Endothelial and smooth muscle cell populations in a decellularized cryopreserved aortic homograft (SynerGraft) 2 years after implantation

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Supplemental material is available online.

Introduction

While the cryopreserved homograft heart valve is an established option for patients requiring replacement of damaged or diseased aortic valves, there is evidence that valve durability may be negatively impacted by the allogenicity of residual viable cells.¹ Decellularization techniques, like the SynerGraft process, reduce the cellular content of homograft valves, in hopes of modifying the host immune response to the graft. Uncertainty remains over the mechanism and extent of “recellularization” occurring in these decellularized grafts after implantation in humans. Characterization of cells populating the implanted decellularized grafts is important in understanding host adaptation and possibly in-vivo longevity of the grafts. This report details the histomorphologic and immunophenotypic features of cells identified in a decellularized (SynerGraft) aortic valve recovered two years after implantation.

Clinical Synopsis

At age 19, a male patient underwent replacement of his congenitally bicuspid aortic valve. At age 52, he underwent insertion of an aortic SynerGraft homograft for prosthetic aortic valve stenosis and an ascending aortic aneurysm. Postoperatively, left ventricular function did not improve significantly, and he underwent cardiac transplantation two years later. The explanted heart showed moderate left ventricular hypertrophy, coronary atherosclerosis, and an old transmural anteroseptal infarct. The homograft walls were pliable and noncalcified. The homograft valve cusps showed minimal fibrous thickening with focal calcification in the non-coronary valve pocket.

Histopathology

Aortic Wall. Histologic sections of the aortic root stained with hematoxylin-eosin and Verhoeff-van Gieson showed that the

SynerGraft tissue was entirely devoid of nuclei, with the exception of the neointimal layer and focal cellular incursions into the superficial elastic media (Figure 1). Foreign body giant cell reaction surrounded the sewing ring, but the remainder of the graft tissue was free of inflammation. Focal microscopic dystrophic calcifications were present at the interface between the graft and the native aorta.

Valve Cusps. Cells were also observed lining the valve cusps and showed focal extension into the subjacent spongy layer. This was more prominent near their annular insertions. The most superficial cells had an endothelial appearance with flattened cytoplasm and elongated nuclei. Deeper cells (both in the aortic elastic media and in the valve spongiosa) had a plumper and more spindled appearance with an eosinophilic cytoplasm suggestive of myofibroblasts or smooth muscle cells.

Immunohistochemistry

Immunohistochemical staining of formalin-fixed paraffin-embedded aortic root sections was performed using antibodies directed against CD31, CD34, factor VIII related antigen, smooth muscle actin (SMA), vimentin, CD68, CD3, CD20, and CD15. Antibody clones and reaction conditions are available on-line (Table E1). Figure 2 illustrates expression of selected antigens.

Staining for endothelial antigens (CD31, CD34, and Factor VIII related antigen) was seen primarily in flattened superficial neo-endocardial cells and focally within valvular capillaries. Staining for smooth muscle and myofibroblastic antigens (smooth muscle actin and vimentin) was confined to the deeper spindled-appearing cells. Macrophage antigen (CD68) expression was seen in scattered cells within the intimal layer and superficial media of the aortic wall and extending up to 1 mm deep to the endothelium of the valve leaflets. No CD3 positive T-cells, CD20 positive B-cells or CD15 positive granulocytes were identified infiltrating the tissues, but reactivity for these antigens was seen in cells circulating within vascular lumens. No staining for any antigen was seen in the acellular homograft wall or deep cusp tissue, which served as negative internal controls.

Discussion

Only one other report has characterized cells within a decellularized homograft in humans - a patient who died 5 weeks after implantation of a pulmonary homograft.² The graft showed infiltration by neutrophils and CD68 positive macrophages into only the most superficial aspect of the neointima. No smooth muscle, fibroblastic, or lymphocytic cells were identified by immunohistochemistry. Animal studies have demonstrated a sequence of early infiltration by macrophages occurring over the first few months, followed by fibroblast colonization (up to 11 months).³

The present case not only affirms the colonization process seen in animal models, but also demonstrates endothelial and smooth

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Figure 1. Gross and microscopic morphology of decellularized aortic homograft. *Upper Left*, Longitudinal section through homograft aortic root. The valve shows thin pliable cusps and focal calcification within valve pocket. Native aortic adventitia, peri-adventitial fat, and single blue suture are subjacent to graft (hematoxylin-eosin stain). *Upper Right*, Elastic media of both graft and native aorta. (Verhoeff-van Gieson stain.) *Bottom*, Cellularity of graft tissue. Absence of nuclei throughout decellularized graft tissue (G), except for neointima (far left) and superficial cell clusters within graft media (arrows). Cellularity of native aortic media (N) and adventitia (Ad) is shown for comparison. (Hematoxylin-eosin stain.)

muscle differentiation in a subset of cells. While recellularization did not involve the full thickness of the aortic homograft wall in this study, there appeared to be a centrifugal propagation of cells, extending from the lumen outward, congruent with theories on circulating progenitor cells.⁴ Also salient was the virtual absence of calcification and inflammation. Calcification is common, even after 2 years, in conventional cryopreserved homografts. Although the relationship between graft allogenicity and in-vivo durability is controversial, the absence of an apparent active cellular immune

Figure 2. Histomorphology and immunohistochemical phenotype of the decellularized aortic homograft. *Upper Left*, Control sample of valve cusp from non-implanted SynerGraft. *Upper Right*, Cusp tissue from explanted SynerGraft. Patient's valve shows formation of cellular layer with infiltration of cells into body of valve cusp (arrow). *Middle and Bottom*, Expression of selected endothelial (CD31), smooth muscle (SM actin), macrophage (CD68), and fibroblastic (vimentin) antigens, as labeled.

response in this case is of interest in light of the minimal calcification and mild fibrosis.

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Table E1. Antibody clones and reaction conditions. [FOR ONLINE VIEWING ONLY]

Antibody	Source*	Clone	Dilution	Antigen Retrieval
CD3	Cell Marque	Polyclonal	1/200	Heat (EDTA)
CD15	Becton Dickinson	MMA	1/10	Heat (Citrate)
CD20	Dako	L26	1/60	Heat (Citrate)
CD31	Dako	JC/70a	1/200	Heat (EDTA)
CD34	Becton Dickinson	My10	1/50	Heat (EDTA)
CD68	Dako	KP-1	1/200	Protease 2
Factor VIII	Dako	Polyclonal	1/500	Protease 2
Smooth Muscle Actin	Dako	1A4	1/150	None
Vimentin	Dako	3B4	1/500	Protease 2

*Sources - Cell Marque: Hot Springs, AR; Becton Dickinson: San Jose, CA; Dako: Carpinteria, CA.