Surgical Technique

Surgical technique: establishing a pre-clinical large animal model to test aortic valve leaflet substitute

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Abstract: To overcome current limitations of valve substitutes and tissue substitutes the technology of tissue engineering (TE) continues to offer new perspectives in congenital cardiac surgery. We report our experiences and results implanting a decellularized TE patch in nine sheep in orthotropic position as aortic valve leaflet substitute. Establishing the animal model, feasibility, cardiopulmonary bypass issues and operative technique are highlighted.

Keywords: Tissue engineering; models, animal; aortic valve; cardiac surgical procedures

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Introduction

Substantial progress has been achieved in treating congenital cardiovascular diseases due to better diagnostics, advance surgical techniques and improvement in postoperative care. Nevertheless, the ultimate goal is to achieve a physiologic and durable repair that has the potential to grow, which has not been attained yet due to the limitations of all the tissue substitutes used. Therefore, there remains the need for surgical re-intervention through the patient's lifetime.

Aortic valve patients suffering from symptomatic aortic stenosis or insufficiency have limited life expectancy (1). Degenerative valve disease has become the most frequent disease leading to aortic valve replacement (AVR) in adults, which has been established as a standard treatment but implies prosthesis-related complications. Generally, the replacement of diseased valves is done by either mechanical prosthesis or bioprosthetic tissue valves. Mechanical valves need anticoagulation, which is contraindicated in the young frequently (relatively), either for planned pregnancy or because their lifestyle is very active. All biological valves are at risk for calcification and structural valve degeneration (SVD), leading to reoperation. The age of the patient at the time of valve implantation was shown to be the most important determinant of SVD (2); besides, it has been demonstrated that neonates and infants undergoing AVR are a high-risk group for mortality (3). All the valve prostheses used have the disadvantage in that they are unable to grow, repair or remodel, and are thrombogenic and pose an increased risk for endocarditis. Techniques like the Ross principle or homograft implantation have been introduced, especially in children and adolescents. Unfortunately, the rate of SVD for homograft—around 30% after 10 years and 59% after 15 years—is similar to bioprosthetic valves (2). Both the Ross principle and homograft do not have the ability to grow, as is expected of an ideal valve substitute, alongside hemodynamic function as the original valve, non-thrombogenic, no antigenicity and no structural degeneration. In contrast to older patients suffering from degenerative valve disease, young adults and children face the risk of either multiple reoperations or the

side effect of lifelong anticoagulation. The lack of an ideal valve substitute has raised interest in alternative techniques, such as repairing the diseased valve rather than replacing it. In the past several years, the percentage of valve repair as compared to AVR has increased (1). For many of the aortic valve repair techniques, a patch is needed as substitute, partial or complete replacement or leaflet extension. So far various cusp tissue substitutes have been tested, but failed to fulfill the requirements for an optimal patch material.

Currently used patch material for valve repair

An optimal patch material should be pliable, hemostatic and resistant to tearing, should not shrink or calcify, and should possibly not induce an inflammatory cascade. Since the late 1960s, attempts have been made to use biologic materials like fascia lata, dura mater and bovine pericardium with poor outcomes (4-6). An easily available source for valve repair is the autologous pericardium, which is widely used for partial or total cusp replacement or leaflet extension (7-10). Some groups propagate pretreatment with glutaraldehyde, which prevents secondary shrinking and calcification (9,10). On the other hand, untreated fresh autologous pericardium might possess true growth, but distensibility as well. Despite good short- and mid-term results, it is associated with a high degeneration rate in the long term (11-14) and is associated with bacterial endocarditis (8,10,15). Bovine pericardium—used as leaflet extension—has been shown to be less effective as compared to autologous pericardium (16,17), and autologous pericardium has greater resistance to retraction. Since polytetrafluoroethylene (PTFE) was successfully used for right ventricular outflow graft reconstruction or in mitral leaflet position with good long-term results (18-20), it was applied as leaflet extension in the aortic valve position. Nosál' and coworkers reported 13 congenitally affected patients, with the youngest being 22 months old, in whom PTFE leaflet extensions were done with a follow-up till 30 months (21). However, there still remains a higher risk of endocarditis, and concerns have been raised about leaflet thickening and decreased mobility (18,22). Technical and scientific achievements in the field of tissue engineering (TE) may offer new perspectives with the potential of an off-the-shelf starter matrix for guided tissue regeneration.

Study rationale

The ultimate goal is to develop an aortic valve leaflet substitute which is physiological, durable and has the potential for growth, and test it in a chronic animal model. Our research group developed an off-the-shelf decellularized TE patch (TEP) as an aortic valve leaflet substitute (23,24). In a step-by-step approach, we want to establish an animal model as proof for feasibility, and, in a further step, we plan to use the chronic animal model as proof of concept.

For decades, different animal models have been a central critical component in the pre-clinical safety evaluation of cardiovascular devices developed for use in humans (25).

Here, we describe our technique of implantation of this decellularized TEP as an aortic valve leaflet substitute in a pre-clinical large animal model in orthotropic position.

Operative technique

We operated on adult Swiss White Mountain sheep (59–65 kg). All the surgical procedures and postoperative care were carried out in accordance with the approved protocol by the Ethics Committee (approval no. 19/2013) of the Canton (state) of Zurich. During the course of the animal experiments, all the animals received humane care in accordance with the Guide for Care and Use of Laboratory Animals (Publication no. 85-23. Bethesda, MD: National Institutes of Health, 1985) as well as with the "Principles of Laboratory Animal-Care".

After general anesthesia and preoperative preparation (invasive blood pressure lines femoral artery and upper body half (ear), venous lines, bladder catheter,) either median sternotomy (n: 6) or right lateral thoracotomy (RLT; n: 4) using 4th intercostal space, without removing a rib, was carried out. The pericardium was opened by protecting the phrenic nerve and stay sutures were placed. For cardiopulmonary bypass (CPB), 10,000 IU of Heparin was given and the activated clotting time (ACT) levels above 300 seconds were maintained throughout the CPB. Arterial cannulation was done via the aortic arch (n: 3) or truncus brachiocephalicus (n: 4) using a 21-F cannula, which allows adequate arterial inflow. Venous cannulation via the right atrium using a single stage 36-F cannula was carried out. A pulmonary VENT catheter was placed with continuous suction (150 mL/minute). Temperature monitoring was done with a rectal probe and mild hypothermia (32 degree Celsius) was sought for the intervention. Calafiore blood cardioplegia was given over the aortic root, followed by aortotomy. The aortic valve was exposed by using three stay sutures just above the upper edge of the commissures. The

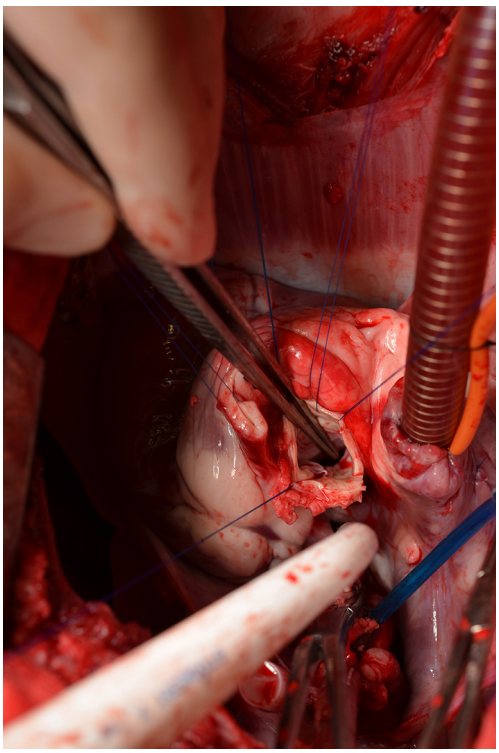

Figure 1 View of the aortic valve after aortotomy showing left and right coronary leaflets in place whereas the tip of the forceps is showing the place where the non-coronary leaflet was explanted.

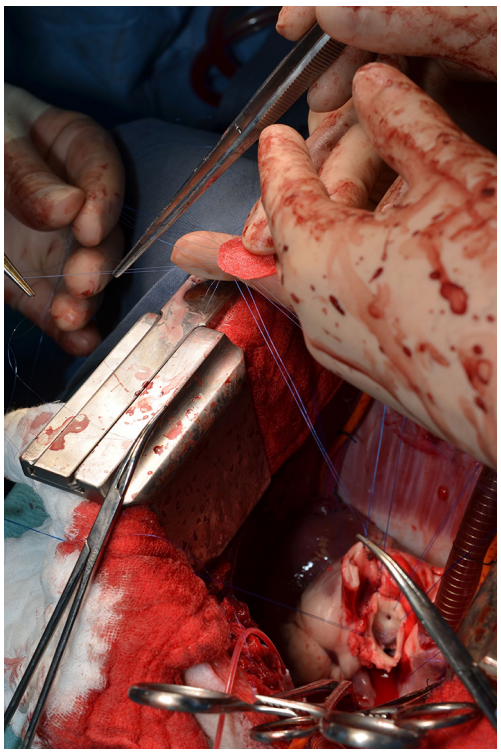

Figure 3 The tissue engineered patch is implanted using single mattress sutures.

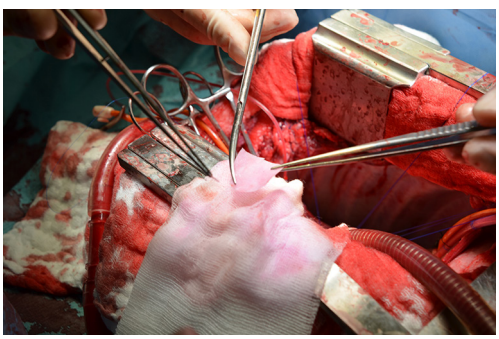

Figure 2 The explanted native non-coronary leaflet is used as a pattern for tailoring the tissue engineered patch.

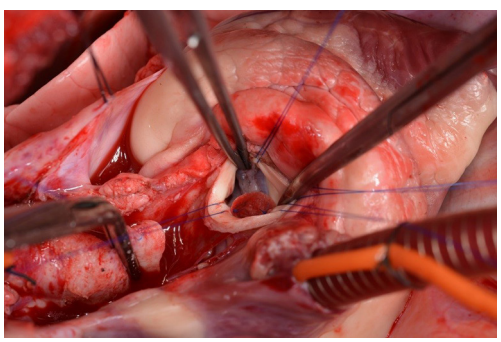

Figure 4 View of the aortic valve after aortotomy showing native left and right coronary leaflets and the implanted tissue engineered patch.

non-coronary leaflet was completely explanted (see *Figure 1*) and used as a pattern for patch tailoring. The TEP was textured to a mild triangular shape of the explanted leaflet by adding 3mm of extra patch material, as compared to the explanted leaflet (see *Figure 2*). The substitute was implanted using six mattress 6-0 polypropylene sutures following the origin of the explant line (see *Figure 3*). The last sutures on each side ended with the upper edge of the commissures. The aim was equal distribution on each side of the created base of the patch to achieve good geometry (see *Figure 4*).

After testing the valve function and successful weaning from CPB, one-third of the full Protamine dose was administered. After chest closure, echocardiography was

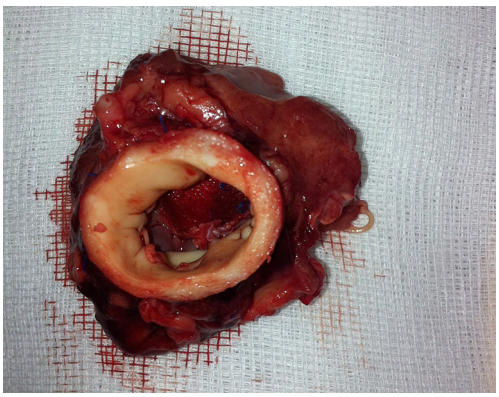

Figure 5 Explanted whole aortic root after scarification of the animal. It shows the aortic valve with native left and right coronary leaflets and the implanted tissue engineered patch.

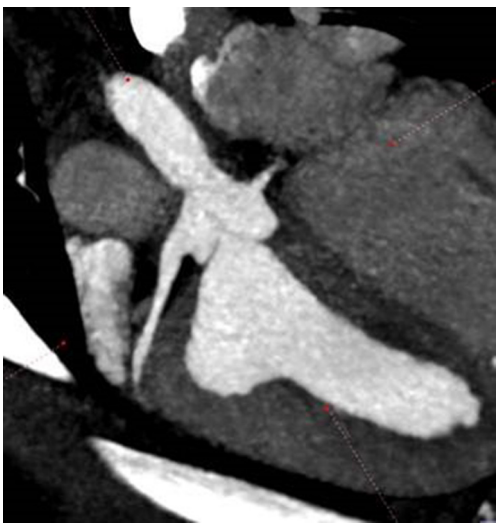

Figure 6 Postoperative CT study image showing co-adaptation of the tissue engineered patch and the native aortic valve leaflet.

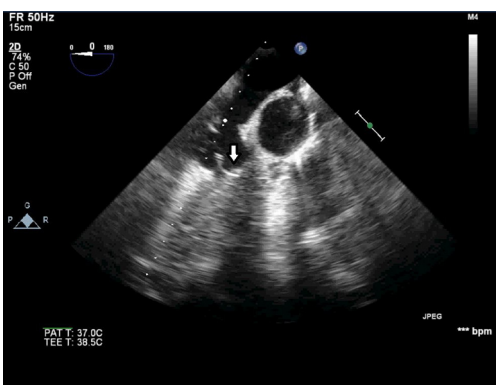

Figure 7 Postoperative echocardiographic study revealing good co-adaptation of the tissue engineered patch (white arrow) and the native aortic valve leaflet.

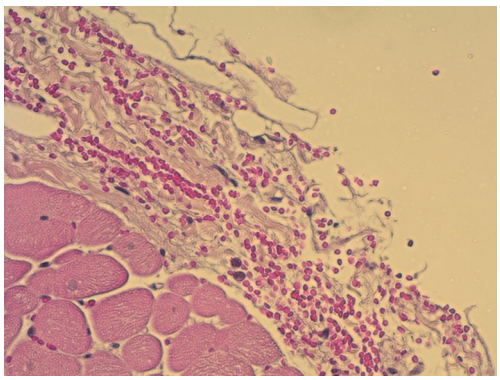

Figure 8 Post-mortem analyses of the implanted tissue engineered patch showing blood cell infiltration into the patch (hematoxylin-eosin stain, 400×).

done followed by computer tomography (CT). Finally, the sheep were sacrificed, and the whole aortic root was explanted (see *Figure 5*) and the TEP was analyzed.

Using this technique, the patch was implanted in nine sheep. The operation was completed successfully in all the nine attempts. The average aortic cross-clamp time was 96 minutes, with the average bypass time over 140 minutes. Postoperative echocardiographic and CT studies (see *Figure 6*) revealed good co-adaptation of the leaflets (see *Figure 7*) without signs of relevant aortic valve stenosis or regurgitation. After post-operative diagnostic (echocardiography and CT), the animals were sacrificed and the TEP was analyzed. The analyses showed no tears within the patch due to systemic blood pressure. Histologic analyses showed infiltration of blood cells into the TEP (see *Figure 8*).

Only the last sheep of the series was kept alive for 24 hours to test feasibility for translation to chronic phase trial.

Comments

There are increasing numbers of scientific publications on the use of animal models in cardiovascular disease research. The advantages of sheep are that they have similarities with humans in the molecular basis of cardiac contraction and in coronary anatomy. Likewise, the physiology is comparable to humans; especially when testing a TEP in the high pressure system, the mean arterial blood pressure in sheep seems to be of approximately 100 mmHg (26). Finally, calcification as one of the main outcomes might occur very early, as one year in a human is equivalent to seven years in a sheep. From our experience and set-up, we would like to

share some crucial issues:

(I) Surgical access: we started with median sternotomy, with the idea that this approach provides the best exposure to the heart; however, the potential benefits of a thoracotomy outweighed those of sternotomy, especially when considering a chronic study. We believe that a thoracotomy leads to decreased morbidity, especially postoperative pain, as compared to that associated with sternotomy. In terms of early extubation and postoperative breathing (as the animals lay on the sternum), thoracotomy is beneficial in comparison to sternotomy. According to our experience, an RLT using the fourth intercostal space offered good exposure to the aorta and truncus brachiocephalicus as well as good access to the aortic valve. Arterial cannulation is easy to achieve using this surgical access. If one chooses to use a left lateral thoracotomy (LLT), which is possible, one has to dissect the main pulmonary artery to get sufficient access to the ascending aorta and the aortic valve.

(II) CPB and cardioplegia: there might be several possibilities to perform arterial cannulation. While we tried to avoid femoral cannulation (lines for arterial blood pressure measurement and venous lines; higher infection risk), one of the shortcomings of right-sided thoracotomy is that one will not have good access to the descending aorta, which is a good option to cannulate when using an LLT. In contrast to humans, where the ascending aorta is the primary target, this part of the aorta in sheep is short, and we tried to preserve the whole ascending aorta for application of cardioplegia (via root) and the cross clamp as well as left enough material for the closure of the ascending aorta. Alternatively, we chose the truncus brachiocephalicus, which is easy to access from RLT, for arterial cannulation.

The mainstays of cardio-protection during cardiac surgery are cardioplegia, hypothermia, and prevention of cardiac distention. We always use antegrade cardioplegia with cannulation of the aortic root as this (still) might be the best way of cardiac protection through the strenuous CPB process. We did not actively cool the animals, but allowed a passive cooling down to 32 degree Celsius. To avoid cardiac distention, a good venous drainage for CPB is essential; and also the placement of the VENT catheter in the main pulmonary artery, which is easy to access and has low bleeding complications after removal might help.

(III) Technique of patch augmentation and implantation: the advantages of using the non-coronary leaflet seem to be obvious, as the risk of complications involving the coronary arteries are much less. We believe that in order to prove the hypothesis of cell immigration into the patch and transformation of the TEP into native leaflet tissue, one should cut out the whole leaflet. The explanted leaflet might give an idea about how big the substitute should be; nevertheless, we decided to add some additional space to the TEP to have a sufficient rim for sutures and a sufficient coaptation height. On the other hand, one has to be careful against adding too much patch material, as this might interfere with the function of the two remaining leaflets. The use of mattress suture allows a more clear view and better placement of the sutures as compared to a running suture; the thorax of sheep is deep (see *Figures 1-4*)!

Our experiences and comments may help other groups using a sheep model in cardiovascular disease research to further increase animal welfare by improving their rates of survival.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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