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EDITORIAL COMMENT

Decellularized Pulmonary Allografts

The Long Path Toward a "Living Graft"

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There is no ideal conduit for right ventricular outflow tract reconstruction in children undergoing surgical correction of complex forms of congenital heart disease, but cryopreserved pulmonary valve allografts, when available, are still considered the gold standard. That fact notwithstanding, it is well documented that after implantation, these allografts are prone to varying degrees of conduit shrinkage and degeneration. This results in progressive degrees of valvular dysfunction, leaving these patients with the possibility of multiple reoperations throughout their lifetime. The mechanisms involved in conduit dysfunction are multifactorial, including patient baseline characteristics and technical factors during the operation, but the immune response of the host after implantation, which is more extreme in infancy and early childhood, plays a central role.[1]

Based on this information, different decellularization protocols were developed to produce an intact acellular matrix scaffold with reduced immunogenicity, capable of being repopulated "in vivo" by host cells with growth potential and regenerative capabilities after implantation. A large amount of "in vitro" and "in vivo" experimental studies in large animals gave supporting evidence for a theoretical advantage of decellularization as a superior processing technique.[2-5]

Initial clinical experience was very promising, demonstrating adequate hemodynamic performance and low incidence of valve-related complications.[6] Some studies confirmed a reduced human leukocyte antigen immunological reaction to decellularized conduits.[7] As experience increased, single and multi-institutional studies, including ours, demonstrated lower rates of reoperations with decellularized allografts in patients undergoing a Ross operation or correction of complex congenital heart defects,[3,5] although 2 meta-analyses failed to show a clear advantage when compared with the conventional cryopreserved counterpart.[8,9] Data from the Hannover group and the group's ESPOIR registry (European Clinical Study for the Application of Regenerative Heart Valves) have even suggested that decellularized allografts were capable of an adaptive growth potential following implantation,[6] a concept about which some are in disagreement.

With longer periods of observation, now extending up to 15 to 20 years, decellularized allografts still confer significant advantages over their cryopreserved counterpart, including lower peak gradients, near absence of calcification, and a lower cumulative incidence of reoperations.[5,10] On the other hand, it also is becoming clear that they also are subject to tissue degeneration, and a late attrition rate is to be expected. Recent studies found that there might be a wide variation in individual immune response to other epitopes in decellularized tissues, indicating that further investigations and improvements in decellularization protocols are warranted.[11]

Histological studies of explanted decellularized allografts or of biopsies during reoperations for other reasons are scarce in the literature. Case reports uniformly describe a well-preserved extracellular matrix and absence of calcification, but repopulation with autologous cells after implantation is scant, suggesting that growth potential should not be a real expectation.[3,6]

In this issue of *JACC: Case Reports*, Kugo et al[12] presented a case of a decellularized pulmonary allograft implanted in a boy at 1 year of age and explanted

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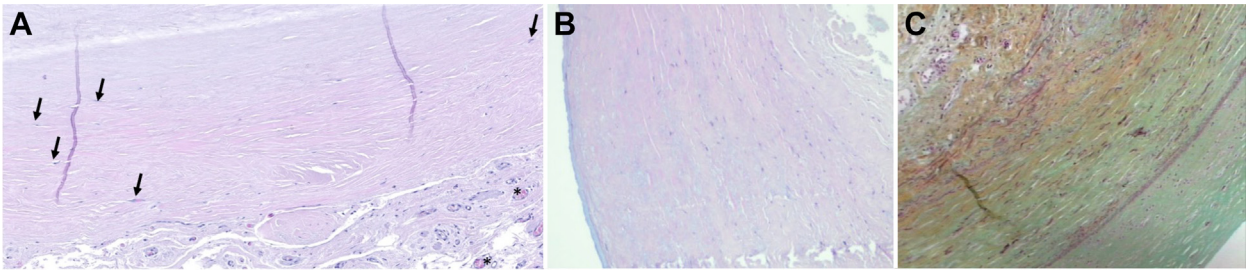
FIGURE 1 Histological Appearance of an Explanted Decellularized Allograft



(A) Sparse myofibroblasts repopulating the media wall of the allograft (arrow). Hematoxylin and eosin staining, (B) Alcian Blue staining, and (C) Movat Pentachromic staining.

4 years later due to stenosis at the distal anastomosis. Their histological findings are similar to those published by others, including our own observations. In 1 explanted allograft and 4 biopsies taken during reoperations for other reasons, we found a well-preserved conduit wall, with mild intimal hyperplasia covered with neo-endothelial cells and partial repopulation of the graft by host cells. Alizarin red staining revealed the absence of calcification. Immunohistochemical analysis was CD3- and CD68-negative, and did not suggest any immune response. However, especially at the proximal conduit, the media of the graft and the valve leaflets were partially replaced by new fibrotic tissue that probably was responsible for the observed conduit shrinkage at this level (Figure 1). Kugo et al's findings[12] help to build evidence for the more favorable remodeling of decellularized allografts when compared with what has been described for standard cryopreserved conduits. The investigators hypothesize that the proximal part of the conduit has a lower cell-density repopulation in comparison with the more distal segments. This is probably because the most proximal part of the pulmonary allograft is compromised by dead muscular tissue and not as amenable to cell invasion as the connective arterial wall tissue. In fact, as described in the preceding text, this is a frequent location of fibrotic retraction at the proximal anastomosis, which may lead to a mild-to-moderate degree of stenosis at later follow-up. We have made a technical modification to avoid this complication. After resecting all allograft muscle, a proximal circular extension of the allograft with decellularized human pericardium is made, that allows for a wider proximal anastomosis and keeps the valve leaflets away from the suture line. This simple modification resulted in lower peak gradients and only mild degrees of valvular insufficiency, if there was any at all at later follow-up.

Kugo et al[12] are to be complimented for their contribution to better understanding the biological behavior of decellularized allografts. We certainly agree with them that further investigations and improvements in decellularization protocols are necessary until the full goals of tissue engineered valves become reality.

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