Tissue Engineered Heart Valve for Aortic Valve Disease. Quo Vadis, Again?

Wilhelm P. Mistiaen

Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium, Department of Healthcare Sciences, Artesis-Plantijn University College, Antwerp, Belgium

Abstract: *Introduction:* Heart valve tissue engineering has been presented as a "promising" solution" for over 20 years. These living devices are supposed to have the capacity to grow, heal and repair or remodel. This would avoid structural valve degeneration of currently used biological heart valve prostheses or the need for life-long anticoagulation or mechanical devices. Especially for patients with stenotic aortic valve disease, which is the third most common cardiovascular condition in Western societies, this solution might be useful.

Methods: A literature search has been performed for the years 2010-2014 with focus for results of tissue engineered valves of in-vitro, in-vivo animal and patient studies

Results: Most experiments were still in-vitro. Especially those experiments which focus on synthetic biodegradable scaffolds have not left the laboratory, because these cannot withstand systemic pressures. The animal studies involved scaffolds of biologic origin with or without reseeding with cells. Cells were harvested from vascular, embryonal tissues or from bone marrow. Large animal studies (ovine, porcine) dealt with implantations in pulmonary position and right ventricular reconstruction, which might be useful in the treatment of congenital heart defects. Implantation in the systemic – high pressure – circulation were only performed in small animals (rat model). One goat model showed some remarkable results, but only on very short time.

Conclusions: Tissue engineered valves seemed very promising, a promise that will not be fulfilled soon. Synthetic bioresorbable scaffolds have not left the laboratory yet. Scaffolds of biologic origin already have been tested in animals, mostly in pulmonary origin. It is by no means certain that behavior of tissue engineered valves in animals reflect the clinical situation, which is much more demanding. Also non-scientific hurdles such as official registration and commercialization of such devices have to be taken.

Keywords: Cell source, Scaffold, Bioreactor, *In vivo, In vitro*, Aortic valve disease.

INTRODUCTION

Calcified aortic valve stenosis, is the most common valve disease in Western societies. Replacement of the diseased valve is the only option to prolong life and alleviate invalidating symptoms. This replacement is possible by mechanical and biological valves. Mechanical are very durable but require life-long anticoagulation. Porcine or bovine pericardial biological valves are more suitable for elderly patients, since immunity and hence inflammation decreases with age. This results in an increased durability in this age group. Especially in the difficult age group of 55 – 70 years, there is a trade-off between life-long anticoagulation with its inherent risk for bleeding and the risk for structural valve degeneration, with need for reoperation [1]. Our own results with pericardial valves in elderly patients were favourable [2-4]. The pericardial valve is very durable and approaches that of the homograft, which can be considered as the gold standard [5]. However, the availability of homograft devices is a problem. In order to avoid the necessity of life-long anticoagulation or the risk for structural valve degeneration, tissue engineered valves (TEHV), have been proposed as a solution. These devices are considered as living structures, capable of growth, remodelling and in-vivo repair. This is not possible with current mechanical or biological devices. TEHV could also be helpful in repairing congenital heart defects. This however is beyond the scope of this manuscript. There are several approaches in use to construct TEHV [6]: 1) cell seeding on biodegradable scaffold with subsequent maturing in a bioreactor; 2) cell seeding on natural biodegradable scaffold; 3) guided tissue regeneration / remodelling of implanted degradable tissues by cells of the host, and 4) implantation of decellularized devices. The question remains: what research has been done so far *in vitro*, in animal studies and what are the results in patients.

METHODS

A systematic review of literature was performed by searching of electronic database "ISI web of knowledge". The time span was from 2010 to 2014 and the used search terms were "tissue engineered heart valve AND animal" (48 items), "tissue engineered heart valve AND ovine" (26 items), and "tissue engineered

*Address correspondence to this author at the Artesis-Plantijn University College of Antwerp, 2610 Antwerp, Belgium; Tel: 32 3 641 82 41; Fax: 32 3 641 82 71; E-mail: wilhelm.mistiaen@uantwerpen.be

E-ISSN: 2410-2822/16 © 2016 Cosmos Scholars Publishing House

heart valve AND patient* (71 items). Forty-one multiple references were identified. Irrelevant papers (not dealing with cardiac tissue engineering), conference proceedings, book series and reviews were also removed (n=47). A search in the references of the identified articles did not result in more useful manuscripts. The papers were divided in pure "*in vitro*" and "*in vivo*" results". Any research papers in which living animals were involved were classified as "*in vivo*".

RESULTS

The search resulted in 29 full articles. There were 18 papers dealing with "*in vitro*" experiments. Table **1** summarizes the main data concerning cells and matrices which are used [7-20]. The main cell sources include valvar interstitial cells, fibroblasts, endothelial cells, smooth muscle cells and mesenchymal or bone marrow stem cells. The matrices most in use are biodegradable polymers such as polyglycolic acid, polyhydroxyalkanoate or poly-4-hydroxybutyrate, or a combination, which are tested for their biomechanical properties. Electrospinning has become a modern mode of fabrication of such synthetic bioresorbable scaffolds [8, 12].

Some papers deal with specific issues, which are not included in the table. Two manuscripts deal with a first issue, namely biocompatibility and immunological considerations such as absence of Gal-epitopes in decellularized pulmonary root conduits, in which the extracellular matrix was partially preserved. This could make such devices potentially suitable as substitute for the right ventricular outflow tract in the Ross procedure [21, 22]. A second issue deals with the interspecies comparison of biomechanical properties. Ovine and porcine valves are much more compliant compared to aged human valves. The latter contain more collagen and elastin compared to ovine valves. Therefore, animal models do not necessarily represent biomechanical properties present in elderly patients, which are most in need for valve replacement [23]. The last issue concerns the development of pulse reactors, hemodynamic and imaging systems [24], which is not the focus of this review.

Ten papers can be qualified as "*in vivo*" since an implantation in animals was involved. The main data are summarized in Table **2** [25-33]. Most of these articles involve implantation, of devices in pulmonary position. Two articles deal with implantation of a U-shaped decellularized device implanted at the level of the infra-renal aorta of a rat. The aorta was ligated and the device which serves as bypass was sutured end-to-side on the aorta. Another remarkable goat model showed that two months after implantation of a mould in dorsal subcutaneous tissue, a semilunar valve like

Table 1: *In Vitro* Studies

Reference	Cell Source	Scaffold Type	Mode of Analysis
7	VIC and MSC	biodegradable tri-layered	biomechanical
8	human VIC	biodegradable*	biomechanical, IHC
9	human fibroblasts	stented tissues	biomechanical
10	none	acellular porcine	IHC pulmonary root
11	ovine umbilical vein	fibrin derived textile reinforced	IHC
12	ovine mitral VIC	biodegradable* hyaluron hydrogrel	biochemical
13	periodontal lig. cells	fibrin-derived	biochemical
14	VIC	composite biodegradable	biochemical & biomechanical
15	non-contractile cells	fibrin-derived	biomechanical & biochemical
16	Aortic VIC & SMC	collagen gel & osteogenic medium	mRNA expression, IHC
17	Ovine myofibroblasts human VSM cells	biodegradable	biomechanical
18	fetal amniotic umbilical blood cells	biodegradable	Biomechanical biochemical, HIS, SEM
19	CD133 positive human bone marrow cells	fibrin-derived & decellularized porcine pulmonary valve	biomechanical, IHC
20	MSC	Decellularized porcine pericardium + PA	IHC

IHC: immunohistochemistry; lig. ligament; PA: pulmonary artery SEM: scanning electron microscope; SMC: smooth muscle cells; VIC: valve interstitial cells; VSM: vena saphena magna; * by electrospinning

Table 2: *In Vivo* Studies

Reference	Animal	Procedure	Scaffold	Cells	Analysis	Follow-up
25	porcine	pulmonary	decellularized	none	IHC, TEM gene expr	6 & 15 months
26	ovine	TPVI	homologous	none	hemodynamic	24 weeks
27	ovine	TPVI	stented valve	art. EC/SMC bm-CD133+	IHC hemodynamic	
28	goat	Ap-Ao	autologous	none	hemodynamic	2 months
			connect. tissue	none	IHC	
29	rat	infrarenal	decellularized	none	IHC, MRI	8 weeks
		aortic	pulmon valve	ovine vascular	Doppler	
30	ovine	TPVI	intest submuc	EC & MFB	angiography, IHC	4 weeks
31	rat	Infrarenal aortic	decell. Aortic valve	isogenic EC & MFB	Doppler, IHC	4 weeks
32	ovine	pulmonary	decell pulmon valve	autologous EPC CD133+ ab	Biomechanical IHC	1 – 3 months
33	ovine	TPVI	synthetic	Autologous vascular & stem cells	echo, angio IHC	8 weeks

ab: antibodies; Ap-Ao: apico-aortic bypass; art: arterial; angio: angiography; coon: connective; decell: decellularized; bm-CD133+: bone marrow derived CD133 pistive cells; EC; endothelial cells; EPC: endothelial progenitor cells; expr: expression; IHC: immunohistochemistry; intest submuc: intestinal submucosa; MFB: myofibroblasts; MSC: mesenchymal stem cells; SMC: smooth muscle cells; PTVI: transcatheter pulmonary valve implantation; pulmon: pulmonary; TEM: transmission electron microscope

construct of connective tissue of type VI can be formed. This proved to be of adequate strength and elasticity. Sufficient opening and coaptation of the leaflets could be demonstrated *in vitro*. The device was implanted as an apical-aortic bypass, which was monitored hemodynamically for 2 months. After explantation, the valves still looked as native aortic valves. Collagen, some elastin, fibroblast and smooth muscle cells could be demonstrated. There was no lining of endothelial cells. Thrombus formation was absent, however [28]. No series involving patients could be identified in the current search.

DISCUSSION

The search for the ideal tissue engineered heart valve is still ongoing. Dozens of scientific manuscripts are produced annually. A considerable part of these papers are reviews. This paper attempts to give a short overview of what has been performed in the last 5 years, from 2010 to 2014. It is immediately clear that most research manuscripts are devoted to in-vitro testing of constructs. There is a wide variation in scaffolds (synthetic and biologic), in cells (vascular, bone marrow and foetal) and in culture media. The majority of in-vivo experiments is focused on devices implanted in pulmonary position. This is useful in paediatric patients with congenital heart defects, but this is a rather small population, with complex and variable pathology. Devices with artificial scaffolds lack stability, which can result in early failure, especially in high pressure systems. For patients with left sided heart valve disease, of which aortic valve stenosis is the most common, experiments with implants in the systemic circulation are of more interest. These experiments are few and all are focused on short term results, *i.e*. up to two months. Last but not least, there are no patient series in whom a tissue engineered valve is implanted. It is also obvious that implantation of glutaraldehyde fixed porcine valves or pericardial bovine valves cannot be considered as “tissue engineering”.

There are still major questions which need to be solved. First, have the components of native valves and their interaction been identified? Although this information is necessary to construct TEHV in an adequate way, it might not be the case since it has been recently deemed to be necessary to compare human valves with porcine and ovine valves. This comparison should have been done long time ago! Second, do properties of *in vitro* constructs correlate with *in vivo* results such as durability, freedom of re-operation and event free survival? Will living cells in these constructs behave in the desired way, if these have been used? Animal studies thus far were limited to a few months, while a valve in clinical practice needs to have a durability of more than 10 years. Moreover, it is by no means certain that an animal model represents clinical situations adequately. In ovine models, for example, fibrosis occurs much more extensively [34]. Third, is the behaviour of a living device such as TEHV predictable enough once it has been implanted? And if

not, can patient-related factors, which are responsible for the variation in behaviour be identified? Fourth, there is a universal bias caused by a tendency not to publish negative results. Much is to learn from failures, but such results have published been rarely. Since one cannot learn from what has not been published, the same futile efforts will be made over and over again. Fifth, there are non-scientific aspects. When a device contains living cells, regulation by FDA and CE marking become much more difficult. Commercialization can also be a major hurdle: one has to compete against established values such as pericardial valves which have a very predictable outcome in terms of survival and adverse events such as structural valve degeneration and thromboembolic events. Even if today, a perfectly working TEHV is produced, the proof of its long-term durability may require 10, 15 or even 20 years. The limitations to produce a viable TEHV device can only be overcome if 1) all failed experiments are published, because one learns most from failure, 2) one source of cells for endothelial lining and possible another source for repopulation of the matrix itself can be found which is superior to all other cell sources, 3) one matrix can be identified which is superior to all other scaffolds in terms of durability, 4) long-term animal results can be produced and 5) agreement can be reached how precisely and by which criteria the devices can be evaluated in-vitro as well as in-vivo. Any tissue engineered device has to compete against established values such as the Carpentier-Edwards pericardial valve which as a durability of 15 years or more in most elderly patients. Contribution of manufacturers can only be obtained when a scientific and commercial viable solution can be demonstrated.

CONCLUSIONS

Tissue engineered have has been promising, but its fulfilment is not about to come in any time soon. Most experiments are in-vitro with an almost endless variety in scaffolds, cells and culturing conditions. In-vivo experiments are mostly limited to pulmonary positions, which has a lower pressure regimen than the systemic circulation. With exception of homograft devices and glutaraldehyde fixed xenografts, which cannot be called tissue engineered heart valves, there are no patient series. It will be difficult for any TEHV to compete with existing durable biological heart valves. There are some limitations in this work: only the last 5 years have been searched and the focus was directed on patients with aortic valve disease. However, experiences of the last 5 years can learn enough about the lack of progress that has been made. This work also excluded decellularized xenografts such as Contegra bovine jugular vein, Shelhigh, Synergraft and Matrix P / Matrix P plus devices. These are in use for reconstruction of the right ventricular outflow tract, in the correction of congenital heart defects. Moreover, one could argue if these really belong to the class of TEHV.

CONFLICT OF INTERESTS AND ACKNOWLEGE-MENTS

There are none to declare.

FUNDING

None

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Received on 25-01-2016 Accepted on 22-02-2016 Published on 11-03-2016

http://dx.doi.org/10.15379/2410-2822.2016.03.01.02

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