

Letter to the Editor

In response to

Gonçalves AC, Griffiths LG, Anthony RV, Orton EC. Decellularization of bovine pericardium for tissue-engineering by targeted removal of xenoantigens. *J Heart Valve Dis* 2005;14:212-217

The authors of the above report assert that a technique for the decellularization of xenogeneically sourced tissues, identified as 'Standard' and likened to the decellularization process used in the manufacture of SynerGraft® heart valves, is insufficient to abolish tissue antigenicity. The data provided by the authors as the basis for the assertion is the persistence of both histochemically stainable nuclei (see their Fig. 2) and the greater detection of a specific epitope, α -gal-1,3-gal (see their Figs. 1 and 4), in Standard processed tissue as compared to tissue treated with ionic detergent extraction.

Data available to us suggest that the reported results may be consequential to the mode of application of a particular technique, and not necessarily to the technique itself. In our laboratory, decellularization of bovine pericardium by a method consisting of hypotonic lysis, nuclease digestion (DNase I and RNase A), and isotonic buffer washout yields a material that is essentially acellular. We find no α -gal-1,3-gal epitope associated with interstitial cells of the native tissue, and any associated with the capillary endothelium is absent following treatment.

Furthermore, we have utilized our lysis-driven decellularization process in the preparation of bovine ureter for use as an arteriovenous shunt for patients with end-stage renal disease undergoing hemodialysis. Over 250 of these grafts have been implanted in patients since 2001, and none has failed due to immune rejection of the implant, as predicted by Gonçalves and colleagues. In fact, the mean time of implant is in excess of 250 days, whilst the longest-standing graft is over 3.5 years old and is still in use for hemodialysis access. Overall, few of the grafts have shown unusual rates of either thrombosis or anastomotic-tissue overgrowth - pathologies that might be construed as having an immune component.

We agree with these authors that suitably chosen endpoints relevant to the intended use of these xenografts must be obtained in products intended for human use. We suggest, however, that the appraisal of the efficacy of any technique must account for the details of its use, as these appear to be critical in achieving these endpoints.

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Reply

We do not have a fundamental disagreement with any of Dr. Goldstein's comments regarding our report in the *Journal of Heart Valve Disease*. First, we cannot know that the hypotonic lysis, nuclease, and washout treatment reported by us is exactly the same as the process used by CryoLife, and there may be differences between the two methods. However, with regard to the α -gal-1,3-gal epitope, we would caution that detection of this known xenoantigen is method-dependent. Galli et al. (1) have demonstrated that the classic method of detection by *Bandeiraea (Griffonia) simplicifolia* IB4 lectin is insensitive compared to the IgM M86 monoclonal antibody. We were unable to detect the α -gal epitope in bovine pericardium when using the classic lectin method, but were able to detect it with both immunohistochemical and Western blot analysis using the M86 monoclonal antibody. In our view, detection of the α -gal epitope is particularly important because an immune reaction to this xenoantigen will not be detected by animal implantation studies prior to clinical implantation into humans.

We cannot disagree that there may be some tolerance to the persistence of xenoantigens in 'decellularized' xenogeneic tissues implanted into humans, and that different degrees of tolerance may exist depending on the clinical application. The major finding of our report was that 'decellularization' on histological sections is not a reliable endpoint if the goal is to remove antigens completely from xenogeneic tissues. The results of our studies suggest that a tissue which is 'essentially acellular' on histological section is very likely to have persistence of known xenoantigens. However, whether or not those xenoantigens are tolerated is a matter for other studies, and was not addressed by our report.

References

1. Galli U, LaTemple DC, Radic M. A sensitive assay for measuring α -gal epitope expression on cells by a monoclonal anti-gal antibody. *Transplantation* 1998;65:1129-1132

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