Bioengineered living cardiac and venous valve replacements: current status and future prospects

Kehl D.¹, Weber B.¹, Hoerstrup S.P.¹

¹ Institute for Regenerative Medicine (IREM), University of Zurich, Center for Therapy Development/GMP, Moussonstrasse 13, CH-8044 Zurich, Switzerland

Correspondence:

Prof. Dr. Dr. Simon P. Hoerstrup

Institute for Regenerative Medicine (IREM)

University of Zurich

Center for Therapy Development/GMP

Moussonstrasse 13

CH- 8044 Zurich / Switzerland

e-mail: simon_philipp.hoerstrup@usz.ch

Abstract

Valvular heart disease remains to be a major cause of death worldwide with increasing prevalence, mortality and morbidity. Current heart valve replacements are associated with several limitations due to their nonviable nature. In this regard, heart valve tissue engineering has shown to represent a promising concept in order to overcome these limitations and replace diseased cardiac valves with living, autologous constructs. These bioengineered valves hold potential for *in situ* remodeling, growth and repair throughout the patient's lifetime without the risk of thromboembolic complications and adverse immune responses. For the fabrication of tissue engineered heart valves several concepts have been established, the "classical" *in vitro* tissue engineering approach, the *in situ* tissue engineering approach, and alternative approaches including 3D printing and electrospinning. Besides first attempts have been conducted in order to produce a tissue engineered venous valve for the treatment of deep venous valve insufficiency. Here we review basic principals and current scientific status of valvular tissue engineering, including a critical discussion and outlook for the future.

Keywords: *heart valve tissue engineering, venous valves, in vitro tissue engineering, in situ tissue engineering, electrospinning, 3D printing*

Abbreviations: BNMCs (bone marrow mononuclear cells), lower extremity chronic venous insufficiency (CVI), ECs (endothelial cells), ECM (extracellular matrix), IL-6 (interleukin-6), MCP-1 (monocyte chemoattractant factor-1), MSC (mesenchymal stem cell), SMCs (smooth muscle cells), dTEHV (decellularized tissue engineered heart valves, TEHV (tissue engineered heart valves), TEVV (tissue engineered venous valves), VEGF (vascular endothelial growth factor), VICs (valvular interstitial cells)

1. Introduction

Valvular heart disease is responsible for a high disease load worldwide, affecting people of all ages and origins. In industrialized countries increasing aging of the population is the major cause of degenerative valve disease, while in developing countries the problem of rheumatic cardiac diseases is still prevalent, resulting in increased mortality and morbidity each year [1, 2]. Although medical progress has been outstanding over the past few decades, the annual number of people requiring heart valve replacements is expected to triple globally from currently approximately 290,000 interventions per year to 850,000 in 2050 [2].

Surgical replacement of the insufficient valves is the most commonly used treatment for end stage valvular heart disease. Nowadays, either mechanical or biological prostheses are used for the replacement of dysfunctional valves. Although dramatically improving the life expectancy of the patients all currently available prostheses are associated with substantial limitations including the principal lack of growth, repair and remodeling capacities. The non-adaptive non-viable materials show substantial surface thrombogenicity (mechanical valves) and progressive calcific degeneration (bioprostheses). Lifelong anticoagulation therapies and repeated reoperations are therefore indispensable, especially for pediatric applications.

Besides also chronic venous insufficiency (CVI) represent a major global health problem with increasing prevalence, morbidity and socioeconomic impact [3]. Over long term, increased blood pressure within the lower extremities and venous return to the heart is impaired. This results from valvular incompetence, perforated veins, venous tributaries, venous obstruction or a combination of these mechanisms [3]. Insufficient valves are associated with venous blood reflux, obstruction, stasis and distal venous hypertension [3]. As in the cardiac field current therapeutic applications are suboptimal with short lasting results using palliative conventional techniques and highly invasive vein reconstructions. Recently bioprosthesis have been investigated, so far with no success in long-term evaluations [4].

Taken together, current prostheses for valvular disease are suboptimal and new approaches need to be considered as alternative therapies. In this regard, tissue engineering has shown

promising results by creating bioengineered, living autologous replacements with the ability for somatic growth, remodeling and repair.

2. Basic principles of heart valve tissue engineering

Tissue engineering is an emerging field of research with the goal to improve and/or restore tissue/organ functionality via the development of engineered, living substitutes. Several strategies of heart valve tissue engineering have been followed: the *in vitro* and the *in situ* approach as well as alternative approaches including three-dimensional (3D) printing and electrospinning. For the traditional *in vitro* approach, autologous cells are isolated from individual patients, seeded onto biodegradable scaffolds, pre-conditioned and matured in a bioreactor system to induce native-analogous neo-tissue formation *ex vivo*, followed by implantation of a living, autologous engineered valve into the diseased patients. This strategy is referred to as *in vitro* tissue engineering and has been the principal experimental approach for heart valve tissue engineering over the last years. The second approach, referred to as *in situ* tissue engineering, aims at stimulating the bodies own regenerative capacity in order to induce tissue formation *in vivo (in situ)*, instead of generating tissue *ex vivo*. Although the design of the scaffold and the potential seeding of isolated cells are still performed *ex vivo*, no extensive and regulatory complex *in vitro* cell expansion and conditioning in a bioreactor system are required prior to implantation. This approach is taking advantage of the intrinsic regenerative potential of the body itself inducing autologous host cell repopulation followed by tissue remodeling and potentially regeneration and growth. It represents a simplified strategy, without the need of extensive *in vitro* cell expansion techniques. Using this approach higher clinical feasibility of the TEHV technology seems possible. Cell-mediated leaflet thickening, retraction and fibrosis induced by the mechanical conditioning of the seeded cells in the bioreactor might be circumvented and *in vitro* cellular manipulation reduced [5]. However it is of note that this approach substantially depends on the regenerative capacity of the host, which might be a critical issue in patients with metabolic diseases or immunologic defects. Therefore over the last years great effort has been made in the inclusion of bioactive compounds into the scaffolds to guide *in situ* tissue remodeling. In this regard the development of novel simplified and fast(er) technologies have been intensively studied. 3D-printing and electrospinning with their different settings and modalities enable to approximate the constructs to the requirements of the ideal valvular substitute on the macro- to microscopic level – including an ideal heart valve anatomy and bioactivity. Ideally the release of bioactive molecules is coupled with the degradation of the polymer fibers, while new ECM

is built *in situ*. However, *in vivo* studies will further need to evaluate these technologies for future TEHV.

FIGURE 1

2.1 *In vitro* heart valve tissue engineering – current status

The requirements for an *in vitro* tissue engineered heart valve compromise I) an autologous, easily accessible cell source, II) a biodegradable scaffold material, designed in the shape of a heart valve, and III) an *in vitro* bioreactor system for the mechanical and biochemical conditioning of the construct. The quality of the tissue depends highly on the individual cell source chosen. In the attempt to define the best suitable cell source, characteristics like their expansion potential, their capability to produce extracellular matrix (ECM) and their regenerative potential are of critical importance. Therefore, several promising cell sources isolated from different donor tissues have already been used for the *in vitro* generation of TEHV such as adipose tissue-derived cells [6], amniotic fluid-derived cells [7, 8], bone marrow-derived cells [9-11], chorionic villi-derived cells [12], umbilical cord-derived cells [13, 14] and dermal fibroblasts [15]. In particular, vascular myofibroblasts and endothelial cells (ECs) have been used for the fabrication of TEHV. After *in vitro* expansion of these cells to high numbers, myofibroblasts are seeded on fully degradable scaffold materials – either synthetic or natural – in its particular design. Ideally, scaffold materials must be biocompatible, supporting cellular ingrowth and interaction for the formation of a continuous tissue layer, and biodegradable, allowing for cellular expansion and matrix formation while the extrinsic material is gradually degraded. Seeded scaffolds are firstly pre-incubated under static conditions before they are placed into a bioreactor system for mechanical conditioning through pulsatile flow and pressure gradients. These physiological conditions are responsible for the production of ECM from the seeded cells and therefore ultimately enhance neotissue formation *in vitro*. Finally the constructs are seeded with ECs to prevent thrombogenicity and resemble a native-like structure with a confluent luminal EC layer. At this point the constructs are ready for implantation. This technology has been investigated by several independent studies. Its principle feasibility has been demonstrated for the first time in 1995 with an *in vitro* engineered single pulmonary leaflet implanted into a lamb model [16]. Since then, the creation of a complete trileaflet TEHV based on synthetic scaffold material has shown to be feasible [17, 18], with *in vivo* functionality up to 120 days [19] and the ability of *in vivo* growth and remodeling [20]. Significant increase in diameter and length demonstrated the

ability of tissue-engineered constructs to grow [20]. Besides the use of vascular derived cells, several different stem cells have been investigated for their use in tissue engineering approaches. In particular, mesenchymal stem cells (MSCs) have shown to be an attractive alternative cell source due to their unique characteristics including I) multilineage differentiation potential, II) extensive *in vitro* proliferation, III) privileged immunological characteristics, and IV) paracrine mechanisms inducing host cell recruitment. In direct comparison to vascular derived cells there is no need to sacrifice intact vascular tissue. MSCs are easily accessible via low risk procedures in a wide variety of tissues, including tissues of fetal origin. This makes them suitable for pediatric *in vitro* heart valve tissue engineering that aims at the fabrication of an autologous, growing heart valve for the repair of congenital malformations prior to birth [21]. Besides the use of the most frequent synthetic biomaterials including poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(ϵ -caprolactone) (PCL) and poly(4-hydroxybutyrate) (P4HB), also natural biomaterials such as collagen and fibrin have been widely used in TE applications. Fibrin-based heart valves seeded with ovine carotid artery-derived cells in an ovine model showed remarkable tissue remodeling [22, 23]. Even augmented collagen production and enhanced retention of glycoaminoglycans within the developing ECM were detected by the use of fibrin in TEHV [24]. Recently, fibrin-based heart valve with a tubular leaflet design have been reported as an alternative to the conventional design of TEHV [25], including their principal feasibility for minimally invasive implantation techniques using self-expandable nitinol stents [26].

However as the process of *in vitro* tissue engineering is logistically complex and highly time consuming, the establishment of new strategies where heart valves could be provided with unlimited supply at every required time point have been initiated. In this regard, the removal of cellular components by combinations of detergents and biological agents results in cell-derived starter matrices composed of ECM and proteins. Minimal criteria for effective decellularization consist of I) <50 ng dsDNA/mg ECM (dry weight), II) <200 bp DNA fragment length, and III) the lack of nuclear material in histological sections (e.g H&E and DAPI) [27]. These homologous decellularized starter matrices are subsequently implanted for the *in situ* host cell repopulation. The decellularization process counteracts acute rejection of the implant by reducing the immune response. The use of allogeneic and xenogenic decellularized heart valves has been reported with long term animal models [28-30] and commercially available heart valve substitutes, including the allograft Cryolife's SynerGraft® or xenografts AutoTissue GmbH Matrix P plus NTM, with highly variable efficacy and outcomes. These initial investigations have led to the combination of *in vitro* tissue

engineering strategies with decellularization techniques. Such decellularized TEHV (dTEHV) have shown principal feasibility in different animal models including non-human primate [31] and ovine models [15, 32, 33], either using synthetic PGA/P4HB [31, 33] or natural fibrin gels in a tubular design [15, 32]. Interestingly dTEHV have shown to exhibit reduced risk of insufficiency through leaflet retraction, cellular repopulation and matrix remodeling. Nevertheless, further enhancement/guidance of the leaflet recellularization seems crucial to prevent adverse remodeling and provide adequate long-term functionality of the constructs (see paragraph 2.2).

FIGURE 2

2.2 *In situ* heart valve tissue engineering – current status

In situ tissue engineering holds great promise due to the simplified fabrication protocols and reduced immunogenicity. As scaffolds are directly implanted without any prior *in vitro* incubation, this involves benefits such as no extensive *in vitro* cell expansion and manipulation as well as no conditioning of the constructs in a bioreactor requiring complex infrastructure and logistics (also involving time consuming regulatory approvals). The principal strategy of *in situ* tissue engineering aims at implantation of a cell-seeded ‘starter matrix’ that triggers autologous host cell recruitment with subsequent vascular remodeling/tissue formation *in vivo*.

In 1999, the *in situ* tissue engineering approach has been used for a first in-man trial for tissue engineered grafts, where human cells harvested from the peripheral vein were directly seeded on synthetic PCL reinforced with woven PGA and implanted successfully [34]. In 2011, clinical trials for tissue engineered vascular grafts have been initiated in the U.S. [35] using the *in situ* tissue engineering approach. This milestone in the field of cardiovascular tissue engineering raised great hope for the extrapolation of this concept to the more complex field of heart valve tissue engineering.

At the same time, this procedure was introduced to the field of heart valve *in situ* tissue engineering. Its principal feasibility has been shown in a non-human primate model [34, 36] as well as in the adult [37, 38] and fetal [39] sheep model. In particular, scaffolds pre-seeded with autologous bone marrow mononuclear cells (BMNCs) have shown to enhance *in situ* remodeling. A higher cellularity indicative for an early cellular infiltration in the acute phase, followed by enhanced layered, endothelialized tissue formation on chronic survival have been reported using the bone marrow-derived mononuclear cell approach [36]. Similar results have

been obtained in first investigations of the high-pressure system (aortic valve position) with acute and chronic survival [37, 38].

As cell harvest and delivery can be performed within the same intervention using the *in situ* tissue engineering strategy, the combination with novel minimally invasive transcatheter-based implantation technologies could provide the opportunity of single-step minimally invasive procedures. In this regard, in a first large-scale ovine animal model, trileaflet heart valves have successfully been implanted using minimally invasive transapical procedures [40]. Furthermore, the prenatal minimally invasive implantation of autologous amniotic fluid stem cell-based TEHV in the ovine fetal model using an in-utero closed-heart hybrid single-step intervention has shown to be feasible [39]. This was performed in order to mimic the prenatal implantation of heart valves in fetuses with congenital heart valve malformations routinely detected by ultrasound examinations.

These results also uniquely exemplified that further characterization of the exact host remodeling cascade initiated by seeded BMNCs needs to be defined to guide *in situ* remodeling / tissue formation and to finally enable a clinical translation of the technology. Initially it was believed that the seeded cells directly contribute to the formation of tissue [41]. However, recent studies have measured a rapid loss of the cells after implantation as the scaffold became repopulated by host-derived cells [42, 43]. No integration of seeded cells into the scaffold could be detected, pointing towards the fact that the seeded cells do not directly contribute to the cellular infiltration and subsequent tissue formation. Nevertheless, seeded cells have shown to release paracrine factors initiating a cascade of tissue remodeling defined by an early monocyte/macrophage attraction (inflammation), followed by the recruitment of ECs and smooth muscle cells (SMCs) and finally leading to tissue formation *in vivo* [44, 45]. The seeded cells therefore seem to create a microenvironment of cytokines and growth factors enhancing the intrinsic remodeling capacity of the body, in particular a healing response with a rapid influx of inflammatory cells. Using a novel rat model, where cellular ingrowth from the adjacent tissue is inhibited, the *in situ* regenerative potential of circulatory cells could be attributed to peripheral immune cells [46]. Infiltrating monocytes support this remodeling cascade by the additional release of multiple pro-angiogenic and immunomodulatory factors such as monocyte chemoattractant protein-1 (MCP-1), interleukin 6 (IL-6) and vascular endothelial growth factor (VEGF). Interestingly, a similar host cell response has been described when comparing unseeded scaffolds releasing MCP-1 with those seeded with BMNCs [45]. MCP-1 is suggested to burst/guide circulating host cells from the bloodstream for tissue formation, regeneration and angiogenesis [45-48]. Besides BMNCs, other cell

sources have been investigated on their pro-angiogenic secretome for the use in TE applications, including MSCs [49], with first promising results. This implies that the cells could be replaced completely in the future. Fast degrading cell-free scaffolds by itself [50] or integrated with a drug delivery system releasing a specific composition and concentration of cytokines [51] might be a ‘ready to use’ solution for future tissue engineering approaches. Taken in mind that effective host remodeling is required for the success of any medical implant, further investigations will need to focus on the exact mechanical, structural and temporal window required.

FIGURE 3

2.3 Alternative and adjunctive approaches for heart valve tissue engineering

Three-dimensional (3D) printing represents a rapid manufacturing technique that allows the construction of a fully customized TEHV with the patient’s specific anatomy. Shortly, a 3D scan of the heart valve is acquired via X-ray or MRI, converted into a stereolithography file for the 3D printer and subsequently printed to generate either a cell-free or cell-encapsulated biological heart valve. In this regard, the group of Butcher has demonstrated that 3D hydrogel printing can fabricate anatomical heterogeneous valve conduits within 45min with accuracy up to 93% [52]. To better mimic the distinct mechanical properties between root and leaflets, different types of photocrosslinkable hydrogels were used accordingly [52]. The fabricated heart valve showed anatomically specific features, including the coronary ostium and sinuses [52]. By crosslinking different striped of PEG hydrogels, varying in stripe width and spacing, the anisotropic degree between the circumferential and radial orientation of a native valve leaflet could be mimic [53]. Furthermore, 3D printing allows to directly engineer heart valves with the desired cell types to promote *in situ* remodeling and deposition of ECM within the construct [54-56]. In particular, human aortic valvular interstitial cells (VICs) encapsulated within bioprint heart valves maintained cellular viability and promoted deposition of collagen and glycosaminoglycans [57]. Also the adhesion behavior, morphology, protein secretion and activation state of encapsulated VICs was modulated by specific bioactive peptides incorporated into the polymer networks [53].

Not only 3D printing but also electrospinning has raised great attention over the last years due to its simplified and fast manufacturing process. Electrospinning uses a high-voltage electrostatic field to generate fine fibers (micro to nano scale) through the ejection of the conductive polymer from a needle. Electrospun poly-(glycerol sebacate):poly(caprolactone)

(PGS: PCL) scaffolds have shown to resemble the ECM of native heart valve leaflets and their mechanical properties [58, 59]. While the PGS component of these hybrid polyester scaffolds accelerates the degradation rate of the scaffold, the seeded VICs remodel and deposit new ECM to consequently maintain the mechanical properties of the leaflet [60]. Native-like leaflet behaviour has also been described with electrospun PEGdma-PLA scaffolds seeded with VICs/ECs under physiological conditions in a bioreactor [61]. Besides electrospun scaffolds composed of poly(ester urethane)ureas (PEUU) have also the ability to mimic heart valve anisotropic mechanical properties [62, 63]. Besides, adjunctive approaches where multiple techniques are combined have been initiated for next generation TEHV. For instance, fibroblast cell sheets have been produced using the self-assembly method and subsequently assembled and molded to generate a stented TEHV [64, 65]. Furthermore the combination of hydrogels with electrospinning might integrate the advantages of both technologies and improve the mechanical properties, scaffold composition, material strength and cellular compatibility of future TEHV [66, 67].

3. Basic principles of venous valve tissue engineering

In comparison to the field of heart valve tissue engineering only few attempts have been conducted in order to produce a tissue engineered venous valve (TEVV) for the treatment of deep venous valve insufficiency. So far only few clinical applications have been initiated for venous valves with either cryopreserved allografts [68] or glutaraldehyde-preserved xenografts [4]. In spite of initial good valvular performance, cryo valve insertion has been associated with high morbidity and high occlusion rates [68]. In a long-term follow-up, also the xenogenic glutaraldehyde-fixed valves resulted in valve incompetence and/or valvular stenosis related with excessive neo-intimal hyperplasia and fibrotic transformation [4]. Besides also surface thrombogenicity mainly owing to the low pressure and flow environment involving static flow phases in the venous circulation branch has been a major issue in valve implants. In summary, no durable and thrombus-resistant venous valvular replacements have been introduced to routine clinical use so far. A tissue engineered venous valve solution could overcome these limitations and mainly allow for autologous thromboresistant (endothelialized) implants. In a first experimental *in vitro* trial, tri- and bicuspid TEVVs have been generated based on the *in vitro* tissue engineering approach [69]. TEVVs were engineered from autologous bone marrow-derived MSCs and subsequently endothelialized showing layered tissue formation analogous to native valvular tissues [69]. The feasibility of using the tissue engineering technique for the generation of future TEVVs for the treatment of

CVI has been demonstrated as part of this first study. However, future *in vivo* studies seem mandatory in order to evaluate their functionality in physiological conditions and their long-term durability.

4. Limitations and future perspectives

Even if still limited to the experimental phase, heart and venous valve tissue engineering has shown to be a potential future alternative therapeutic option for the replacement of diseased valves. Taking in consideration the demographic development, the prevalence of heart and venous valve disease will remain to be on the rise as the global population increases in age. Besides there is still no ideal therapy for congenital disease as the currently available prostheses lack the potential of growth. Since the introduction of vascular tissue engineering technologies, significant improvements have been made. First clinical trials on tissue-engineered vascular grafts have been initiated, the transition from animal models to the clinical reality on a broader scale seems within our grasp.

Nevertheless, several questions have to be addressed before a clinical application of tissue engineering will be feasible. For the *in vitro* tissue engineering approach the most favorable cell source needs to be defined. Therefore an exact characterization of the cells is indispensable, taking their proliferation capacity and cell survival in account. The impact of the ex vivo expansion on the cells has to be defined, given that their ex vivo manipulation might result in adverse biochemical, genomic and immunological characteristics. In addition, xenogenic factors, such as fetal calf serum within the proliferation medium, need to be replaced for a safe clinical application. For the design of the valves, in all tissue engineering approaches, further adaptations will have to be considered in order to minimize radial shortening of the leaflets and central valvular regurgitation. In this regard TEHV need to better resemble the complex geometry of native heart valves, which includes sinuses, ostia, individual layers of valve leaflets (ventricularis, fibrosa and spongiosa) and a heterogeneous cellular composition [70]. Another important aspect concerns the scaffold material. Exact definition of degradation rates, mechanical properties, thrombogenicity and surface interaction with cells (cellular adhesion and ingrowth) need to be specified. Ultimately, cellular ECM production needs to be exactly timed with degradation of the actual scaffold to maintain structural integrity and counteract chronic inflammation, which might result in fibrosis and inappropriate functional behavior of the valve. This chronic inflammation is characterized by an accumulation of macrophages in the attempt to phagocytize the foreign biomaterial. Interestingly, the balance between M1 and M2 macrophages has shown to

influence the remodeling capacity following implantation. In this regard several groups have established composite scaffolds for the controlled release of active biomolecules to influence macrophage phenotype. As to the *in situ* tissue engineering approach particular concerns have been raised given the highly heterogeneous *in vivo* remodeling responses among different individuals. This implies the definition of clinical guidelines for the use of tissue engineering technologies, where exact inclusion and exclusion criteria are of central importance. Moreover it requires an improved understanding of the cellular cascades evident throughout the *in vivo* remodeling phase. Summarized, improved outcomes for valve tissue engineering will require a systemic understanding of the multiple interacting factors to allow a safe clinical translation of such technologies in the future [70, 71].

Figure legend

Figure 1: Main strategies of heart valve tissue engineering. **(B)** The concept of *in vitro* tissue engineering comprises the harvest of autologous cells from the patient, *in vitro* expansion to high cell numbers (1), seeding onto a biodegradable scaffold (2) and conditioning in a bioreactor (3). When tissue formation is achieved, tissue engineered heart valves (4) are ready for implantation into the particular patient (5). **(A)** For the possibility of storage, the concept of decellularization removes cellular components by detergent solutions (6) to create decellularized starter matrices (7) with long-term availability (8). These off-the-shelf constructs are subsequently implanted (9) for the *in situ* host cell repopulation. **(C)** The concept of *in situ* tissue engineering takes advantage of the intrinsic remodeling capacity of the body. The implantation of starter matrices triggers autologous host cell recruitment with subsequent vascular remodeling and tissue formation *in vivo* (10). **(D)** This process can be enhanced by seeding bone marrow mononuclear cells (BMMCs) onto the starter matrices. Therefore bone marrow is obtained by sternal puncture (11), BMMCs are isolated by a density gradient centrifugation (12,13) and finally seeded and finally seeded onto the stented heart valve starter matrices (14).

Figure 2: *In vitro* tissue engineered heart valves. **(A)** Distal view of a TEHV based on vascular-derived cells. **(B)** The crimped TEHV, integrated into a nitinol stent (b), is loaded into the delivery system and inserted transapically into the right ventricle (a). Deployment into the pulmonary position is conducted by slowly advancing the inner pusher (c) into the sheath system (d). Adapted and reprinted from Schmidt et al., 2010.

Figure 3: *In situ* tissue engineered heart valves. **(A)** Bone marrow-derived TEHV. Isolated bone marrow mononuclear cells were seeded on stented polyglycolic acid scaffold matrices (a,b) using fibrin as a cell carrier (c,d). **(B)** Macroscopic view of a decellularized TEHV for implantation in a non-human primate model. Adapted and reprinted from Weber et al., 2011 and 2013.

References

1. Go, A.S., et al., *Executive summary: heart disease and stroke statistics--2014 update: a report from the American Heart Association*. Circulation, 2014. **129**(3): p. 399-410.
2. Yacoub, M.H. and J.J. Takkenberg, *Will heart valve tissue engineering change the world?* Nat Clin Pract Cardiovasc Med, 2005. **2**(2): p. 60-1.
3. Eberhardt, R.T. and J.D. Raffetto, *Chronic venous insufficiency*. Circulation, 2014. **130**(4): p. 333-46.
4. Zervides, C. and A.D. Giannoukas, *Historical overview of venous valve prostheses for the treatment of deep venous valve insufficiency*. J Endovasc Ther, 2012. **19**(2): p. 281-90.
5. Parvin Nejad, S., et al., *Biomechanical conditioning of tissue engineered heart valves: Too much of a good thing?* Adv Drug Deliv Rev, 2016. **96**: p. 161-75.
6. Colazzo, F., et al., *Extracellular matrix production by adipose-derived stem cells: implications for heart valve tissue engineering*. Biomaterials, 2011. **32**(1): p. 119-27.
7. Schmidt, D., et al., *Prenatally fabricated autologous human living heart valves based on amniotic fluid derived progenitor cells as single cell source*. Circulation, 2007. **116**(11 Suppl): p. I64-70.
8. Schmidt, D., et al., *Cryopreserved amniotic fluid-derived cells: a lifelong autologous fetal stem cell source for heart valve tissue engineering*. J Heart Valve Dis, 2008. **17**(4): p. 446-55; discussion 455.
9. Hoerstrup, S.P., et al., *Tissue engineering of functional trileaflet heart valves from human marrow stromal cells*. Circulation, 2002. **106**(12 Suppl 1): p. I143-50.
10. Sutherland, F.W., et al., *From stem cells to viable autologous semilunar heart valve*. Circulation, 2005. **111**(21): p. 2783-91.
11. Gottlieb, D., et al., *In vivo monitoring of function of autologous engineered pulmonary valve*. J Thorac Cardiovasc Surg, 2010. **139**(3): p. 723-31.
12. Schmidt, D., et al., *Living autologous heart valves engineered from human prenatally harvested progenitors*. Circulation, 2006. **114**(1 Suppl): p. I125-31.
13. Schmidt, D., et al., *Engineering of biologically active living heart valve leaflets using human umbilical cord-derived progenitor cells*. Tissue Eng, 2006. **12**(11): p. 3223-32.
14. Sodian, R., et al., *Tissue engineering of autologous human heart valves using cryopreserved vascular umbilical cord cells*. Ann Thorac Surg, 2006. **81**(6): p. 2207-16.
15. Syedain, Z., et al., *6-month aortic valve implantation of an off-the-shelf tissue-engineered valve in sheep*. Biomaterials, 2015. **73**: p. 175-84.
16. Shinoka, T., et al., *Tissue engineering heart valves: valve leaflet replacement study in a lamb model*. Ann Thorac Surg, 1995. **60**(6 Suppl): p. S513-6.

- 422 17. Hoerstrup, S.P., et al., *Functional living trileaflet heart valves grown in vitro.*
423 Circulation, 2000. **102**(19 Suppl 3): p. III44-9.
- 424 18. Sodian, R., et al., *Tissue engineering of heart valves: in vitro experiences.* Ann Thorac
425 Surg, 2000. **70**(1): p. 140-4.
- 426 19. Sodian, R., et al., *Early in vivo experience with tissue-engineered trileaflet heart*
427 *valves.* Circulation, 2000. **102**(19 Suppl 3): p. III22-9.
- 428 20. Hoerstrup, S.P., et al., *Functional growth in tissue-engineered living, vascular grafts:*
429 *follow-up at 100 weeks in a large animal model.* Circulation, 2006. **114**(1 Suppl): p.
430 I159-66.
- 431 21. Weber, B., S.M. Zeisberger, and S.P. Hoerstrup, *Prenatally harvested cells for*
432 *cardiovascular tissue engineering: fabrication of autologous implants prior to birth.*
433 Placenta, 2011. **32 Suppl 4**: p. S316-9.
- 434 22. Flanagan, T.C., et al., *The in vitro development of autologous fibrin-based tissue-*
435 *engineered heart valves through optimised dynamic conditioning.* Biomaterials, 2007.
436 **28**(23): p. 3388-97.
- 437 23. Flanagan, T.C., et al., *In vivo remodeling and structural characterization of fibrin-*
438 *based tissue-engineered heart valves in the adult sheep model.* Tissue Eng Part A,
439 2009. **15**(10): p. 2965-76.
- 440 24. Alfonso, A.R., et al., *Glycosaminoglycan entrapment by fibrin in engineered heart*
441 *valve tissues.* Acta Biomater, 2013. **9**(9): p. 8149-57.
- 442 25. Weber, M., et al., *Tissue-engineered fibrin-based heart valve with a tubular leaflet*
443 *design.* Tissue Eng Part C Methods, 2014. **20**(4): p. 265-75.
- 444 26. Moreira, R., et al., *Tissue-engineered heart valve with a tubular leaflet design for*
445 *minimally invasive transcatheter implantation.* Tissue Eng Part C Methods, 2015.
446 **21**(6): p. 530-40.
- 447 27. Crapo, P.M., T.W. Gilbert, and S.F. Badylak, *An overview of tissue and whole organ*
448 *decellularization processes.* Biomaterials, 2011. **32**(12): p. 3233-43.
- 449 28. Iop, L., et al., *Decellularized allogeneic heart valves demonstrate self-regeneration*
450 *potential after a long-term preclinical evaluation.* PLoS One, 2014. **9**(6): p. e99593.
- 451 29. Theodoridis, K., et al., *Successful matrix guided tissue regeneration of decellularized*
452 *pulmonary heart valve allografts in elderly sheep.* Biomaterials, 2015. **52**: p. 221-8.
- 453 30. Gallo, M., et al., *Physiological performance of a detergent decellularized heart valve*
454 *implanted for 15 months in Vietnamese pigs: surgical procedure, follow-up, and*
455 *explant inspection.* Artif Organs, 2012. **36**(6): p. E138-50.
- 456 31. Weber, B., et al., *Off-the-shelf human decellularized tissue-engineered heart valves in*
457 *a non-human primate model.* Biomaterials, 2013. **34**(30): p. 7269-80.

- 458 32. Syedain, Z.H., et al., *Implantation of completely biological engineered grafts*
459 *following decellularization into the sheep femoral artery*. Tissue Eng Part A, 2014.
460 **20**(11-12): p. 1726-34.
- 461 33. Driessen-Mol, A., et al., *Transcatheter implantation of homologous "off-the-shelf"*
462 *tissue-engineered heart valves with self-repair capacity: long-term functionality and*
463 *rapid in vivo remodeling in sheep*. J Am Coll Cardiol, 2014. **63**(13): p. 1320-9.
- 464 34. Shin'oka, T., Y. Imai, and Y. Ikada, *Transplantation of a tissue-engineered pulmonary*
465 *artery*. N Engl J Med, 2001. **344**(7): p. 532-3.
- 466 35. Vogel, G., *Tissue engineering. Mending the youngest hearts*. Science, 2011.
467 **333**(6046): p. 1088-9.
- 468 36. Weber, B., et al., *Injectable living marrow stromal cell-based autologous tissue*
469 *engineered heart valves: first experiences with a one-step intervention in primates*.
470 Eur Heart J, 2011. **32**(22): p. 2830-40.
- 471 37. Emmert, M.Y., et al., *Transapical aortic implantation of autologous marrow stromal*
472 *cell-based tissue-engineered heart valves: first experiences in the systemic circulation*.
473 JACC Cardiovasc Interv, 2011. **4**(7): p. 822-3.
- 474 38. Emmert, M.Y., et al., *Stem cell-based transcatheter aortic valve implantation: first*
475 *experiences in a pre-clinical model*. JACC Cardiovasc Interv, 2012. **5**(8): p. 874-83.
- 476 39. Weber, B., et al., *Prenatally engineered autologous amniotic fluid stem cell-based*
477 *heart valves in the fetal circulation*. Biomaterials, 2012. **33**(16): p. 4031-43.
- 478 40. Schmidt, D., et al., *Minimally-invasive implantation of living tissue engineered heart*
479 *valves: a comprehensive approach from autologous vascular cells to stem cells*. J Am
480 Coll Cardiol, 2010. **56**(6): p. 510-20.
- 481 41. Matsumura, G., et al., *First evidence that bone marrow cells contribute to the*
482 *construction of tissue-engineered vascular autografts in vivo*. Circulation, 2003.
483 **108**(14): p. 1729-34.
- 484 42. Harrington, J.K., et al., *Determining the fate of seeded cells in venous tissue-*
485 *engineered vascular grafts using serial MRI*. FASEB J, 2011. **25**(12): p. 4150-61.
- 486 43. Hibino, N., et al., *Tissue-engineered vascular grafts form neovessels that arise from*
487 *regeneration of the adjacent blood vessel*. FASEB J, 2011. **25**(8): p. 2731-9.
- 488 44. Hibino, N., et al., *A critical role for macrophages in neovessel formation and the*
489 *development of stenosis in tissue-engineered vascular grafts*. FASEB J, 2011. **25**(12):
490 p. 4253-63.
- 491 45. Roh, J.D., et al., *Tissue-engineered vascular grafts transform into mature blood*
492 *vessels via an inflammation-mediated process of vascular remodeling*. Proc Natl Acad
493 Sci U S A, 2010. **107**(10): p. 4669-74.
- 494 46. Talacua, H., et al., *In Situ Tissue Engineering of Functional Small-Diameter Blood*
495 *Vessels by Host Circulating Cells Only*. Tissue Eng Part A, 2015. **21**(19-20): p. 2583-
496 94.

- 497 47. Ballotta, V., et al., *Synergistic protein secretion by mesenchymal stromal cells seeded*
498 *in 3D scaffolds and circulating leukocytes in physiological flow*. Biomaterials, 2014.
499 **35**(33): p. 9100-13.
- 500 48. Capoccia, B.J., A.D. Gregory, and D.C. Link, *Recruitment of the inflammatory subset*
501 *of monocytes to sites of ischemia induces angiogenesis in a monocyte chemoattractant*
502 *protein-1-dependent fashion*. J Leukoc Biol, 2008. **84**(3): p. 760-8.
- 503 49. Smits, A.I., et al., *Shear flow affects selective monocyte recruitment into MCP-1-*
504 *loaded scaffolds*. J Cell Mol Med, 2014. **18**(11): p. 2176-88.
- 505 50. Wu, W., R.A. Allen, and Y. Wang, *Fast-degrading elastomer enables rapid*
506 *remodeling of a cell-free synthetic graft into a neoartery*. Nat Med, 2012. **18**(7): p.
507 1148-53.
- 508 51. Jana, S., et al., *Drug delivery in aortic valve tissue engineering*. J Control Release,
509 2014. **196**: p. 307-23.
- 510 52. Hockaday, L.A., et al., *Rapid 3D printing of anatomically accurate and mechanically*
511 *heterogeneous aortic valve hydrogel scaffolds*. Biofabrication, 2012. **4**(3): p. 035005.
- 512 53. Zhang, X., et al., *Integrating valve-inspired design features into poly(ethylene glycol)*
513 *hydrogel scaffolds for heart valve tissue engineering*. Acta Biomater, 2015. **14**: p. 11-
514 21.
- 515 54. Duan, B., et al., *Stiffness and adhesivity control aortic valve interstitial cell behavior*
516 *within hyaluronic acid based hydrogels*. Acta Biomater, 2013. **9**(8): p. 7640-50.
- 517 55. Duan, B., et al., *3D bioprinting of heterogeneous aortic valve conduits with*
518 *alginate/gelatin hydrogels*. J Biomed Mater Res A, 2013. **101**(5): p. 1255-64.
- 519 56. Xu, T., et al., *Complex heterogeneous tissue constructs containing multiple cell types*
520 *prepared by inkjet printing technology*. Biomaterials, 2013. **34**(1): p. 130-9.
- 521 57. Duan, B., et al., *Three-dimensional printed trileaflet valve conduits using biological*
522 *hydrogels and human valve interstitial cells*. Acta Biomater, 2014. **10**(5): p. 1836-46.
- 523 58. Sant, S., et al., *Hybrid PGS-PCL microfibrinous scaffolds with improved mechanical*
524 *and biological properties*. J Tissue Eng Regen Med, 2011. **5**(4): p. 283-91.
- 525 59. Masoumi, N., et al., *Electrospun PGS:PCL microfibers align human valvular*
526 *interstitial cells and provide tunable scaffold anisotropy*. Adv Healthc Mater, 2014.
527 **3**(6): p. 929-39.
- 528 60. Sant, S., et al., *Effect of biodegradation and de novo matrix synthesis on the*
529 *mechanical properties of valvular interstitial cell-seeded polyglycerol sebacate-*
530 *polycaprolactone scaffolds*. Acta Biomater, 2013. **9**(4): p. 5963-73.
- 531 61. Hinderer, S., et al., *Engineering of a bio-functionalized hybrid off-the-shelf heart*
532 *valve*. Biomaterials, 2014. **35**(7): p. 2130-9.
- 533 62. Fan, R., et al., *Optimal elastomeric scaffold leaflet shape for pulmonary heart valve*
534 *leaflet replacement*. J Biomech, 2013. **46**(4): p. 662-9.

- 535 63. Amoroso, N.J., et al., *Microstructural manipulation of electrospun scaffolds for*
536 *specific bending stiffness for heart valve tissue engineering*. Acta Biomater, 2012.
537 **8**(12): p. 4268-77.
- 538 64. Dube, J., et al., *Progress in developing a living human tissue-engineered tri-leaflet*
539 *heart valve assembled from tissue produced by the self-assembly approach*. Acta
540 Biomater, 2014. **10**(8): p. 3563-70.
- 541 65. Tremblay, C., et al., *A new construction technique for tissue-engineered heart valves*
542 *using the self-assembly method*. Tissue Eng Part C Methods, 2014. **20**(11): p. 905-15.
- 543 66. Eslami, M., et al., *Fiber-reinforced hydrogel scaffolds for heart valve tissue*
544 *engineering*. J Biomater Appl, 2014. **29**(3): p. 399-410.
- 545 67. Tseng, H., et al., *Anisotropic poly(ethylene glycol)/polycaprolactone hydrogel-fiber*
546 *composites for heart valve tissue engineering*. Tissue Eng Part A, 2014. **20**(19-20): p.
547 2634-45.
- 548 68. Neglen, P. and S. Raju, *Venous reflux repair with cryopreserved vein valves*. J Vasc
549 Surg, 2003. **37**(3): p. 552-7.
- 550 69. Weber, B., et al., *Living-engineered valves for transcatheter venous valve repair*.
551 Tissue Eng Part C Methods, 2014. **20**(6): p. 451-63.
- 552 70. Cheung, D.Y., B. Duan, and J.T. Butcher, *Current progress in tissue engineering of*
553 *heart valves: multiscale problems, multiscale solutions*. Expert Opin Biol Ther, 2015.
554 **15**(8): p. 1155-72.
- 555 71. Usprech, J., W.L. Chen, and C.A. Simmons, *Heart valve regeneration: the need for*
556 *systems approaches*. Wiley Interdiscip Rev Syst Biol Med, 2016. **8**(2): p. 169-182.
557

Figure 1: Main strategies of heart valve tissue engineering. **(B)** The concept of *in vitro* tissue engineering comprises the harvest of autologous cells from the patient, *in vitro* expansion to high cell numbers (1), seeding onto a biodegradable scaffold (2) and conditioning in a bioreactor (3). When tissue formation is achieved, tissue engineered heart valves (4) are ready for implantation into the particular patient (5). **(A)** For the possibility of storage, the concept of decellularization removes cellular components by detergent solutions (6) to create decellularized starter matrices (7) with long term availability (8). These off-the-shelf constructs are subsequently implanted (9) for the *in situ* host cell repopulation. **(C)** The concept of *in situ* tissue engineering takes advantage of the intrinsic remodeling capacity of the body. The implantation of starter matrices triggers autologous host cell recruitment with subsequent vascular remodeling and tissue formation *in vivo* (10). **(D)** This process can be enhanced by seeding bone marrow mononuclear cells (BMMCs) onto the starter matrices. Therefore bone marrow is obtained by sternal puncture (11), BMMCs are isolated by a density gradient centrifugation (12,13) and finally seeded onto the stented heart valve starter matrices (14).

Figure 2: In vitro tissue engineered heart valves. **(A)** Distal view of a TEHV based on vascular-derived cells. **(B)** The crimped TEHV, integrated into a nitinol stent (b), is loaded into the delivery system and inserted transapically into the right ventricle (a). Deployment into the pulmonary position is conducted by slowly advancing the inner pusher (c) into the sheath system (d). *Adapted and reprinted from Schmidt et al., 2010.*

A Bone marrow mononuclear cell approach

B Decellularization approach

Figure 3: In situ tissue engineered heart valves. (A) Bone marrow-derived TEHV. Isolated bone marrow mononuclear cells were seeded on stented polyglycolic acid scaffold matrices (a,b) using fibrin as a cell carrier (c,d). (B) Macroscopic view of a decellularized TEHV for im-plantation in a non-human primate model. *Adapted and reprinted from Weber et al., 2011 and 2013.*