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Tissue engineered aortic valve

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ABSTRACT

Several prostheses are available to replace degenerative diseased aortic valves with unique advantages and disadvantages. Bioprotheses show excellent hemodynamic behavior and low risk of thromboembolic complications, but are limited by tissue deterioration. Mechanical heart valves have extended durability, but permanent anticoagulation is mandatory.
Tissue engineering created a new generation heart valve, which overcome limitations of biological and mechanical heart valves due to remodelling, regeneration and growth potential. Several publications are available in using tissue engineered heart valves in right ventricular outflow tract reconstruction. Limited experiences are available on these heart valves implanted into the systemic circulation.
This overview shows the current state on the development of tissue engineered aortic heart valves.

Keywords: *tissue engineering, aortic valves, decellularization, remodeling potential, systemic circulation.*

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INTRODUCTION

Treatment of aortic valve diseases is a common therapy. Some of these valves can be reconstructed; however this is not always feasible due to tissue degeneration and therefore replacement is needed.
Several suitable prostheses are available to replace the aortic valve, with specific advantages and disadvantages.
Biological heart valves, of either allogenic or xenogenic origin, show excellent hemodynamic behavior and low risk of thromboembolic complications, but their use is limited due to tissue deterioration. Mechanical heart valves have extended durability, but permanent anticoagulation is mandatory. Therefore a new generation heart valve is needed to overcome these limitations, showing the advantages of a healthy viable tissue valve with remodelling, regeneration and growth potential (1). A concept to create a patient specific viable aortic heart valve can be performed with tissue engineering (TE) techniques, in which three essential components are included.

Creating a tissue engineered heart valve

First of all a sufficient extracellular matrix, the so-called scaffold, is needed to utilize a three-dimensional structure.
These scaffolds are generally made out of polymers or decellularized allo- or xenogenic materials.

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A sufficient scaffold should fulfil mechanical and biological integrity, provide dynamic and biochemical signals, showing cell attachment and migration, secure diffusion of vital cell nutrients and expressed factors and allow dynamic changes of the scaffold's architecture (2).

The generally used synthetic scaffolds are simple tubes created by polyglycolic acid (PGA) (3) or PGA in combination with poly-4-hydroxybutyrate (P4HB) (4). The recently developed scaffolds were created from P4HB, including sinuses to support the leaflet closure (5). Nevertheless none of these TE aortic heart valves have yet been clinically implanted and evaluated.

Another option to create a TE aortic heart valve is based on decellularized scaffolds. The important difference between aortic valves and pulmonary valves is the discrepancy between the thickness of the aortic wall and the pulmonary valve in relation with the leaflet of the valves.

Complete decellularization of the aortic wall is therefore more demanding since the leaflets will be more easily decellularized but should not show structural deterioration and need to withstand the systemic blood pressure. Additionally, validated sterilization should be performed on the decellularized scaffolds. In the past allograft irradiation was used.

Unfortunately, Cohen et al. (6) showed that irradiation completely destroyed the extracellular matrix, with a minimal valve survival and early deterioration of these treated allografts.

This study compared the implantation of γ irradiated allografts ($n = 41$) versus β-propiolactone ($n = 39$) treated allografts. At a 15 year follow-up 84 % of the γ irradiated and β-propiolactone irradiated heart valves needed to be replaced and therefore only 22 % and 12.5 % respectively remained implanted. In these patients the dosage of γ irradiation was 22.5 kGy; however the generally excepted dose to γ irradiate to sterilize tissue is 25 kGy (7). Gouk et al. (8) showed in a study the changes of decellularized tissue matrices by low dosage of γ irradiation: increase of tensile strength and a decrease of elasticity. The reason for this is the cross-linking of α1 and α2 subunits of the collagen (9).

Somers et al. (10) studied the dosage influence of γ irradiation on tissue, with significant stiffness increase at 1 Gy. Leaflet deterioration showed a statistically significant increase in calcium content after 2 weeks of implantation in the subcutaneous rat model, showing 100 Gy γ irradiation compared with 10 Gy γ irradiation, respectively 651 ± 57 µg/mg vs. 118 ± 54 µg/mg; $p = 0.005$. Additionally the optical density values of IgG antibodies were also in linear relation with the radiation dose. Important to notice in this study are the 100 times lower doses compared with doses used to sterilize tissue.

When an appropriate scaffold is available, valvular cells are needed to allow the heart valve to remodel, regenerate and eventually grow. Cells which are needed include endothelial valvular cells and interstitial valvular cells. These cells can be seeded *in vitro* or *in vivo*. In the last situation the host will be the bioreactor for creating the complete TE heart valve.

Several studies showed that both concepts are possible to perform. Dohmen et al. (11) compared *in vitro* seeded and non-seeded heart valves in the sheep model. In this study no evidence was shown to seed TE heart valves in the laboratory; however, if decellularized scaffolds were seeded preoperatively the repopulation of the tissue was accelerated.

In some studies only vascular endothelial cell were used for seeding (12); in other studies also interstitial valvular cells were used (13). Endothelial cells are demanding to multiply *in vitro* and therefore alternati-

ve cell sources are being investigated, such as bone marrow derived cells (14) or umbilical cord cells (15), to improve the growth rate without compromising cell function. Studies are still going on to identify the optimal cell source if *in vitro* cell seeding is needed, mostly in synthetic scaffolds.

Experimental experience of TE aortic valves

Meanwhile there are some experimental studies performed on TE heart valves implanted in the aortic position. Since this is a demanding surgical procedure, with a high mortality rate, there were several implantation techniques performed.

Dohmen et al. (16) started implanting decellularized equine pericardium into the descending aorta to investigate the stability of this material under systemic circulation conditions.

There was no operative mortality and the authors could prove that there was not only no tissue deterioration but also complete ingrowth of interstitial cells and an overgrowth of endothelial cells.

These initial studies were performed to develop a TE heart based on decellularized pericardium.

Baraki et al. (17) implanted decellularized ovine aortic heart valves in a juvenile sheep model as a root replacement with a maximum follow-up of 9 months. These valves were compared with "homovital" ovine aortic valves as a control group. Significant differences between the two groups were seen, since control valves showed massive degeneration and thrombotic formation as early as 3 months after implantation, compared with the decellularized with only minimal calcification at the anastomosis side and micro-thrombotic formation in only one leaflet surface.

Functional analyses showed significant differences in aortic valve regurgitation between the decellularized and control group (0.5 ± 0.5 versus 2.5 ± 0.0 respectively; $p = 0.002$).

Dohmen et al. (18) implanted decellularized porcine heart valves in the aortic position, using a subcoronary technique. At explantation, gross examination showed smooth and pliable leaflets with complete recellularization as early as 4 months after implantation.

During follow-up no function distortion was seen and regurgitation was absent. This study was also able to show that decellularized valve leaflets are able to withstand the systemic circulation.

Honge et al. (19) examined decellularized stented porcine aortic valves implanted orthotopically in the aortic position. As a control valve a commercially available gluteraldehyde treated Carpentier-Edwards (Edwards Lifesciences, Irvine, CA) valve was used and compared after 6 months of implantation.

The porcine model was used since the anticoagulation system is more similar to the human compared with the juvenile sheep model (20).

The reason for using a stented heart valve is that today 90 % of all bioprosthetic valves implanted are stented and in adult patients no growth potential is needed. The study showed no stenosis and no calcification or regurgitation in the decellularized stented aortic heart valves; however the Carpentier-Edwards was completely destroyed at 6 months of implantation.

This study confirmed superiority of the decellularized stented aortic heart valve compared with the commercially available Carpentier-Edwards valve. Emmert et al. (21) demonstrated, in an initial report, a transapical approach of implanting a TE heart valve transcatheter in the descending aortic position. In the next years more studies will follow this approach combining tissue engineering with transcatheter valve application.

Clinical studies with TE aortic valves

Meanwhile a limited number of experiences have been published of clinically implanted TE aortic valves.

These results show not only the resistance of the systemic pressure but additionally regeneration and remodelling potential of these valves. Zehr et al. (22) implanted in total 22 decellularized aortic allografts as root replacements.

No reoperations due to valve dysfunction were needed at a mean follow-up of 30.3 ± 5.2 months.

Echocardiographic examination showed a mean gradient of 8.8 ± 6.3 mmHg and maximum aortic valve regurgitation was trivial in all patients. Furthermore panel reactive antibody testing was negative in 19 out of 20 at 1 year follow-up.

Da Costa et al. (23) used a decellularized aortic allograft in 41 patients to perform root replacement.

This group reports no valve related reoperation up to 53 months of follow-up; however, one patient was reoperated on due to severe mitral valve regurgitation.

At this time a tiny biopsy was taken from the TE aortic heart valve wall.

Histological examination showed a well preserved extracellular matrix at 18 months of follow-up but also some degree of intimal hyperplasia was seen. Hemodynamic evaluation showed no or trivial regurgitation in all except one patient who showed mild to moderate regurgitation.

The mean pressure gradient at discharge ($n = 34$) was 5 mmHg, (range 1-17 mmHg) and at the latest follow-up ($n = 31$) 2 mmHg (range 1-11 mmHg).

CONCLUSION

Experimental data support the feasibility to implant a TE heart valve, based on a decellularized scaffold, into the systemic circulation. First clinical results are promising, however long-term results are needed.

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