fontan	10 (100)
Patch not explanted	2 (17)
Median age at implant, months	6
Median age at explant, months	27
Median time in vivo, months	21

* Values may not sum to expected total because of rounding.

Data are n (%) unless otherwise noted.

AVSD = atrioventricular septal defect; DILV = double inlet left ventricle; DIRV = double inlet right ventricle; DORV = double outlet right ventricle; HFP = hemi-Fontan procedure; HLHS = hypoplastic left heart syndrome; HLV = hypoplastic left ventricle; HRV = hypoplastic right ventricle; IVC = inferior vena cava; PS = pulmonary stenosis.

right atrial tissue. Specimens were photographed, and the histopathologic results were recorded. An unused CorMatrix patch was analyzed as a control after being prepared according to the manufacturer's instructions (Figs 2A, 2B).

Results

CorMatrix hemi-Fontan patches were implanted in 12 infants, ranging in age from 4 to 8 months (Table 1). Time spent in vivo ranged from 18 to 26 months (median, 21 months). Two patients did not undergo completion Fontan because of unfavorable hemodynamics.

Fibrosis, acellular material, chronic inflammation, and foreign body giant cell reaction were seen in all explanted specimens (Table 2; Figs 3A, 3B). Disorganized elastic fibers (2 specimens), dystrophic calcification (2 specimens), and eosinophils (2 specimens) were less frequent findings (Figures 4–6). No explanted CorMatrix material showed evidence of ingrowth of native cells or transformation into cardiac tissue at a median of 21 months after implantation.

Comment

The potential for regeneration of native cardiac tissue in humans with CorMatrix remains unclear. To our

Table 2. Histopathologic Variables (n = 10)

Patient	Diagnosis	Age at Implant (months)	Time in Vivo (months)	Histopathologic Finding
1	HLHS	8	26	Fibroelastic tissue and acellular material with chronic inflammation, and foreign body giant cell reaction
2	DILV	4	20	Fibrous tissue with elastic fibers, focal chronic inflammation, and foreign body giant cell reaction
3	HLHS	5	26	Foreign material surrounded by palisading chronic inflammation and dense fibrosis
4	HLHS	6	20	Acellular material and small portion of arterial wall with chronic inflammation, fibrosis, and foreign body giant cell reaction
5	HLHS	5	20	Dense fibrosis and focal chronic inflammation
6	HLHS	6	18	Acellular material, chronic inflammation, eosinophils, fibrosis, and foreign body giant cell reaction
7	DORV, HRV, PS	6	20	Acellular material with calcifications, chronic inflammation, fibrosis, and foreign body giant cell reaction; Movat and trichrome special stains confirmatory
8	HLHS	6	22	Acellular material and fragment of arterial wall with chronic inflammation, multinucleated giant cell reaction; and fibrosis by trichrome and Movat special stains
9	DIRV, DORV	7	25	Acellular material with chronic inflammation, fibrosis, eosinophils, and foreign body multinucleated giant cells; Movat and trichrome stains confirmatory
10	DORV, unbalanced AVSD, HLV	6	26	Membranous fibroconnective tissue with mild chronic inflammation and dystrophic calcification

AVSD = atrioventricular septal defect; DILV = double inlet left ventricle; DIRV = double inlet right ventricle; DORV = double outlet right ventricle; HLHS = hypoplastic left heart syndrome; HLV = hypoplastic left ventricle; HRV = hypoplastic right ventricle; PS = pulmonary stenosis.

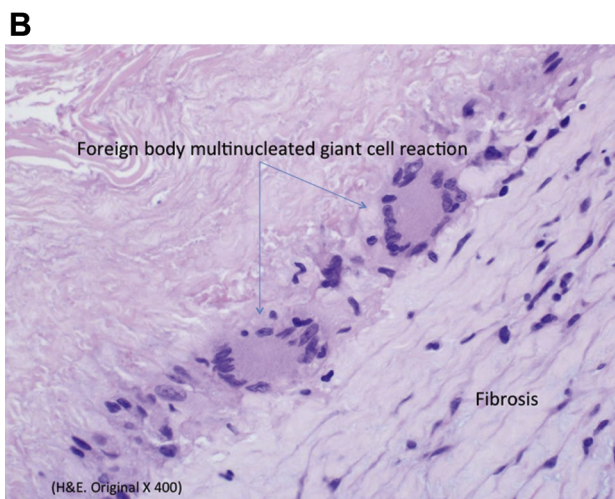
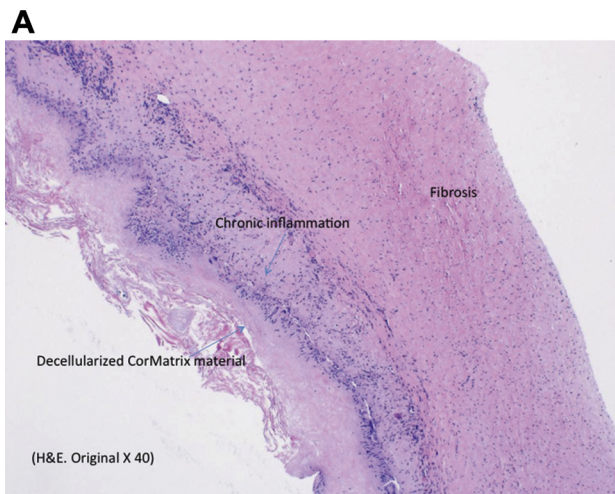


Fig 3. (A) Low-power magnification with hematoxylin and eosin (H&E) stain showing section of the CorMatrix patch with a band of chronic inflammation and aggregates of histiocytes. (B) High-power magnification with H&E stain showing a section of the CorMatrix patch. This photomicrograph highlights the foreign body multinucleated giant cell reaction.

knowledge, this study represents the first prospective evaluation of explanted intracardiac CorMatrix patches in children with congenital heart disease. In 100% of explanted patches, chronic inflammation and scar were identified with no evidence of native tissue ingrowth or regeneration. However, patches performed well in the HFP baffle application and remained pliable and largely without calcification.

CorMatrix ECM has been marketed as an intracardiac patch material that can generate new healthy tissue because it is completely resorbed over time [7]. The proposed mechanism is that the SIS ECM elicits a regenerative pathway that is immune-mediated and provides a scaffold for native tissue ingrowth [1].

Early animal studies of SIS products were encouraging [1, 8–12]. Badylak and colleagues [1] used circular patches created from sheets of ECM derived from

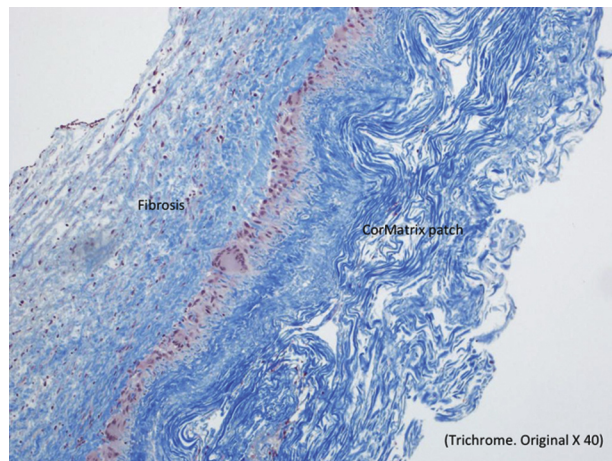


Fig 4. Trichrome stain at low-power magnification highlighting the acellular CorMatrix and the associated fibrosis.

porcine small intestinal submucosa or porcine urinary bladder matrix to repair full-thickness defects of the right ventricular anterior walls of six pigs. They reported complete replacement of the ECM with a mixture of tissue types, including cartilage and contractile myocardial tissue [1].

In general, experimental animal models with SIS ECM for cardiac use have involved suturing the patch material to an area of induced full-thickness injury [1]. In our study, as would be the case in a typical atrial septal defect closure in humans, patches are sewn to uninjured tissue with an intact endothelium. The process of creating a cardiac defect in animal models initiates an inflammatory pathway that could potentially contribute to a difference in findings. Future studies to investigate the role of suturing the patch to an edge of cut myocardium, as opposed to an intact endothelialized surface such as the right atrial wall, may be useful.

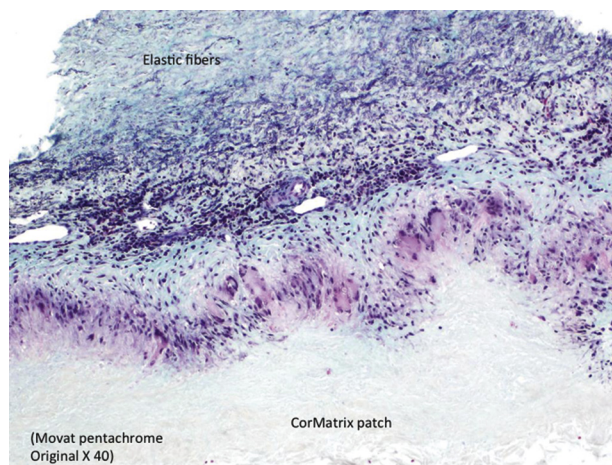


Fig 5. Movat pentachrome stain at low-power magnification with disorganized elastic fibers (stained black).

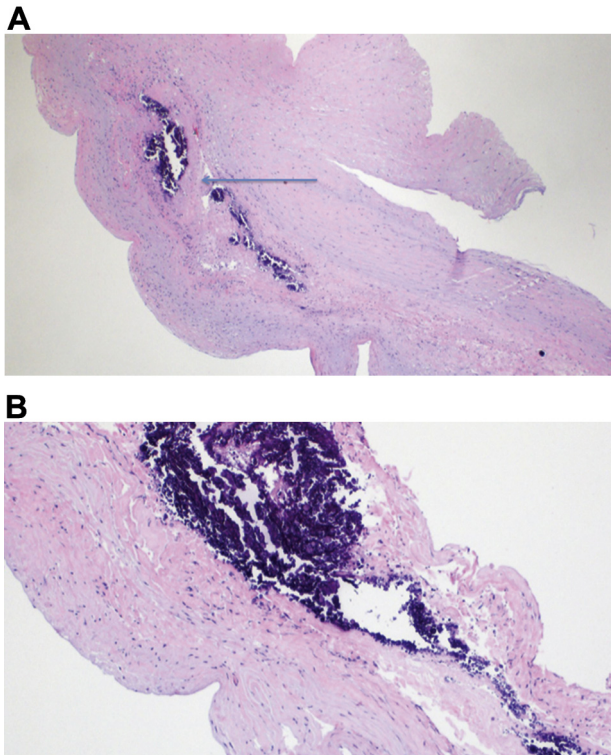


Fig 6. (A) Low-power and (B) high-power hematoxylin and eosin stain showing membranous fibroconnective tissue with mild chronic inflammation and dystrophic calcification (arrow).

Most specimens in this study demonstrated a robust foreign body giant cell reaction to the CorMatrix patch, similar to the findings of Woo and colleagues [2] and Rosario and colleagues [3]. The appearance of the aggregate of histiocytes (Figs 3A, 3B) is similar to what would be expected with arteritis within the media of a large vessel wall. Although elastic fibers were seen in two cases on pentachrome staining, they were disorganized, not well defined, and did not form a distinct layer (Fig 5). In addition, the background of mucin-containing ground substance, staining light blue on pentachrome stain, has an appearance similar to a fibrinous exudate. These findings do not support the theory that the CorMatrix was in the process of forming an organized, elastic, or muscular tissue layer such as atrial wall.

Badyalak and colleagues [13] suggested that the forces present in the mechanical environment might affect the ability of SIS ECM products to remodel. Because our patches were exposed to a low-pressure system, it is possible that the lack of mechanical loading contributed to a microenvironment that was not conducive to remodeling in the time frame studied.

In the current study, the median time patches spent in vivo (21 months) is greater than previously published reports, and all were removed electively [4, 14, 15]. In general, time in vivo in prior reports has been short, and most patches were removed for failure [4, 5, 14–16]. Additional strengths of this study are the homogeneity of

the patient population, the standardization of the implantation technique, and the consistent site of implantation within the right atrium. Prior studies of explanted CorMatrix represent a mix of patient ages and implantation sites [2–4, 15, 17]. Although CorMatrix is only approved as a pericardial replacement or for intracardiac repair, sites of implantation from prior reports of explanted CorMatrix vary widely from the right ventricular outflow tract, the mitral valve, pulmonary artery, pulmonary valve, and aortic arch [2–4, 14, 15, 18, 19].

As has been demonstrated, CorMatrix may be a clinically suitable patch material for the closure of septal defects and reconstruction of the great vessels [15, 19]. Note that, in some studies, the tissues were not examined for transformation, only for clinical efficacy [16, 18]. Although some investigators have noted suboptimal functional performance of CorMatrix when used for pulmonary artery wall reconstruction, for example, most studies have found that CorMatrix patches remained pliable and did not exhibit calcification at mid-term follow-up, much like our experience [3, 15, 18–20]. In the current study, CorMatrix performed well as a HFP baffle, but there is no evidence in our study that CorMatrix demonstrates regenerative properties in humans.

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DISCUSSION

DR ALI DODGE-KHATAMI (Jackson, MS): That was a beautiful presentation. Congratulations on your results. As you mentioned, a lot of the things that have been published on CorMatrix are kind of retrospective and have almost the feel of the believers and the nonbelievers of CorMatrix; some saying that it is great stuff, and others saying that it is just as good as any other patch material.

Based on the study that you did, which is very controlled, the patches are in a low-pressure system. That may somehow influence your results. To the believers of CorMatrix, do you think that part of the success would be the fact that they are implanting CorMatrix as part of aortic valve repairs or conduit substitutes? Do you think pressure or volume can play a role as to how CorMatrix behaves?

DR NELSON: In other words, do I think that it would be more successful in native tissue regeneration in a higher pressure system?

I think there is a lot of evidence that looks at explanted valve repair specimens which does not show native tissue remodeling either. For the low-pressure example, in the dog studies that were referenced in the CorMatrix product brochure, there were ASD patches placed in 2 dogs and right ventricular free wall patches in 2 other dogs. When explanted, by that report, they were completely indistinguishable from otherwise normal cardiac tissue.

The high-pressure example was looked at by Zaidi in 2014, and those aortic and mitral valve specimens were basically the results we have shown here. So no, I do not think so.

DR ANDREW LODGE (Durham, NC): I have always been concerned that implanting this material as a patch where it is not sewn to the cut edge could affect the ability for native tissue to replace the patch. So the first question I have is: Do you think that the fact that this patch was sewn to an endothelialized surface could have had an impact on what you saw?

And the second is: Do you have any experience explanting patches that are sewn to a cut surface, such as a ventriculotomy or an arterial wall, that have shown different results?

DR NELSON: I will answer your second question first. No experience in the evaluation of explanted patches that were sewn to a cut edge.

To answer your first question, I think that it is possible. And in those studies I mentioned in dogs, those ASDs had been created for that model; therefore, there was a cut edge. Whereas in our example, you are right, with the perpendicular attachment of the

patch to the right atrial wall it is an otherwise undisturbed, uncut endothelialized surface. So I suppose that is a possible explanation, although I reserve my enthusiasm.

DR MUHAMMAD MUMTAZ (San Antonio, TX): I have two questions. One is that in your histopathologic studies did you see any evidence of neovascularization of this tissue?

DR NELSON: We did not.

DR MUMTAZ: Did you look for that?

DR NELSON: We did.

DR MUMTAZ: And the second question is, which I'm assuming the answer would be yes, is that at the time when you were putting this patch, at the time of hemi-Fontan, let's suppose that patch size was X, then by the time you took it out the child had grown and so the actual diameter of the patch is now larger than when it was placed. Did you look at that? And this may not represent growth, I understand that, but just in terms of plain geometry of the patch, the size that was put in and the size that was explanted, was there a difference?

DR NELSON: There was a difference. The explanted patches were slightly smaller. They appeared whitish on the gross inspection, and they did appear slightly contracted.

DR MUMTAZ: So that is interesting. My own observation is that they are not smaller but they have actually grown to the size of whatever structure they are sutured to. Whereas if it was actually smaller, would you expect the hemi-Fontan to develop a gradient? I'm a little surprised by your answer.

DR NELSON: I would not expect a gradient in the hemi-Fontan pathway just by nature of the flow patterns and the position of the baffle which just really creates a barrier between the superior venocaval-pulmonary artery junction and the body of the right atrium. So contraction of this patch in that location would not cause obstruction to flow there. I would also say that the degree of contraction was almost imperceptible, but we were looking really hard at that. And the difference in the size of the right atrium was not of clinical significance.

DR CONSTANTINE MAVROUDIS (Orlando, FL): This was a delightful presentation with excellent data. The fact that you only have a few patients, 10 or 12, should not matter at all. I think you

presented your data in a forthright manner, and let the science speak for itself.

However, I have a question on what you are doing now, because that might be telling to the audience. I think there is a bias either for or against these kinds of modified pericardial patches. So the question for you is, what are you using now?

DR NELSON: We have returned to using PTFE for this patch in this position.

DR JOHN E. MAYER (Boston, MA): I would say I am not surprised by your findings. I think the question about whether

the tissue is going to shrink or stretch, or I hesitate to use the word, grow, is clearly going to depend on the hemodynamic forces to which the material is subjected. I think the important thing is that you have demonstrated quite clearly this is nonliving tissue. Nonliving tissue that is continuously flexed or stretched is going to stretch out and get bigger. If it is not subjected to those forces, then it is likely to shrink as a part of the inflammatory response to this foreign body. I think the results that you show are exactly predictable if viewed from that perspective.

DR NELSON: Thank you for your comments.