

stent-grafting via a median sternotomy: the frozen elephant trunk procedure. *Ann Thorac Surg.* 2002;74(suppl):S1821-4.
doi:10.1016/j.jtcvs.2007.09.081

Reply to the Editor:

We would like to thank Drs Asano and Okita for their comments regarding our study,¹ in which we demonstrated that the majority of arch aneurysms could be repaired with a long elephant trunk (LET) anastomosed at the base of the innominate artery without distal anastomosis in the descending aorta. They raised the issue of distal fixation of the LET in our technique. Although the proximal end of the LET is sutured at the base of the innominate artery and proximal endoleak is prevented, the distal end of the LET is not fixed, and distal endoleak is possible with our method. As demonstrated in Figure 2, C, in our article, the aneurysmal sac outside the LET was enhanced with contrast material in 9% of our patients, and they required distal fixation of the LET.

However, it is important to note that the majority (91%) of our patients did not show contrast material outside the LET, and none had expansion or rupture of the aneurysm occur during the average follow-up of 33 ± 18 months. Furthermore, it was encouraging for us to find that the nonenhanced aneurysmal sac around the LET shrank more than 5 mm in 82% of the patients and more than 10 mm in 50% at 1 year after total arch replacement with the LET, as shown in Figure 2, B, of our article. The follow-up period might not have been long enough to rule out the possibility of late expansion of a nonenhanced aneurysm, and we consider that long-term follow-up of our patients is mandatory.

In their letter they referred to their article,² in which they insist that arch aneurysms with extension to 1 cm below the level of the carina are accessible from a median sternotomy. Interestingly, they demonstrate that an increased depth of distal anastomosis is a risk factor for prolonged distal anastomosis, even in Japanese patients, whose chest cavities are generally smaller than those in white patients.² Based on my personal experience as a clinical fellow for 3 years in United States, I wonder how many surgeons are comfortable to do the distal anastomosis 1 cm below the level of the carina in a typical barrel-chested white patient. On the other hand, with our technique, the descending aorta in the deep

posterior mediastinum does not need to be exposed, but rather only a distal anastomosis is performed in the distal ascending aorta. Because of this simplicity, we required only 23 ± 8 minutes of hypothermic circulatory arrest in the lower body to complete the insertion and anastomoses of the LET. Thus we conceive that the benefit of our technique might be even more evident in white patients with a large chest.

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References

1. Toda K, Taniguchi K, Hata H, Shudo Y, Matsue H, Kuki S, et al. Single-stage repair of arch aneurysms with a long elephant trunk: medium-term follow-up of thromboexcluded aneurysms. *J Thorac Cardiovasc Surg.* 2007; 134:47-52.
2. Asano M, Okada K, Nakagiri K, Tanaka H, Kawanishi Y, Matsumori M, et al. Total arch replacement for aneurysm of the aortic arch: factors influencing the distal anastomosis. *Interact Cardiovasc Thorac Surg.* 2007;6: 283-7.

doi:10.1016/j.jtcvs.2007.10.076

Tissue-engineered heart valves: Bioreactor—yes or no?

To the Editor:

I read with great interest the article by Vincentelli and associates.¹ In this article the authors present the results of the effects of in situ injection of autologous bone marrow-derived mononuclear cells and mesenchymal stem cells (MSCs) on the outcome of xenogenic decellularized scaffolds in a lamb model. The main focus of this article is on a new method to reseed the cells in valve scaffolds consisting of an in situ injection of bone marrow cells into a porcine decellularized scaffold before implantation. Tissue-engineered heart valves created from MSCs and injected directly in a decellularized xenograft scaffold exhibited satisfactory hemodynamic aspects after 4 months.

Even if the authors demonstrate that in the MSC group the global organization of collagen fibers was preserved, a few fusi-

form cells were observed in the subendothelial area and the adventitia.

Pulmonary leaflets present few recolonizing cells positive for α -actin staining, with a typical organization in 3 layers, fibrosa, spongiosa, and ventricularis, as previously reported by others,² who showed that decellularized pulmonary valves revealed a well-preserved 3-dimensional network of collagen fibers in the extracellular matrix.

As we know, maintenance of the structural valve properties is fundamental to preserve the extracellular matrix with its complex of glycosaminoglycans and collagen fibers produced from valve fibroblasts and active fibroblasts. This appears to be fundamental for the mechanical valve's capability and its long-term durability.²⁻⁴ Repopulation of a decellularized valve is crucial for valve vitality. How this could be achieved still remains controversial. As a matter of fact, at this time, it is not completely clear whether a bioreactor is fundamental for the valve repopulation. Lichtenberg and colleagues⁵ clearly demonstrated that in vitro conditioning of engineered tissues in a bioreactive system stimulates and enhances the production of extracellular matrix and the related tissue strength. On the contrary, Dohmen and associates,⁶ studying seeded or nonseeded decellularized valves implanted into right ventricular juvenile sheep outflow tracts, showed that a difference was identified in the recellularization density of in vitro seeded and nonseeded valves up to 3 months, but no such difference was seen after 6 months.

Nevertheless, a moderate pulsatile flow with small increments seems to be able to stimulate endothelial cell proliferation on the decellularized valve scaffold. A rapid increase in bioreactor flow to physiologic levels leads to significant damage of the reseeded endothelium and complete loss of cusp cellularity. This effect might be responsible for the in vivo failure of static, reseeded, tissue-engineered valves exposed to physiologic hemodynamic forces.

However, whether MSCs injected in the scaffold induced in situ differentiation into myofibroblasts and endothelial cells remains uncertain. The injection of the stem cells in the inner side of the valve before valve implantation must be able to produce a colonization not only by recipient endothelial cells but above all by active

fibroblasts. These should be able to continually remodel the valve matrix and produce its components (eg, glycosaminoglycans) with very high turnover.

On this basis, this work is a great contribution in the complex field of engineered tissue valves, and the announcement is impressive because it should be able to offer a different and easier way to reseed valves before implantation. The authors are congratulated for their current achievements and should be encouraged to validate their results in other in vivo models.

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References

1. Vincentelli A, Wautot F, Juthier F, Fouquet O, Corseaux D, Marechaux S, et al. In vivo autologous recellularization of a tissue-engineered heart valve: are bone marrow mesenchymal stem cells the best candidates? *J Thorac Cardiovasc Surg.* 2007;134:424-32.
2. Cebotari S, Lichtenberg A, Tudorache I, Hilfiker A, Mertsching H, Leyh R, et al. Clinical application of tissue engineered human heart valves using autologous progenitor cells. *Circulation.* 2006;114:1132-7.
3. Dainese L, Barili F, Topkara VK, Cheema FH, Formato M, Aljaber E, et al. Effect of cryopreservation techniques on aortic valve glycosaminoglycans. *Artif Organs.* 2006;30:259-64.
4. Stamm C, Steinhoff G. When less is more: go slowly when repopulating a decellularized valve in vivo!. *J Thorac Cardiovasc Surg.* 2006;132:735-7.
5. Lichtenberg A, Cebotari S, Tudorache I, Sturz G, Winterhalter M, Hilfiker A, et al.

Flow-dependent re-endothelialization of tissue-engineered heart valves. *J Heart Valve Dis.* 2006;15:287-94.

6. Dohmen PM, da Costa F, Yoshi S, Lopes SV, da Souza FP, Vilani R, et al. Histological evaluation of tissue-engineered heart valves implanted in the juvenile sheep model: is there a need for in-vitro seeding? *J Heart Valve Dis.* 2006;15:823-9.

doi:10.1016/j.jtcvs.2007.09.080

Reply to the Editor:

We thank Dr Dainese for this comment. Several groups have demonstrated that in vitro seeding of cells on artificial scaffolds or on natural matrices in a biomimetic environment succeeded in the generation of functional tissue-engineered heart valves.^{1,2} More recently, Yacoub³ has said in an interview about mesenchymal stem cells that “if you subject these cells, in a 3 dimensional matrix, to programmes of conditioning using cyclic pressure of a predefined nature, they change their phenotype to one that is very similar to valve tissue.” Yacoub and his team still believe that the future of the tissue-engineered heart valve is dependant of an in vitro seeding in a bioreactor. To our knowledge, for the first time in this field, our work⁴ suggests that direct injection of mesenchymal stem cells seems to induce almost physiologic recolonization of valvular matrix under physiologic shear stress. Re-endothelialization of a decellularized xenogeneic matrix can be obtained in vivo without the cumbersome step of bioreactor recellularization. This has the evident advantage of simplifying the recellularization procedure and guaranteeing that recolonizing cells will stand the actual high shear conditions of a cardiac valve. However, we have to underline the evident limits of our work. First, we do not know

at the present time whether the recolonizing cells directly derive from the injected cells or from autologous cells migrating from blood or neighboring tissue. Second, as suggested by Dainese et al, a longer follow-up period is mandatory and should be prolonged for more than 1 year to confirm our results.

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References

1. Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, et al. Functional living trileaflet heart valves grown in vitro. *Circulation.* 2000;102:III44-9.
2. Dohmen P, Lembcke A, Holinski S, Kivelitz D, Braun J, Pruss A, et al. Mid-term clinical results using a tissue-engineered pulmonary valve to reconstruct the right ventricular outflow tract during the Ross procedure. *Ann Thorac Surg.* 2007;84:729-36.
3. Yacoub M. Viewpoint: heart valve engineering. Interview by James Butcher. *Circulation.* 2007;116:F44-6.
4. Vincentelli A, Wautot F, Juthier F, Fouquet O, Corseaux D, Marechaux S, et al. In vivo autologous recellularization of a tissue-engineered heart valve: are bone marrow mesenchymal stem cells the best candidates? *J Thorac Cardiovasc Surg.* 2007;134:424-32.

doi:10.1016/j.jtcvs.2007.12.033