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Review Article

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

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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 three-dimensional 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.

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 the problem of rheumatic cardiac diseases is still prevalent in developing countries, 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 nonadaptive nonviable 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) represents 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 theses 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 bioprostheses 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

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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, preconditioned, and matured in a bioreactor system to induce native-analogous neotissue 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 tissue-engineered heart valve (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 has 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 macroscopic level to the 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 extracellular matrix (ECM) is built *in situ*. However, *in vivo* studies will further need to evaluate these technologies for future TEHV (Fig. 1).

2.1. In vitro heart valve tissue engineering — Current status

The requirements for an *in vitro* TEHV 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 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 preincubated 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

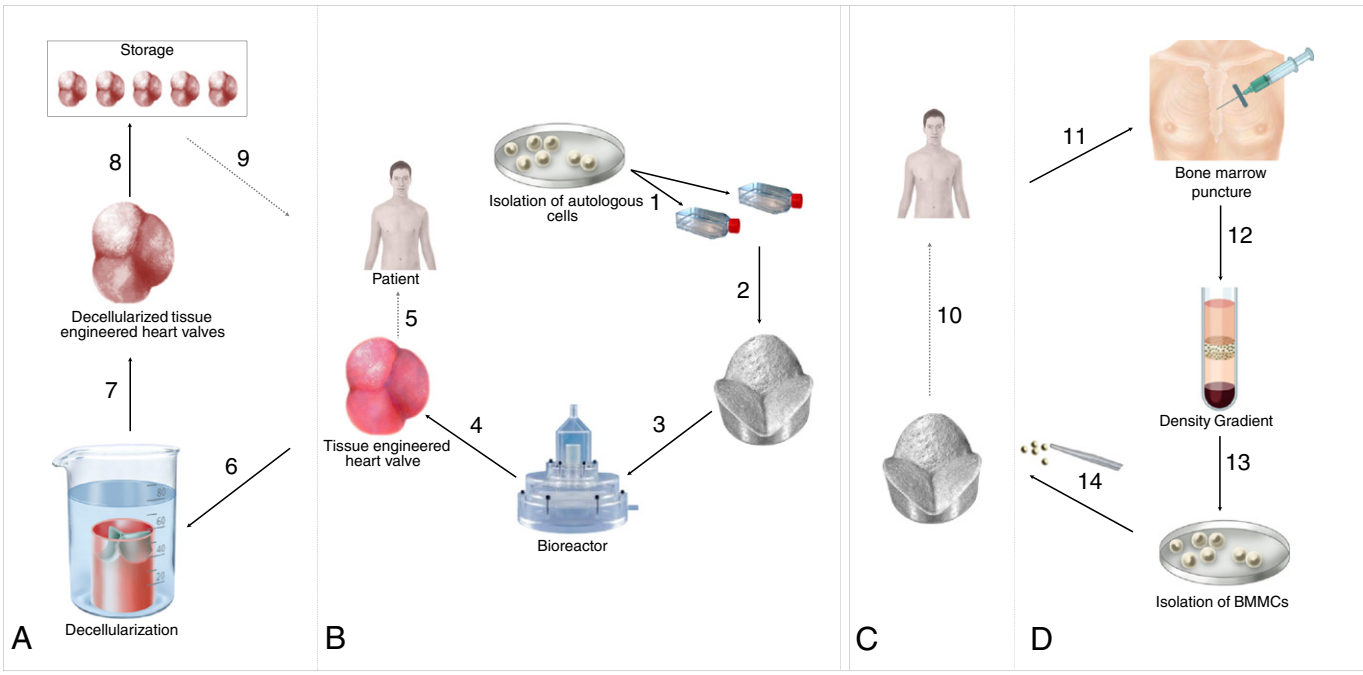

Fig. 1. Main strategies of heart valve tissue engineering. **(B)** The concept of *in vitro* tissue engineering compromises the harvest of autologous cells from the patient, *in vitro* expansion to high cell numbers (*1*), seeding onto a biodegradable scaffold (*2*), and conditioning in a bioreactor (*3*). When tissue formation is achieved, TEHV (*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 BMNCs onto the starter matrices. Therefore, bone marrow is obtained by sternal puncture (*11*), and BMNCs are isolated by a density gradient centrifugation (*12* and *13*) and finally seeded onto the stented heart valve starter matrices (*14*).