Tissue-Engineered Devices in Cardiovascular Surgery

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Key Words

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Abstract

Manufacturing life-long functional cardiovascular (CV) implants is the ultimate goal for researchers and clinicians in the cardiothoracic field. Tissue engineering (TE) is an opportunity to create ideal prostheses that are vital, growing, adaptive, autologous and functionally optimally performing. Today, initial translation from basic science to first clinical trials has begun. The article depicts the state of the art in TE techniques for CV products and describes milestones in the ongoing development of tissue-engineered myocardial, valvular and vascular devices from an experimental and clinical point of view. Artificial CV implants still reveal remarkable limitations but promising advances regarding optimal structural design, the prevention of intimal hyperplasia and the reduction of antigenicity and thrombogenicity. Where applicable, the implantation of vascularized autografts should still be preferred. Apart from that, decellularized allogeneic bioprotheses currently represent most promising matrix scaffolds that can be autologously cellularized in vitro prior to or in vivo after implantation. Capable biologic alter-

natives have been described like the decellularized porcine small intestinal submucosa. Rising evidence suggests that in vitro endothelialization might be the minimal requirement for improved long-term results of biological tissue-engineered CV grafts.

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Introduction

Functional reconstruction and replacement of cardiovascular (CV) structures with the means of tissue engineering (TE) is evolving dynamically. The implantation of ideal prostheses has not been described yet. However, TE could enable the generation of vital, growing, adaptive, autologous and functionally competent CV tissues and organs. Understanding the roles and interactions of parenchymal, stromal and stem cells with highly defined biologic and artificial matrices and scaffolds will smoothen the way to success [1]. Herein, translational approaches facilitate bridging the latest results from laboratory benches to surgical theaters. The article describes current advances in TE of myocardial, valvular and vascular products and estimates their availability and feasibility for the clinical surgeon.

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The biocompatibility and long-term performance of biological and artificial devices, in particular xenografts and artificial small-diameter tubular grafts, are clearly limited due to complications like degradation, fibrosis, calcification, immunologic rejection, infection and/or thrombogenicity [20–22]. As a consequence, special decellularization processing was recommended for biological grafts improving their immunogenicity and engraftment [23, 24]. Today, autologous cellularization of TE products is feasible for various multipotent stem and dif-

Fig. 1. General principles in cardiovascular TE. The scheme illustrates the processing of vascular, valvular and myocardial grafts generated from biological and artificial scaffolds and cellularized by in vitro bioreactor-driven conditionings, direct tableside coating strategies or in vivo engraftment following implantation. Consequently, these TE products could replace primarily the function of a diseased structure and secondly the complete structure by autologous and functional reconstructed tissue.

ferentiated mature cell types [1]. It was postulated that endothelial cell seeding could eliminate thrombogenicity of tissue-engineered CV implants [25]. For superior endothelial engraftment, different in vitro cellularization techniques were introduced including bioreactor-driven conditionings [26–29] or direct coating strategies [30]. For the enhancement of the angiogenic microenviron-

Table 1. Milestones in the development of vascular TE devices

Device type	Graft origin/scaffold	In vitro cellularization	Functionalization	Implantation	Availability	Reference
Biologic	Porcine detergent decellularized aorta	Scaffold cell seeding and bioreactor conditioning	None	Orthotopic in vivo	Experimental	Bader et al. [27] Teebken et al. [37]
	Hemodialysis shunt graft	Autologous in vitro grown	None	Arteriovenous shunt in vivo	Clinical study	McAllister et al. [39]
Artificial	Small-diameter polycaprolactone	None	+/- Paclitaxel-eluting	Rat aorta in vivo	Experimental	Innocente et al. [35] Pektok et al. [41]
	ePTFE graft	Scaffold cell seeding	RGD-peptide coating	In vitro analysis	Experimental	Walluschk et al. [29]
	Different polymer grafts	None	None	Porcine coronary artery in vivo	Experimental	van der Giessen et al. [22]
	Copolymer (polycaprolactone, polylactid, polyglycolic acid)	Scaffold cell seeding and bioreactor conditioning	None	Pulmonary artery, cavopulmonary conduit in vivo	Clinical study	Shin'oka et al. [38] Hibino et al. [42]

The table summarizes highly relevant advances in experimental and clinical research developing tissue-engineered vascular devices with respect to graft origin, cellularization technique and possible implantation sides.

ment, autologous biomaterials like fibrin could be used in cell-plus-matrix molded or coated CV devices [30, 31]. As an alternative, tissue-engineered CV implants could be cellularized in vivo (i.e. in situ) with possible regulation by cytokines or special surface coating strategies [29, 32, 33]. The incorporation of local drug delivery systems into the matrix of a graft could remarkably modulate in vivo stem cell recruitment and the recellularization process [32]. Intense investigation is currently ongoing in order to create smart TE products that are stimulus-sensitive and therefore adaptive to multiple microenvironmental situations.

Vascular Tissue Engineering

The transplantation of arterial and venous autografts still represents the gold standard for vascular replacement of small-diameter vessels like coronary arteries. Dacron and polytetrafluoroethylene prostheses, xenografts or allograft are commonly used for the reconstruction of larger vessels like the ascending aorta, the pulmonary trunk and arteries, the aortic arch and its branch vessels. However, these artificial or xenogenic vascular grafts show unsatisfactory long-term results with lower prosthetic diameters (below 6 mm) because of intimal hyperplasia and other above-mentioned reasons [20–22]. Apart from that, the availability of autografts can be limited due to former surgery or vascular disease and harvesting of autografts will definitely increase the surgical

trauma. Consequently, TE concepts aim at the generation of anytime-available, adaptive, immunologically accepted and functionally long-term competent prostheses [34]. Three-dimensional artificial or biologic cell-free matrices could be tissue-engineered employing in vitro or in vivo cellularization reducing long-term complications especially in small-diameter artificial prostheses [26, 30, 32, 35] (table 1). These cellularization technologies demonstrate the potential to promote complete autologous replacement of implanted vascular grafts with cellular organization and structural integrity of a native vessel. First in-man trials are currently ongoing [36–39].

The highly precise fabrication of artificial tubular constructs can be accomplished by computer-controlled rapid prototyping or electrospinning beside other methods. Biomechanical, biochemical as well as micro- and nanostructures (e.g. porosity) can be regulated [10, 40]. However, biodegradable vascular prostheses are currently not yet commercially available for the clinical surgeon because of long-term insufficiency [34]. Polymers with prolonged in vivo biodegradation time like polycaprolactone demonstrated at least promising results. Six months after experimental in vivo transplantation into the arterial circulation small-diameter vascular polycaprolactone grafts were found still functional and completely engrafted with autologous endothelialization [41]. More recently, advanced approaches even introduced biodegradable polymer-based vascular grafts into the clinical practice by implanting extracardiac cavopulmonary conduits made from polymer sheets in children [42].

Table 2. Milestones in the development of valvular TE devices

Device type	Graft origin/scaffold	In vitro cellularization	Function-alization	Implan-tation	Availability	Reference
Biologic	Porcine cryopreserved decellularized pulmonary graft	Direct cell-plus-matrix scaffold coating	None	In vitro analysis	Experimental	Kaminski et al. [30]
	Porcine fibrin hydrogel-based valvular grafts	Complete cell-plus-matrix molding and bioreactor conditioning	None	Pulmonary graft in vivo	Experimental	Flanagan et al. [31]
	Porcine pulmonary graft coated with small intestinal submucosa for pulmonary valved stent	Scaffold cell seeding and bioreactor conditioning	None	Orthotopic in vivo	Experimental	Lutter et al. [4]
	Detergent decellularized aortic and pulmonary grafts (porcine, sheep)	Scaffold cell seeding and bioreactor conditioning	None	Orthotopic in vivo	Experimental	Rieder et al. [23] Kasimir et al. [24, 25] Bader et al. [26] Steinhoff et al. [33] Lopes et al. [55]
	Titanium-coated glutaraldehyde-fixed xenografts	None	Titanium-coating	In vitro analysis	Experimental	Guldner et al. [62]
	Human detergent decellularized pulmonary graft	Scaffold cell seeding and bioreactor conditioning	None	Orthotopic in vivo	Clinical study	Cebotari et al. [45]
	Human detergent decellularized aortic graft	None	None	Orthotopic in vivo	Experimental/clinical study	da Costa et al. [56] (clinical) Akhyari et al. [61] (experimental)
	Human cryopreserved decellularized pulmonary graft	None	None	Orthotopic in vivo	Clinical study	Brown et al. [50] Konuma et al. [51]
Artificial	Polycaprolactone three-leaflet valve	None	None	In vitro functional assessment	Experimental	Del Gaudio et al. [46]
	Polymer (SIBS) three-leaflet valve	None	None	In vivo	Experimental	Wang et al. [49]

The table summarizes highly relevant advances in experimental and clinical research developing tissue-engineered valvular devices with respect to graft origin, cellularization technique and possible implantation sides.

Valvular Tissue Engineering

Long-term results after heart valve replacement using allogenic and xenogenic biological heart valves are influenced by tissue degeneration, calcification and infection. Unless optimal hemodynamics valvular allografts and xenografts as well as vital autografts in Ross procedures even show limited late postsurgical outcomes [2, 43, 44]. TE may be used to generate valve tissue undergoing vital regeneration from autologous cell source and tissue adaptation including endothelial recovery and infectious compatibility. In particular, pediatric and younger patients could be treated with vital, growing and adaptive implants preventing reoperations [2]. In accordance with vascular TE, valvular TE requires a three-dimensional biologic or artificial matrix scaffold as well as in vitro or in vivo cellularization [4, 26, 30, 45]. In addition, there are necessities mainly attributed to valvular TE that have not yet been accomplished today. The regional different load conditions

at a performing semilunar valve set strong demands to the fabrication of an optimal TE valve [46]. Soft but sufficient valve closure requires a balance of structural stability, tensile strength and elastic modulus for both the three-dimensional scaffold as well as the single polymer fibers. Moreover, the creation of vital TE valves most probably needs a highly dynamic processing with the maintenance of special active signals [47, 48]. Apart from that, polymer biodegradation (of implanted biodegradable valves) and in vivo restoration of a long-term functional vital valve must run simultaneously and equally efficient [49].

Decellularized allografts and xenografts seem almost optimal scaffolds for valvular TE [30, 50, 51] (table 2). However, different decellularization protocols show varying efficiency for decellularization, recellularization capacity and removal of antigenicity [20, 23, 24]. The cryopreserved decellularized valves show a highly preserved extracellular matrix structure resulting in adequate midterm hemodynamic performance of allografts

Table 3. Milestones in the development of myocardial TE devices

Device type	Graft origin/scaffold	In vitro cellularization	Function-alization	Implantation	Availability	Reference
Biologic	Porcine extracellular matrix from urinary bladder	None	+/- growth factors	Myocardial patch in vivo	Experimental	Badylak et al. [64] Ota et al. [66]
	Small intestinal submucosa	Scaffold cell seeding and in vitro cultivation	None	Myocardial patch in vivo	Experimental	Tan et al. [3]
	Decellularized bovine extracellular matrix from pericardium	+/- scaffold cell seeding	+/- growth factors	Myocardial patch in vivo	Experimental	Chang et al. [65, 67]
	Vascularized grafts (small intestine submucosa, gastric submucosa)	None	None	Myocardial patch in vivo	Experimental	Tudorache et al. [69] Ruel et al. [70]
	Skeletal muscle (M. latissimus dorsi) grafting	None	None	Ventricle (re-) construction in vivo	Experimental	Acker et al. [72]
	Porcine decellularized myocardial	+/- scaffold cell seeding and in vitro cultivation	None	Myocardial patches and pouches in vivo	Experimental	Wang et al. [75] Yildirim et al. [76] Zimmermann et al. [77]
	Combined autografting	None	None	Cardiomyoplasty in vivo	Case study	Taheri et al. [74]
Artificial	Polyester urethane urea patch	Direct laser cell printing	None	Myocardial patch in vivo	Experimental	Gaebel et al. [68]

The table summarizes highly relevant advances in experimental and clinical research developing tissue-engineered myocardial devices with respect to graft origin, cellularization technique and possible implantation sites.

that is even superior to conventional allografts [50, 51]. These cryopreserved decellularized allografts are commercially available anytime [51] but the overall supply is limited due to the donor dependency. Unfortunately, these valves revealed a seemingly low recellularization capacity complicating in vitro and in vivo TE [30, 52, 53]. Moreover, several studies documented a remarkable number of residing donor cells and immunogenic epitopes like alpha-Gal that had led to strong foreign body reactions in xenografts [20, 24, 54]. In contrast, detergent-mediated decellularized valves seem less immunogenic and more engraftable while maintaining adequate mid-term hemodynamic performance even in aortic valve position [50, 51, 55, 56]. By applying TE, these allografts and possibly xenografts might become optimal substitutes for a diseased native valve [57].

However, the feasibility and necessity of in vitro recellularization of decellularized valves is currently in question. In vitro preconditioning in bioreactors requires several weeks of complex laboratory processing with contamination risks and high costs [58]. Direct cell-coating strategies performed bedside during valve replacement surgeries seem more simply applicable, anytime available and adaptive to individual specialties [30]. Apart from that, the availability of autologous cells for valvular in vitro cellularization is critical especially in pediatric cases.

The importance of umbilical cord-derived endothelial cells as well as stem cells has to be emphasized for these special patients [1]. Moreover, valvular cells seem highly differentiated. Consequently, the appropriateness of using endothelial and interstitial cells from peripheral vessels for valvular TE is also questioned [59]. Regarding the necessity for in vitro cellularization prior to implantation, the majority of studies support the need for superficial endothelialization of decellularized valves that might improve long-term results of grafts at least in pulmonary valve position [25, 60]. On the contrary, the more demanding interstitial in vitro cellularization might be dispensable for the prevention of explained long-term complications [61].

Promising alternatives are exclusively in vitro manufactured autologous cell-plus-matrix molded valves that have already been tested in vivo in a pioneer study [31]. However, bioreactor conditioning is intensely time consuming and the concept is currently not yet applicable for clinical use. Another biologic opportunity might be the construction of valves with the decellularized matrix from small intestinal submucosa. These xenografts would be commercially available anytime and seemingly demonstrate high in vivo recellularization potential [3, 4]. Apart from that, coating of conventional glutaraldehyde-fixed xenografts with titanium might accelerate in vivo endothelialization and improve long-term outcomes [62].

Myocardial Tissue Engineering

The bioartificial generation of myocardial tissues similarly employs artificial or biologic cell-free matrices that will be cellularized *in vitro* or *in vivo*. In contrast to other TE products, myocardial prostheses demonstrate special requirements like a synchronized activation and contraction of its cardiomyocytes, adequate mechanical strength, elastic modulus and force development as well as electrophysiological integration after *in vivo* implantation. Apart from that, cell-plus-matrix myocardial TE grafts (thicker than 100 μm) need vascularization for the survival of cardiac myocytes [63].

Various biological substrates like the urinary bladder, the small intestinal submucosa or the pericardium have been tested (table 3). These cell-free matrices were vascularized and recellularized *in vivo* with cardiomyocytes, myofibroblasts and smooth muscle cells beside others. Some of the *in vivo* tissue-engineered prostheses even demonstrated remarkable contractility [3, 64, 65]. The addition of growth factors and/or stem cells could further accelerate the recellularization process [66, 67]. In our group, we found that precisely directed cell seeding on artificial matrices with the use of laser cell printing ('endothelial network printing') could faster facilitate a functional connection of the grafts' preformed microvasculature to the host's microvasculature [68]. Apart from that, the use of vascularized autotransplants from small intestinal or gastric submucosa, peritoneum or omentum enables the application of transplants with immediate sufficient vascularization. At present, they have been predominantly tested experimentally as epicardial patches for the repair of ischemic myocardium [69–71]. Prostheses from skeletal muscle seemingly still demonstrate the greatest potency for myocardial support or replacement [72]. However, the transdifferentiation potential and electrical coupling of myoblasts and myocytes to cardiomyocytes is limited [73]. Clinically, pioneer surgeons have treated severe heart failure patients with combined biological TE products [74].

A decellularized heart muscle logically seems as the most favorable biomatrix for myocardial TE products. The macroscopic and microscopic three-dimensionality is preserved and cell-matrix interactions after autologous recellularization could work optimally [75]. However, the limited availability of allografts, the immunogenicity of xenografts as well as the difficulties in isolation and cultivation of an adequate number of autologous contractile cells currently impede clinical translation. Nevertheless, highly advanced approaches experimentally tested in vi-

tro tissue-engineered myocardial pouches for ventricular assist devices following physical conditioning in bioreactors [76]. This research group even showed efficient electrical coupling of tissue-engineered cardiomyocyte cell sheets after pericardial transplantation to the heart [77]. These encouraging results will hopefully soon find translation into clinical practice for the regeneration and replacement of diseased myocardium.

Conclusion

Clinical translation has begun in cardiac surgery. TE promises the creation of vital, growing, adaptive, autologous and functionally optimally performing CV implants. However, basic technology and clinical translation is still in an initial phase. Small-diameter vascular grafts, life-long functional valvular grafts without the need for anticoagulation and sufficiently force-developing myocardial grafts will not be clinically available in a short-term development. The article cites landmark studies in the evolution of TE in the CV field. Artificial grafts still show several limitations but remarkable improvements in structural design, engraftment and hemocompatibility. Apart from that, techniques for vascularized autograft transplantation have been described. If autografting is not applicable or feasible, decellularized allogeneic bioprostheses can be applied that are recellularized *in vitro* or *in vivo*. Promising biological alternatives have been portrayed like the decellularized small intestinal submucosa. Noteworthy, *in vitro* endothelialization might be obligatory for the improvement of long-term results of decellularized biological transplants. The importance of regulating *in vivo* cellularization by active signals via biofunctionalization is under intense investigation.

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Disclosure Statement

None of the authors have a conflict of interest to declare.

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