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Tissue engineered heart valves based on human cells

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Summary

Valvular heart disease is still a significant cause of morbidity and mortality worldwide. Clinically used valve replacements including mechanical valves as well as fixed biological xeno- or homografts are associated with several major disadvantages. Alternatively, tissue engineering aims at the fabrication of autologous living cardiovascular replacements with the potential to grow and to repair, particularly for paediatric applications. Therefore, autologous cells are harvested and seeded onto three-dimensional matrices followed by biomimetic in vitro conditioning enabling the development of the neo-heart valve tissue. Here, we review different human cell sources such as vessels, bone marrow, umbilical cord tissue and blood, and chorionic villi with particular regard to cell phenotypes and their suitability for extracellular matrix production for tissue engineering purposes.

Key words: tissue engineering; heart valves; bone marrow cells; umbilical cord cells; chorionic villi cells

Introduction

Progress in medical treatment of cardiovascular diseases and defects has been significant; particularly tissue substitution has shown that functional replacements of tissue and organs could be lifesaving. In the United States, for example, 60,000 heart valve replacements are performed annually [1]. Nevertheless, heart valve disease is still a significant cause of morbidity and mortality worldwide and leads in approximately 20,000 cases per year to death [1]. Furthermore, 60% of substitute valve recipients develop serious prosthesis-related complications within 10 years postoperatively [2]. Thromboembolisms as well as increased rates of infections and immunological reactions against the foreign material are the major problems with currently available heart valve replacements, which are either artificial valves or chemically treated biological xeno- or homografts. Often lifelong anti-coagulation therapy is necessary, burdened with a substantial risk of spontaneous bleeding and embolism, particularly in patients over 70 years [3].

Remaining inherently different from the tissue it replaces, the currently available prostheses do not actively adapt to the physiological environment such as to pressure changes and mechanical demands as they represent non-living materials. Furthermore, when implanted into the immature heart of a child, these materials do not grow with the paediatric patient, which is a disadvantage for the repair of congenital defects. Regarding this major limitation, there is a special need for living, growing cardiovascular replacements for paediatric applications. Paediatric treatment of cardiac defects commonly requires non-autologous valves or conduits [4, 5] with many disadvantages including obstructive tissue ingrowths and calcification of the replacements [6]. This typically causes re-operations over the lifetime of paediatric patients with cardiovascular defects, associated with increasing morbility and mortality. Living tissue replacements with the capacity of growth and regeneration would have fundamental advantages over the currently available cardiovascular replacements.

The above mentioned limitations have motivated the exploration of novel approaches towards valve replacement. A series of studies have been undertaken to determine if tissue engineering principles could be used to develop viable, valve substitutes with a thromboresistant surface and a viable interstitium with repair, remodelling and growth capabilities. Several groups demonstrated the feasibility of creating living cardiovascular structures by cell seeding on synthetic polymers, collagen, or xenogeneic scaffolds [7–10]. As to heart valve tissue engineering the first milestone was the successful replacement of a single pulmonary valve leaflet by a tissue engineered autologous leaflet [11].

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Strategies in heart valve tissue engineering

Two strategies have been used to generate living autologous heart valve replacements. One requires an *in vitro* phase generating the replacement ex vivo [12]. The other bypasses the *in vitro* tissue culture phase by direct implantation of natural tissue-derived heart valve matrices for potential cell ingrowth and remodelling *in vivo* [13]. Matrices used for the latter approach included decellularised tissues derived from pericardium or valves, cell free porcine small intestine submucosa [13] or synthetic biodegradable polymeric scaffold such as collagen or fibrin gel. However, decellularised scaffolds implanted in humans demonstrated ingrowth of host cells, no calcification but a strong inflammatory response [14]. The structural failure of these materials inhibited further use.

Concept of *in vitro* heart valve tissue engineering

Following the approach of *in vitro* tissue engineering, the successful fabrication of autologous living cardiovascular replacements similar to their native counterparts is supported by three main elements: (1) autologous cells that resemble their native counterparts in phenotype and functionality, (2) a temporary supporter matrix (biodegradable scaffold) which promotes tissue strength until the extracellular matrix produced by the autologous cells guarantees functionality on its own, and (3) culture conditions enabling tissue formation and maturation by *in vitro* conditions similar to a physiological environment.

Figure 1 summarises the concept of *in vitro* tissue engineering. Concretely, in a first step cells are harvested from an autologous donor structure.

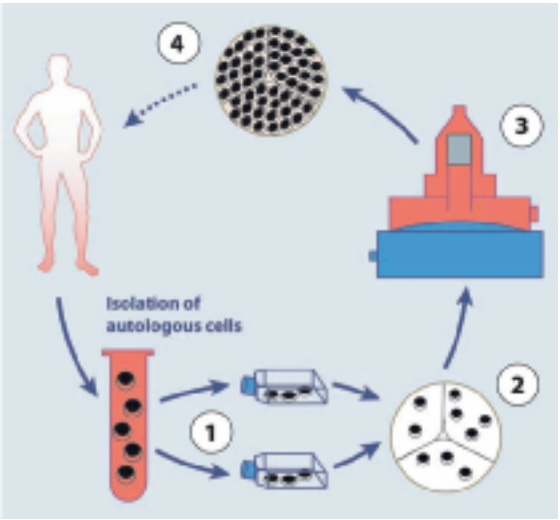

Figure 1

Concept of in vitro heart valve tissue engineering. Autologous cells are harvested from the patient and expanded in vitro (1). When sufficient numbers are reached, cells are seeded onto a biodegradable heart valve scaffold (2). Constructs are positioned in a bioreactor (3) and conditioned. When tissue formation is sufficient, tissue engineered heart valves are ready for implantation (4).

After expansion *in vitro*, cells are seeded onto a biodegradable heart valve scaffold, which should ideally be at least 90% porous [15]. Scaffolds could be fabricated from polymers and the use of these different synthetic materials has already been broadly demonstrated for cardiovascular tissue engineering. An overview about the most common scaffold types applied in cardiovascular tissue engineering is given in table 1. After seeding, the constructs are cultured in nutrient media supplemented with ascorbic acid and 10% foetal calf serum in a pulse duplicator *in vitro* system (bioreactor) mimicking the *in vivo* environment. In order to improve cell migration, proliferation and extracellular matrix production mechanical load by means of shear stress have been applied to the seeded valves in pulsatile flow bioreactors [16–18]. There, the tissue formation takes place and after several days the constructs are ready for implantation.

This concept for *in vitro* heart valve tissue engineering was earlier applied in an animal model using completely autologous tissue engineered heart valves based on polyglycolic acid coated with poly-4-hydroxybutyrate (PGA/P4HB) (figure 2) starter matrices [9]. In this "proof of principle" study trileaflet heart valve scaffolds were fabricated from PGA/P4HB bioabsorbable polymers and sequentially seeded with autologous ovine myofibroblasts and endothelial cells. The constructs were grown for 14 days in a pulse duplicator *in vitro* system under gradually increasing flow and pressure conditions and implanted into a growing sheep model (n = 6 lambs; mean weight at cell

Table 1

Examples of different scaffolds for heart valve tissue engineering.

Scaffold	Source	Examples	Reference
synthetic	biocompatible and biodegradable polymers	polyglycolic acid (PGA) polylactic acid (PLA) polyhydroxyalkanoates (P3HB) PGA and PLA (PGLA) PGA and P4HB	Shinoka T, et al., Circulation 1996 Shinoka T, et al., JTCS 1998 Sodian R, et al., Circulation 2000 Zund G, et al., EJCTS 1997 Hoerstrup SP, et al., Circulation 2000
biological	xenogenic or allogenic	decellularized porcine pulmonary heart valves pulmonary heart valves on allogenic acellular matrix conduits	Schenke-Layland K, et al. Cardiovasc Research 2003 Steinhoff G, et al., Circulation 2000
gels	fibrin	heart valves based on fibrin-myofibroblast cell suspension	Jockenhövel S, et al., EJCTS 2001
hybrid	decellularised heart valves coated with synthetic polymer	porcine aortic heart valves dip coated with biodegradable poly(hydroxybutyrate)	Stamm C, et al., Ann Thorac Surg 2004

harvest 9 ± 2.8 kg). Echocardiography demonstrated mobile, functioning leaflets without stenosis, thrombus or aneurysm. These autologous tissue engineered valves functioned up to 5 months *in vivo* and resembled normal heart valves as to microstructure, mechanical properties and extracellular matrix formation.

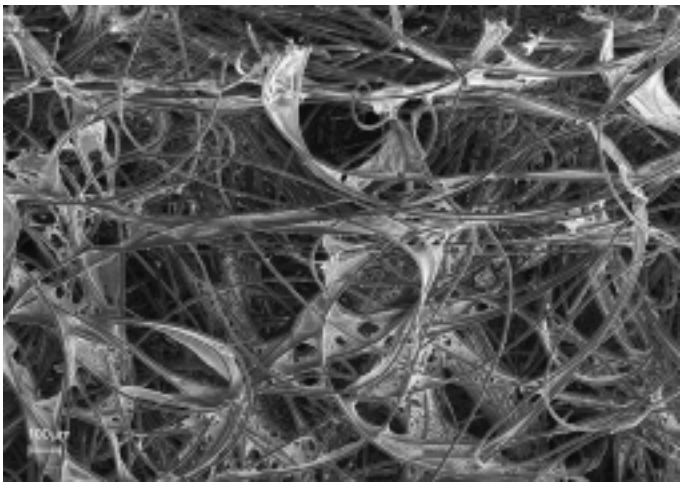

Figure 2

Example of a biodegradable scaffold (PGA/P4HB) before seeding.

Evaluation of human cell sources for tissue engineered heart valves

Following extensive studies using ovine vascular derived cells [9, 19, 20] and regarding future human application the suitability of human cells derived from various human cell sources have been investigated. Among the most promising are vascular-derived cells, bone marrow-derived cells, blood-derived cells, umbilical cord-derived cells and chorionic villi-derived cells, particularly for paediatric application (table 2).

Human vascular-derived cells

Experiments with human aortic myofibroblasts and endothelial cells demonstrated easy isolation and *in vitro* culture [9, 21]. Sequentially seeded on biodegradable scaffolds, the human aortic cells showed layered tissue formation [22]. Cells from saphenous veins showed comparable excellent growth properties and tissue formation after seeding on biodegradable scaffolds as aortic cells [23] and mammary artery cells (unpublished data). Saphenous vein cells can be obtained by a minor surgical intervention in local anaesthesia and therefore represent an attractive cell source for cardiovascular tissue engineering. In recent experiments we studied the influence of the cell donor age on the suitability of human myofibroblasts derived from saphenous vein for tissue engineering purposes. Neither the growth as monolayer cell culture nor the three-dimensional growth as tissue engineered constructs was influenced by the age of the cell donor (unpublished data). Extracellular matrix protein production and mechanical properties of the tissue engineered constructs were comparable among the different age groups (unpublished data). Based on these results we conclude that the cardiovascular tissue engineer-

Table 2

Examples of different cell sources for heart valve tissue engineering.

Human cell source	Cardiovascular construct	Reference
dermal fibroblast (covered with bovine endothelium)	valve leaflets	Sinoka T, et al. Ann Thorac Surg 1995; Circulation 1996
foreskin fibroblast (covered with human endothelial)	Patch	Zund G, et al. EJCTS 1998
marrow stromal cells	trileaflet heart valve	Hoerstrup SP, et al. Circulation 2002
aortic myofibroblasts	patch/trileaflet heart valve	Ye Q, et al, EJCTS 2000 Jockenhoevel S, et al. EJCTS 2001
aortic myofibroblasts and venous cells	Patch	Hoerstrup SP, et al. ASAIO 2002 Schnell A, et al. Thorac Cardiovasc Surg 2001
venous myofibroblast	Leaflets	Mol A, et al. Thorac Cardiovasc Surg 2003
umbilical cord myofibroblast from vein	pulmonary artery conduits	Hoerstrup SP, et al. Ann Thorac Surg 2002
umbilical cord myofibroblast (covered with human endothelial cells derived from umbilical cord blood endothelial progenitor cell)	Patch	Schmidt D, et al. EJCTS 2005
chorionic villi-derived cells (covered with human endothelial cells derived from umbilical cord blood endothelial progenitor cell)	Leaflets	Schmidt D, et al. Circulation 2006

ing concept may be independent of cell donor age and therefore be suitable also for elder patient populations.

Human marrow stromal cells

With regard to future routine clinical realisation of the tissue engineering concept, human marrow stromal cells are a promising cell source. In contrast to vascular cells, these cells can be obtained without surgical interventions representing an easy-to-access cell source in a possible routine clinical scenario. The usage of marrow stromal cells may offer several advantages in i) easy collection by a simple bone marrow puncture avoiding the sacrifice of intact vascular structures, ii) showing the potential to differentiate into multiple cell lineages, and iii) demonstrating unique immunological characteristics allowing persistence in allogenic settings. Recently, human marrow stromal cells have been used for the fabrication of trileaflet heart valves (figure 3) [24]. Histology of the tissue engineered valve leaflets revealed viable tissue organised in a layered fashion with extracellular matrix proteins characteristic of heart valve tissue such as collagen I and III, and glycosaminoglycans. However, the typical three-layered structural composition of native valve leaflets comprising a ventricularis, spongiosa and fibrosa layer was not achieved. The ultra-structural analysis of the tissue engineered heart valves supported this observation demonstrating cell elements typical of viable, secretionally active myofibroblasts such as actin/myosin filaments as well as collagen fibrils and elastin. The quantitative extracellular matrix protein analysis revealed values significantly lower compared to human native valve tissue.

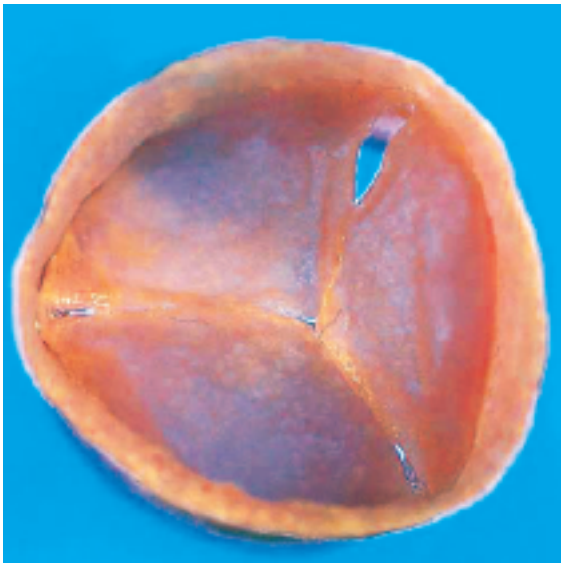

Figure 3

Tissue engineered trileaflet heart valve based on human stromal cells. Reprinted with permission from: Hoerstrup SP, et al. Circulation. 2002;106:I-143–50.

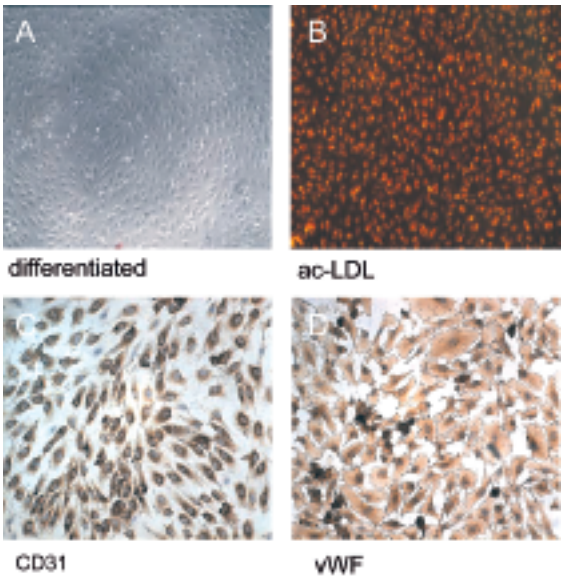

Figure 4

Umbilical cord blood-derived endothelial progenitor cells. 21 days after isolation, endothelial progenitor cells demonstrated cobble-stone morphology (A) and up-take for acetylated human Low Density Lipoprotein (ac-LDL; B). Furthermore, cells showed endothelial phenotype expressing cluster of differentiation 31 (CD31; C) and von Willebrand factor (vWF; D). Reprinted with permission from: Schmidt D, et al. Ann Thorac Surg. 2002;78:2094–8.

Human umbilical cord-derived cells

In order to provide tissue engineered constructs for congenital heart defects, alternative cell sources have been investigated, with particular attention to preserving the intact donor structure of the newborn patients. Human umbilical cords are readily available, easy to obtain and by means of modern cell and tissue banking technologies they might be used as an individual cell pool for the patient's lifetime. Furthermore, the presence of mesenchymal progenitor cells in the Wharton's jelly of human umbilical cords with multilineage potential [25, 26] and the possibility to obtain these cells prenatally using ultrasound guided sampling technology make this cell source even more attractive. In culture, human umbilical cord cells demonstrated excellent growth properties. Recently, umbilical cord-derived cells have been successfully utilised to generate paediatric tissues *in vitro* demonstrating excellent production of extracellular matrix [27, 28].

Blood derived endothelial progenitor cells

It has been shown that the presence of endothelium on heart valves reduces the risk for both coagulation and inflammatory complications. Therefore, to improve the functional capacities, the tissue engineered heart valve constructs are covered with a layer of autologous human endothelial cells. Endothelial cells from different vascular sources have been investigated demonstrating promising results [21, 22]. When typical endothelial antigen expression of endothelial cells from different sources including arteria radialis, arteria mammaria, vena saphena, umbilical cord was compared by flowcytometry, no significant cell-source dependent differences were detected (unpublished data). Also functional assays such as adhesion assays using peripheral blood-derived mononuclear cells have shown comparable functionality (unpublished data). However, the harvest of endothelial cells from vessels requires an invasive procedure. Furthermore, for paediatric application prenatal endothelial cell harvest from vessels would not be possible without substantial risks for the unborn child. Blood-derived endothelial progenitor cells are an attractive alternative cell source as they can be isolated from peripheral blood as well as from umbilical cord blood. The

latter one can already be obtained during pregnancy using the well-established method of ultrasound guided percutaneous blood sampling. Thus, harvesting endothelial progenitor cells prior birth is possible without substantial risks. The feasibility of using human umbilical cord blood-derived endothelial progenitor cells for tissue engineering of cardiovascular replacements for paediatric application has been demonstrated (figure 4) [27, 28]. When differentiated endothelial progenitor cells were co-cultured with non-endothelial cells as well as when exposed to mechanical stimuli they showed stabile phenotypes [29]. The extracellular matrix production of undifferentiated endothelial progenitor cells was demonstrated to be insufficient whereas the differentiation into endothelial cells on biodegradable scaffolds was observed [30]. In the overall tissue engineering concept, endothelial progenitor cells represent a promising cell source for the endothelialisation of heart valves. Since endothelial progenitor cells are easily accessible current research aims at their transdifferentation into myofibroblast cells in order to enable blood as a sole cell source for paediatric applications.

Chorionic villi-derived cells

Of particular importance is cell-harvesting at early perinatal stage that allows having the tissue-engineered replacement ready for implantation at the birth of the patient in order to prevent secondary damage to the immature heart. As prenatal tissue harvest from human placenta by chorionic villi sampling is already a routine procedure for prenatal genetic diagnostics the human placenta represents a promising prenatal cell source. Particularly, its chorionic villi provide extra-embryonically situated foetal mesenchymal cells including progenitor cells. The obtained tissue samples could also serve as a cell source for tissue engineering. Theoretically, one specimen could then be used for both diagnostics and the tissue engineering application. Recently, the successful use of chorionic villi-derived myofibroblast-fibroblast like cells obtained from routine prenatal tissue sampling combined with umbilical cord blood-derived endothelial progenitor cells for heart valve tissue engineering has been demonstrated [31]. The generated heart valve tissues showed cell phenotypes similar to their native counterparts and production of glycoaminoglycans and collagen as major extracellular matrix elements of native heart valves.

Limitations

Besides a few occasional pilot studies based on decellularised heart valves [14, 32] no systemic evidence that the heart valve tissue engineering concept can be applied in the clinical routine has been reported so far. However, as shown by Simon et al. [14] some of the decellularised scaffolds implanted in humans exhibited a strong inflammatory response resulting in dramatic failure.

Regarding the use of biodegradable scaffolds, local inflammation and systemic toxicity due to degradation products is a possible problem. Furthermore, there is a potential risk that ex vivo treatment of cells or the use of immature cells such as progenitors might lead to tumour development by uncontrolled cell growth or differentiation via genetic alterations. Future *in vivo* studies will focus on these important aspects.

Conclusion

In summary, various cells seem to be suitable for tissue engineering purposes. Among the most promising are progenitor cells either obtained from bone marrow, umbilical cord or chorionic villi, particularly for paediatric applications. This review was undertaken to provide more detailed understanding of cell phenotype and extracellular matrix development during the tissue engineering process, in order to define quality criteria for future clinical use. Although having first indications as to the influence of age, cell sources and *in vitro* conditions, knowledge on biochemical and immunological characteristics of the cells / tissues undergoing *in vitro* growth is still very limited. In addition, little is known about the influence of endothelial cells on extracellular matrix formation and on the quality of *in vitro* engineered tissue.

However, heart valve tissue engineering is a promising approach for living, functional autologous replacements. Particularly paediatric patients will benefit from growing replacement materials for the repair of congenital heart defects. Nevertheless, before clinical application of the tissue engineering heart valve concept will be routine several issues will have to be addressed.

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