Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: tissue-engineered vascular grafts, autologous pericardium, pulmonary artery, aortic valve plasty, pediatric cardiovascular surgery

Discussion

Presenter: Dr Hisayuki Hongu

Dr Toshiharu Shinoka (Columbus, Ohio). I would like to thank the AATS, Dr Brown, and Dr Romano for the opportunity to discuss this report today. Congratulations to Dr Hongu, Dr Yamagishi, and colleagues for your exciting and unique preclinical procedural techniques. I would also

like to thank Dr Hongu for providing me with your manuscript well in advance. I have several questions.

The idea to create autoregressive vascular graft *in vivo* by subcutaneously implanting a silicone manual was originally reported by Dr Sparks in 1969. They used Dacron reinforced grafts for the replacement of pulmonary arteries. This concept was abandoned soon because of the high incidence of infection, low patency, and aneurysmal formation?

I also have experience with dermal *in vivo* tissue engineering for the abnormal aorta replacement by using the same method as your method, in a sheep model in 1995. We used a fresh graft without any fixation. Unfortunately, our sheep died within 48 hours, because of the graft rupture. Your external fixation would be a great idea to strengthen the materials.

Dr Hongu, do you believe these dermal tissue constructs would be able to tolerate a systemic blood pressure? If the PA pressure is high, what would be the pressure limit that this growth can tolerate? How do you consider that this material could be used in aortic arch repair? And you have various periods of mold implantation from 6 months to 2 years, what do you think is the optimal duration for this graft to incubate in the body? Do you think a longer incubation period is better to create stronger tissue?

Dr Hongu (Kyoto, Japan). Thank you Professor Shinoka. I'm really grateful for the opportunity to present in such a honored meeting and would like to thank Dr Brown and Dr Romano very much.

I will answer from the first question.

In our research, of course, the detailed *in vivo* data whether the TEVG can withstand the arterial pressure was not obtained. However, the result of mechanical property tests showed that the clinically harvested TEVG fragments were stronger than IMA with ethanol treatment, indicating that they may be used as an arterial graft.

So, theoretically, TEVG can be tolerated for any pulmonary hypertension.

In our clinical case, TEVG could be safely used for moderate pulmonary hypertension cases. The patient's pulmonary artery pressure was approximately 70% of arterial

pressure due to hypoplastic MAPCA. But TEVG did not show any aneurysmal change in a 1.2-year follow-up.

In our test, glutaraldehyde fixation showed almost the same, or very slightly stronger than ethanol treatment. So, take into consideration the toxicity and the calcification, we apply the ethanol treatment.

In an in vitro trial, TEVG can be made at least 4 weeks in the animal body. However, to use in vivo, we have longer incubation period for safety margin. In addition, the degree of the TEVG was not associated with the incubation period.

Dr Shinoka. Thank you. My next question is related to the application to the valve leaflet. In 1995, when I was working as a research fellow in at Boston Children's Hospital, we tried to create a tissue-engineered pulmonary valve leaflet in a sheep model. I compared dermal fibroblast seeded leaflet with the vascular cell seeded valve leaflet in the pulmonary valve position in a sheep model. Unfortunately, all of the dermal valve leaflets shrunk down within 6 months while the vascular cell tissue engineered showed better performance. Could you describe in more detail about the long-term results of your prepared valve?

Dr Hongu. Thank you for your question. We used TEVG for valve leaflet extension. Because we had no experience with using TEVG for valvuloplasty, we did not resect the leaflet and replace it with TEVG, but only for shortening the valve leaflet extension. Furthermore, because we have experience of valve replacement with autologous pericardium in cases such as truncus arteriosus, we planned to use autologous pericardium as the first option in this case as well. However, in the case of re-sternotomy, as expected, it was not possible to extend all 3 leaflets with only the autologous pericardium, which led to the use of TEVG for the right coronary cusp. The follow-up period of this case was only 6 months. So careful follow-up is needed, especially for the right coronary cusp using TEVG.

Dr Shinoka. My final question is regarding the histological results that you show in your manuscript. We reported a long-term histological result of autologous pericardium

implanted in the pulmonary artery position in 2 patients. This report was published in the *New England Journal of Medicine* in 2003. Both patients had redo surgery after PA angioplasty and we had the opportunity to harvest the previously implanted pericardium tissue. The histology in both patients showed a well remodeled pulmonary artery structure, including an abundance of the smooth muscle cell layer reuse and a single layer of endothelial cells after the 3 years post implementation.

We concluded that autologous fresh pericardium is a good scaffold material to create a tissue-engineered pulmonary artery in vivo in some patients. Your longest follow-up is 3 years in this series. I am wondering why your graft histology had no smooth muscle cells. Did you see any endothelial cells on the surface of the graft? Or do you have any large animal data that supports later smooth muscle cell migration on your graft?

Dr Hongu. Thank you for your question. Because the histological image shown in this presentation is the image before implantation, we don't have accurate evidence of histological data after implantation. Before implantation, it consists of collagen fibers and fibroblasts, which are common to small animals, large animals, and humans.

In small and large animals, endothelium of the lumen surface and formation of the vascular media layer by smooth muscle cells have been observed several months after implantation in blood vessels. The TEVG histology of the aorta has not been published. Also, examination of tissue histological change after implantation in humans is a future theme. We have some experiences of autologous pericardium implantation for pulmonary coarctation, and the histological examination showed complete endothelialization, tiny smooth muscle cells, and a rich collagen fiber layer.

I have no idea why our harvested tissue was different from your study. Sorry.

Dr Shinoka. Okay. Congratulations again, to Dr Hongu and Dr Yamagishi for your brave clinical challenge.