

Tissue-Engineered Heart Valve Scaffolds

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Since the first heterotopic implanted biological heart valve in 1956 by Murray, many improvements have been made. For allografts, different methods have been evaluated and modified to stabilize and preserve tissue. Xenografts were fixated to cross-link the connective tissue and to overcome immunogenic reactions. Nevertheless, glutaraldehyde fixation leads to structural deterioration, which can be partially reduced by different kinds of antimineralization treatments. Because of preservation and fixation, allografts and xenografts become nonviable bioprostheses with a lack of remodeling, regeneration, and growth. Tissue engineering is a possible key to overcome these disadvantages because it will provide a living tissue with remodeling, regeneration, and growth potential. This overview will issue the key points to provide such a tissue-engineered heart valve by creating a sufficient scaffold where cells can grow, either in vitro or in vivo, and remodel a neoscaffold that will lead to a functional autologous heart valve. (Ann Thorac Cardiovasc Surg 2009; 15: 362–367)

Introduction

Since the first heterotopic implantation of a biological heart valve in 1956 by Murray,¹⁾ many improvements have been made. Currently available bioprostheses are nonviable heart valves. For many years it was thought that allografts were viable; however, histological evaluation showed the absence of endothelial cells, and only a few fibroblasts were observed after years of implantation.²⁾ Those limited available specific interstitial cells are not functional because previous studies showed an absence of remodeling and regeneration potential of the extracellular matrix, notwithstanding the potential of immunogenic reactions.³⁾ Furthermore, no host endothelial or interstitial cells can recellularize these structures.⁴⁾ Potential donor endothelial cells left on the inner surface of an allograft are highly immunogenic, and this can contribute to structural degeneration of allografts.^{5,6)}

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Xenogenic heart valves are normally glutaraldehyde cross-linked to mask antigens, which will prevent rejection and stabilize the connective tissue.^{7,8)} On the other hand, glutaraldehyde is toxic and avoids repopulation, which was also seen in previous studies about bioprosthetic heart valves.⁹⁾ The absence of glutaraldehyde treatment from xenografts could improve quality and long-term durability without tissue degeneration or calcification, and decellularization and sterilization should avoid the transmission of animal diseases to humans.^{10–13)}

Viable heart valves have the possibility to grow, remodel, and regenerate, influencing their long-term durability, as previous studies showed.^{14,15)} Tissue engineering is a tool that could provide autologous viable heart valves.¹⁶⁾ To create tissue-engineered valves, two elementary key points are needed, namely, a sufficient scaffold and an ingrowth of host vascular cells.

Metaphilosophy of Tissue Engineering

Several concepts have been studied to create a tissue-engineered heart valve; however, all concepts must include the two elementary key points. First, there is the three-dimensional supporting tissue, the so-called scaffold, which is sufficient (1) if mechanical and biological integrity is given, (2) if it provides dynamic and biochemical signals,

(3) if it shows cell attachment and migration, (4) if it secures diffusion of vital cell nutrients and expressed factors, and (5) if it allows dynamic changes of the scaffold architecture. These scaffolds can be divided into two groups based on synthetic and biological sources.

Second, the cellular components are either obtained *in vitro* by harvesting from autologous origin or initiated by a dynamic and chemically stimulated environment that *in vivo* provides cell signals to encourage autologous progenitor cells or valvular endothelial and interstitial cells to migration differentiate, if needed, and bind to the scaffold.

The Scaffold Sources

The two kinds of scaffold sources, synthetic- and biological-based materials, are evaluated to construct a tissue-engineered heart valve.

Synthetic-based scaffold

The original synthetic polymer scaffolds were made of polyglycolic acid (PGA) and polylactic acid (PLA).¹⁷⁻¹⁹⁾ These are polyesters that degrade by hydrolysis within the human body to form lactic acid that will be secreted in urine or enter the tricarboxylic acid cycle.²⁰⁾ Thus the advantages of these materials were based on biocompatibility, absorbability, and the possibility for *in vitro* seeded cells to attach their surfaces and remodel. Major limitations, however, were noticed. Generally, such aliphatic polyesters as PGA and PLA belong to this family and are thick, nonpliable, and stiff, which results in difficulties when they are handled. The absorbability of these scaffolds showed an overshooting fibrosis, resulting in leaflet retraction that leads to incompetence.¹⁷⁻¹⁹⁾ Furthermore, these early results showed only the behavior of leaflets and not a complete trileaflet heart valve.

Modifications were made by using polyhydroxyalkanoates (PHAs). Sodian et al.^{21,22)} used PHAs and poly-4-hydroxybutyrate (P4HB) to create a valve scaffold. The advantage of these thermoplastic polyesters to create a trileaflet heart valve were due to better handling by molding a trileaflet heart-valve shape. Limitations in these studies were the prolonged biodegradation, resulting in a synthetic scaffold persistence *in vivo*. Therefore no complete replacement of the scaffold was seen by autologous tissue during the follow-up period, which could lead to side effects.

Hoerstrup et al.²³⁾ combined the high porosity of PGA with the thermo-properties of P4HB for fabrication of a complete trileaflet heart valve. This valve showed no

leaflet degradation, absence of thrombus, or aneurysm for up to 20 weeks. The biomechanical optimized scaffolds were completely degraded 6 to 8 weeks after implantation. Furthermore, experimental data showed a progression of stenosis and regurgitation over time under low pressure circumstances. DNA levels showed higher at 20 weeks of implantation compared to native tissue, which needs to be observed as overshooting that has previously led to fibrosis by using only PGA.¹⁷⁻¹⁹⁾ This overshooting of ingrowth of valvular interstitial cells could be a result of the lack of effective biochemical signals and secures diffusion of vital cell nutrients and expressed factors that are not yet sufficiently controlled by the environment.²⁴⁾ Moreover, Schoen²⁵⁾ showed the dynamic tissue architecture and cellular evaluation development of a native heart valve during maturation, with a decrease of available valvular cells.

At the time, further limitation of the approach that uses a synthetic scaffold to create heart valves is the simplification of the valve wall to be a tube, which underestimates the integrity of a trileaflet heart valve. The native valve wall, however, is much more complicated and innovative than a simple tube, having sinuses that support the valve closure,²⁶⁾ but also it is important for the long-term durability of a heart valve, as shown by recent studies on developing new tissue valves.^{27,28)}

Another concern was made by Lutolf and Hubbel,²⁹⁾ who noted that biological scaffolds do not degrade through hydrolysis, which is the resorbability process used in synthetic scaffolds. The natural bioresorbability is rather through proteolysis, performed by proteolytic enzymes.

Biologic-based scaffold

Independent of the origin of the tissue and the ingrowth of valvular cells, two important issues need to be considered. The first is to create a sufficient three-dimensional supporting scaffold, which needs to fulfill the criteria as mentioned previously. As soon as this goal has been completed successfully, the scaffold must be sterilized to make it available for clinical use.

Decellularization

The approach to using a biological-based scaffold was initiated by respecting the complexity and integrity of the extracellular matrix given by nature. Donor-cell components, which do not allow host cells to migrate into the extracellular matrix to start the development of a neo-scaffold, need to be removed. Furthermore, these cells are immunogenically active, as mentioned previously, and are potential carriers of diseases that can be transmitted from

the donor to the host. Scaffolds available for this purpose can be from either allogenic or xenogenic origin.

The advantage of allogenic materials is that the tissue is of the species origin, which theoretically leads to less immunogenic reactions and also to the absence of potential xenogenic disease transmission.

Our group was able to show favorable results by using seeded allogenic scaffolds with a specific decellularization treatment.³⁰⁾ Unfortunately, allografts are rare especially in small sizes; therefore availability is limited as known from classical cryopreserved allografts. Leyh et al.,³¹⁾ however, showed different results by using a different decellularization technique, comparing xenogenic and allogenic tissue in an experimental model. Surprisingly, results were favorable for xenogenic compared to allogenic acellular valve matrix conduits in this model. Therefore not only is the origin of tissue important, but also the decellularization treatment that will be performed.

Large available quantities of xenogenic materials make these materials more attractive. But the disadvantages are the potential immunogenic reactions and the potential to transmit diseases from animal to human.^{32,33)}

The potential for immunogenic reactions from xenogenic materials was studied by Lynn et al.,³⁴⁾ showing potential immunogenic responses of bovine collagen, and was reported to be from 1 to 8% in patients treated with bovine derma. In contrast to these findings, Nakamura³⁵⁾ was able to show no adverse reactions by using porcine collagen.

The second potential problem is the transmission of xenogenic diseases, such as the porcine endogenous retrovirus (PERV)^{36,37)} or bovine spongiform encephalopathy (BSE), which can transfer in Creutzfeld-Jakob disease.³⁸⁾ Leyh et al.³⁹⁾ was able to show in an immunogenic study that even if a complete decellularization was not performed and 2% of residual deoxyribonucleic acid (DNA) was available, no risk was seen for the transmission of PERV to another species. Nevertheless, one should be careful to reduce DNA as far as possible.¹³⁾

Different techniques have been used to decellularize valve scaffolds; however, mostly a combination of different treatments is used. The most commonly used single elements to perform tissue decellularization are divided into the following:

1) Nonionic detergents

Nonionic detergents disrupt lipid-lipid connections as well as lipid-protein connections, but they do not interfere with protein-protein connections.⁴⁰⁾ Triton X-100, which

removes completely nuclear material, is the most widely known. The disadvantage of it is that cellular debris was still found in the valve wall,⁴¹⁾ and an almost complete loss of glycosaminoglycans (GAGs) was seen.

2) Ionic detergents

Ionic detergents are effective for complete removal of the cytoplasmatic and nuclear cellular materials.⁴²⁾ Sodium deoxycholate acid and sodium dodecyl sulfate are well known and most often used. Deoxycholic acid is able to perform a complete decellularization of heart valve tissue showing structural proteins been retained and appear to be intact.^{13,43,44)} Similar results can be achieved with sodium dodecyl sulfate; however, a disruption of the native tissue structure and causes of a decrease in GAGs concentration with loss of collagen integrity was previously reported.^{45,46)}

3) Chelating agents

Chelating agents, such as ethylenediaminetetracetic acid (EDTA), form a ring-shaped molecular complex that firmly binds and isolates a central metal ion. It has been shown that bivalent cations, such as Mg^{2+} and Ca^{2+} , are necessary for cells to attach to collagen of the extracellular matrix.⁴⁷⁾ Because of the binding of the bivalent cations, these agents remove the cellular material from the tissue.⁴⁸⁾

Enzymatic methods

Enzymatic techniques of decellularization include the use of protease digestion and nucleases.⁴⁹⁾ Trypsin is the most commonly used proteolytic enzyme. It is a highly specific enzyme that cleaves the peptide bonds.

Nucleases such as endonucleases catalyze the disruption of the interior bonds of the ribonucleotide or deoxyribonucleide chains, whereas exonucleases catalyze the disruption of the terminal bonds of ribonucleotide or deoxyribonucleide chains, resulting in a degradation of ribonucleic acid (RNA) and DNA.⁵⁰⁾ Enzymatic methods showed adverse effects at the extracellular components.⁵¹⁾ Furthermore, these enzymes are commonly from bovine sources (e.g., trypsin, DNase, and RNase) with the potential disadvantages of submitting typical bovine diseases to human and an adverse immune response to the hosts. These disadvantages are well known.³⁴⁾

Sterilization

At the time we have a sufficient decellularized scaffold, the next issue is sterilization, which is no less important. If the biologic scaffold source is from human origin, the

preparation will be similar to cryopreserved allograft.^{52,53} In the use of xenogenic material, a validated sterilization technique (ISO EN 1174-1-3:2000) is mandatory. Few data, however, have been known about this complex issue until now.

Previous studies showed that physical techniques, such as freeze-drying and irradiation, showed poor long-term results.⁵⁴ Cohen et al.⁵⁵ demonstrated that irradiation can lead to leaflet alterations, including rupture and calcification. Chemical agents, e.g., β -propiolactone, showed decreased durability, which also cannot be used.⁵⁶ Our group,¹³ however, showed that it is possible to perform a validated sterilization without destruction of the decellularized scaffold.

Clinical availability

The clinical availability of synthetic-based scaffolds for tissue-engineered heart valves is at this time unsure, regarding published in vitro and in vivo data. Tissue-engineered heart valves based on a decellularized scaffold, the so-called Matrix P[®] and Matrix P Plus[®] manufactured by AutoTissue GmbH, Berlin, Germany, have been commercially available in Europe since 2004. In European countries, such as Great Britain, France, Italy, and Germany, more than 1000 patients suffering from either congenital or acquired heart valve disease have been treated with these tissue-engineered heart valves.

Summary

In this overview, different concepts have been issued to develop a sufficient scaffold for a tissue-engineered heart valve, for which the possibility to remodel, regenerate, and grow is a demanding fundamental goal. Two concepts have been presented for reaching this goal. First, there are the synthetic-based scaffolds that have great advantages, such as easy manufacture, easy sterilization, easy production, and no potential risk of transmitting diseases. On the other hand, there are several disadvantages, such as the lack of a dynamically and chemically stimulated environment that can provide cell signals to encourage autologous valvular cells to bind the scaffold. Furthermore, the tubular shape of the valve design should be modified to a natural shape. Experimental data until now have been performed under low-pressure circumstances, which reveal the difficulties of withstanding this pressure. More modifications will be needed for a high-pressure environment.

The biological scaffold showed more potential to develop a scaffold that can be used in the high-pressure

system if some conditions are respected, namely, (1) use of an adequate source of tissue, either xeno- or allogenic; (2) performing a complete decellularization so that the scaffold will no longer transmit disease; (3) having an environment that will function as in native heart valves, stimulate valvular cell ingrowth, and function; and (4) using a validated sterilization technique. Since 2004, tissue-engineered heart valves (Matrix P[®] and Matrix P Plus[®], AutoTissue GmbH, Berlin, Germany) based on a decellularized scaffold are commercially available in Europe and have been implanted in more than 1,000 patients.

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