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Tissue engineering of heart valves – challenges and opportunities

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Abstract

Background: Heart valve disease is a clinically serious condition. The replacement of damaged valves practiced since the 1950's is the ultimate treatment for end-stage heart failure caused by severe valve dysfunction. The choice of adequate prosthesis is challenging. Unfortunately, the treatment options available today do not satisfy completely physicians and scientists' needs. Mechanical valves require long-term anticoagulation therapy because of poor hemocompatibility. Biological substitutes have better hemodynamics, but need replacement in ~ 10 years due to calcification and degeneration. In order to overcome the shortcomings of current treatment options many researches are motivated to fabricate a functional, living heart valve replacement by tissue engineering.

Conclusions: Tissue engineering is a promising approach that may lead novel constructs that will satisfy the need and overcome the limitations of current valve prosthetics. Scaffolds, fabricated from synthetic or biological materials, do not require donor tissue, but have struggled to recreate the macro- and micro valve anatomy and mechanical properties of native valve. Decellularized cardiovascular grafts have the opportunity to improve patients care by reducing the risk of sensitization to donor antigens, calcify and stenosis and providing with a good graft that will grow (especially important in children). In this way the emotional and financial drain on the patient and family of enduring multiple surgeries may be significantly minimized. The choice of decellularization method can be rational if mechanism of action is contemplated and clearly understood.

Key words: tissue engineering of heart valve, decellularized scaffolds, hybrid starter matrices.

Introduction

Valvular heart diseases remain a serious clinical condition, a major health problem and one of the main causes of morbidity and mortality worldwide. Even if the prevalence and incidence of valvulopathies increase with age, it represents an important problem for pediatric patients too (1% -2% of all live birth are affected by congenital heart diseases, the most common of which affects the heart valves) [1].

Etiology of heart valve diseases is various [2]**, including:**

a. Congenital defects (bicuspid aortic valve, tetralogy of Fallot, congenital pulmonary valve insufficiency, pulmonary artery hypoplasia).

b. Inflammatory/immunological disorders (rheumatic fever, syphilis, antiphospholipid syndrome, angiosarcoma of the aorta or pulmonary artery angiosarcoma).

c. Heritable disorders of connective tissue (Marfan syndrome).

d. Endocardial disorders with valvular involvement.

e. Diseases and disorders of other organs (such as, chronic renal failure).

f. Aging (valve calcification).

g. Post interventional valvular diseases.

For better understanding of valve condition and produced pathophysiological disturbances it is necessary to know the role of each heart valve.

The heart consists of four chambers: two atria and two ventricles, and four flap-like membranous structures, namely valves. Valves determine the direction of blood flow from the atria to the ventricles and from the ventricles to the great vessels.

The valves located between the atria and ventricles, or atrioventricular valves, are:

- Tricuspid valve, between the right atrium and right ventricle.
- Mitral valve, between the left atrium and the left ventricle.

The valves located between the ventricles and great arteries, or the semilunar valves, are:

- Pulmonary valve, between the right ventricle and pulmonary artery.
- Aortic valve, between the left ventricle and aorta.

As it is known, the cardiac cycle consists of two phases: diastole phase and systole phase. During the diastole phase, the atrioventricular valves are opened and semilunar valves are closed, and during the systole phase, the atrioventricular

valves are closed and semilunar valves are opened, passive openings and closings being determined by a transvalvular pressure gradient. When the valve is damaged or diseased, it does not open or close properly and the blood flow becomes disrupted [3].

Valvular heart diseases can be broadly characterized by the following pathological disorders:

- Stenosis: the valve opening becomes restricted and the blood flow out is prevented. In order to move blood, the heart needs to contract with increased force;
- Regurgitation: the valve does not fully close, causing the blood flowing back instead of forward flow through the valve;
- And heart valves can have both malfunctions at the same time [4, 5].

The contemporary medicine offers a few strategies for the treatment of heart valve diseases: special medications that help to control the symptoms and to avoid further valve damage (diuretics, anti-arrythmic medications, vasodilators, etc.), valve repair, and valve replacement.

Since the first mitral valve repair in 1923 and the first successful prosthetic valve replacement in 1960 described by Starr and Edwards, surgery for valvular diseases has advanced significantly [6].

Due to better long-term results of valve repair and lower morbidity and mortality this procedure is used in preference when possible [7]. However, when heart valve repair is not possible, open-heart surgery with removing of damaged valve and implantation of an artificial one in its place is recommended. During the last decades more than 80 models of prostheses have been developed, however, none of them corresponds completely to the criteria of an "ideal" product, described in cardiovascular surgical literature, such as [8-10]:

- Non-thrombogenicity,
- Excellent hemodynamics,
- Availability in a range of sizes,
- Excellent handling characteristics,
- Long-term valve function,
- Low-to-moderate price,
- Low infections potential,
- Potential for growth (in particular in pediatric patients).

Mechanical and biological valve substitutes used currently have struggled to recreate the macro- and microvalve anatomy and mechanical properties of native valve [11]. As a result, their long-term performance is associated with major limitations. Thus, none of them may be considered "ideal" solution.

Mechanical valve substitutes: general characteristic

Three types of mechanical valvular prosthesis are available now: ball valves, disc valves, or monoleaflet valves, and bileaflet valves.

Even if mechanical valves remain the most structurally durable replacements, they have poor hemocompatibility because of their non-physiological surfaces and flow abnormalities. As a result, life-long anticoagulation therapy is necessary for prevention of thromboembolic complications. At the same time, anticoagulation therapy can cause serious spontaneous bleeding and embolism [12]. In addition, mechanical valve substitutes are noisy and susceptible to infection [13, 14].

Biological valve substitutes: general characteristic

By application in practice of biological heart valve replacements the hazards of anticoagulation treatment were avoided.

Different types of bioprosthetic valves are described, such as autografts, xenografts (for example, porcine aortic valves or bovine pericardial valves) and homografts, or allografts (valves taken from human donors) [8, 15].

In 1967 Donald Ross [16] described a new procedure for the treatment of aortic valvular disorders. It involves replacement of patient diseased aortic valve with his own pulmonary valve and then installation of a mechanical or bioprosthetic valve in the hemodynamically weaker pulmonary position. The procedure is associated with a significant surgical risk and risk of postoperative complications, transforming the patient with one pathological valve into a patient with two diseased valves.

Even if cryopreserved, donor valves are closest to the natural valve, have low thrombogenicity, superior hemodynamic performance and resistance to infection. Their main disadvantages are limited availability and failure to regenerate and grow *in vivo*. Moreover, the recipient can become sensibilized to the donor Major Histocompatibility Complex (MHC) antigens, which are present in endothelial cells linking the luminal surface (MHC I) and smooth muscle cells in the media of the arterial wall (MHC II) [17]. Also, when compared to mechanical valves, the structural degeneration of bioprosthetics due to inflammatory/immune response and calcification occurs earlier (in about 10-20 years).

None of currently available biological substitutes shows any potential to grow, regenerate and develop *in vivo*. All these characteristics are important especially in the treatment of pediatric patients [18].

Even the progress in the field of development of new types of valve replacements is undoubted, tissue engineering is the unique approach that may propose a promising strategy to overcome the limitations mentioned above and to provide the surgeons with alternative suitable substitutes, which are able to grow and remodel as the age of the patients advances [19, 20].

Material and methods

Articles containing the keywords "Valvular diseases", "Heart valve replacement", "Tissue Engineering of Heart Valves", "Polymeric starter matrices", "Decellularization", "Decellularized scaffolds", "Biological/Polymeric starter matricies" were selected from PubMed and SpringerLink databases.

The following filters were used: articles published since January 2008 in English. After a preliminary analysis the bibliography of the identified articles has been studied also

in order to find other relevant articles on this topic. Subsequently, information was systematized highlighting the main aspects of contemporary vision on advantages and disadvantages of existing heart valve replacements, scaffolds used in fabrication of a tissue engineered heart valve, improving the procedures of scaffolds development, main characteristics of new valvular prostheses.

Discussion

Being motivated by the lack of adequate replacements pediatric surgeons were the first who introduced the concept of tissue engineering of heart valve [12] and, perhaps, Grim *et al* were the first who presented an example of a tissue-engineered heart valve at the University of Vienna in 1990's. They demonstrated the possibility of including and growing of endothelium on glutaraldehyde-fixed bovine pericardium [21]. Between February 1986 and February 1992, 144 patients received 149 bovine pericardial valve bioprostheses. Even short-term results were satisfactory, long-term results were as follows – 10 patients required reoperation because of valvular dysfunction (valvular stenosis – 7, valvular regurgitation – 2, paravalvular leakage - 1), defect bioprosthesis being removed 34 to 81 months after implantation [22].

The advancement in the field of heart valve tissue engineering since the first published study till today is undoubted. Future development of TEHV needs elaboration of appropriate starter matrices that are able to support cell growth and cell-to-cell interaction with tissue formation. Apart from standard requirements for general tissue-engineered scaffold, like biodegradability, biocompatibility and non-immunogenicity, scaffolds used for tissue engineered heart valve (TEHV) should correspond to several other important criteria [4, 23-26]:

- Non-thrombogenicity.
- Mechanically resistance.
- Growth with patient.
- Anatomically-shaped.
- Non-obstruction.
- Ability to close promptly and completely.

According to these criteria, three main types of starter vehicles are applied in TEHV:

- Polymeric (synthetic) bioresorbable starter matrices (such as polyglatin, polyglicolic acid, polylactic acid etc.),
- Decellularized allogeneic starter matrices,
- Biological / Polymeric hybrid starter matrices [4].

A. Characteristics of Polymeric Scaffold

The concept of use of polymeric starter matrices in tissue engineering is simple – the cells of a particular phenotype seeded on a porous material are expected to generate the tissue growth and organ formation as the scaffold degenerates (important, the degeneration rate of the scaffold should be controllable and proportional to the rate of tissue formation). Except being biocompatible and biodegradable, the vehicles used should match the mechanical properties of the native tissue, exhibit a cell-favourable surface chemistry and to be at least 90% porous (interconnected pore network is essential for cell growth, nutrient supply and removal of metabolic waste products) [27].

The first models of synthetic biodegradable scaffolds were constructed from aliphatic polyester like polyglatic (in 1995), polyglicolic acid (PGA, in 1996), polylactic acid (PLA, in 1998) and copolymer of PGA and PLA (PGLA, in 1997) [25, 26, 28, 29]. Because these materials demonstrated to be too stiff, new more compliant scaffolds, like polyhydroxyalkanoate (PHAs, in 2000) and poly-4-hydroxybutyrate (P4HB, in 2000) have been investigated [30] to create trileaflet heart-valve conduits. Combination of aliphatic polyesters and PHAs, as alternative composite polymers, has demonstrated promising results in TEHV [31].

As conclusion, the use of polymeric starter matricies has been already broadly demonstrated for cardiovascular tissue-engineering [12] with good results at short-term follow-up. Unfortunately, the mid- to long-term results are not clear yet.

B. Characteristics of decellularized starter matrices

It has been supposed that by decellularization of cryopreserved cardiovascular grafts and removal of donor cells and cell membrane associated MHC I/MHC II proteins the immunogenic potential may be reduced. The main challenge remains elaboration of an appropriate processing method.

According to the definition, decellularization is the process of removing cellular (including nuclear) material from the extracellular matrix (ECM) with its' preservation. Unaltered extracellular matrix and proteins play an important role in promoting tissue regeneration and repair and serve as a native scaffold for cell migration growth and differentiation [32, 33].

The first clinical implantation in pediatric patients of decellularized homografts engineered with autologous endothelial progenitor cells for pulmonary valve replacement was performed in 2002 (since 2005 only non-seeded decellularized allografts have been implanted). The first clinical application in humans of decellularized aortic homografts for aortic valve replacement was performed in February 2008 in Chisinau, the Republic of Moldova [15].

There are different methods used for tissue decellularization, such as [34]:

a. Chemical agents:

- Acids and bases.
- Hypotonic and hypertonic solutions.
- Detergents: ionic – sodium dodecyl sulphate (SDS), sodium deoxycholate (SDC), N-Lauroylsarconsinate (NLS); non-ionic – Triton X-100, Tween-20; and – zwitterionic detergents.
- Other solvents – alcohols, acetone, tributyl phosphate (TBP).

Complete removal of residual chemicals from ECM after decellularization is obligatory, because even low residual concentration may influence negatively on ECM-scaffold properties [35].

b. Biological agents [34]:
- ♦ Enzymes: nucleases – DNase and RNase; trypsin; collagenase; lipase; dispase, etc.
- ♦ Non-enzymatic agents: chelating agents – ethylenediaminetetraacetic acid (EDTA), ethyleneglycoltetraacetic acid (EGTA).

c. Physical and miscellaneous agents [36-38]:
- ♦ Temperature (freeze-thaw processing).
- ♦ Force and pressure: mechanical abrasion.
- ♦ Non-thermal irreversible electroporation.

Because of a variety of techniques, in the context of heart valve decellularization the following criteria were elaborated [17, 31, 32]:
- ➢ It should be stringent enough to ensure completely cellular material removal (DNA, mitochondria, membrane lipids, cytosolic proteins) in order to avoid any adverse cellular immune response post-implantation.
- ➢ It should be gentle enough to preserve the biomechanical strength and structural properties of the remaining ECM, because the conduits and leaflets are under extreme environmental demands.
- ➢ It should preserve potential for recellularization.
- ➢ It should reduce of immunogenicity and thrombogenicity.

Broadly speaking, the choice of the method of processing is of key importance in decellularization strategy.

The most often employed decellularization combinations for cardiovascular tissue

It's very important to understand the effects of the decellularization technology on the properties of donor heart valve.

a. Biological agents [32, 39, 40]

♦ ***Nucleases (DNase/RNase)*** cleave nucleic and sequences into shorter segments, expediting their removal from the ECM.

♦ **Trypsin** (a serine protease) cleaves proteins hydrolytically and is used to digest cellular proteins in the decellularization process. Because the structural proteins of ECM have limited resistance to trypsin cleavage, visible histological damage to the ECM is often determined. As conclusion, even it is known that tyrosine cleaves proteins at the arginine or the lysine amino acid residue on the carboxyl side, except when followed by proline; it is capable of degrading the extracellular matrix and cannot be considered a "perfect" strategy for decellularization of cardiac tissue.

♦ **Trypsin+ EDTA**, most often employed enzyme-based combination. Intracellular proteases released as the cells are being trypsinized are inactivated by EDTA. In this way degradation of extracellular matrix by proteases can be avoided, but, unfortunately, all the proteolytic activity of the intracellular proteases cannot be inhibited by it.

Thus, biomechanical integrity of ECM could be adversely affected due to aggressive effect of biological agents.

b. Chemical agents

Detergents have a hydrophilic head and hydrophobic tail, and by reducing the surface tension of the local environment they can penetrate the extracellular matrix and cell membranes [41]. They are classified into three main categories based on the property of the hydrophilic head group: non-ionic, ionic, and zwitterionic.

Detergents are very effective agents because they are able to solubilize cell membrane, lyse cells, and dissociate DNA.

Characteristics of ionic detergents

Ionic detergents contain a head group with a net charge that can be either negative (anionic) or positive (cationic). Ionic detergents can disrupt protein-protein interactions along with lipid-lipid and lipid-protein interactions, and they may denaturate proteins [42].

Anionic detergents (SDS, SDC) are stronger solubilizing agents than non-ionic detergents and are often used in valve decellularization for cells and DNA removing from ECM [32].

♦ **SDS** (Sodium-dodecyl-sulphate) is a good candidate detergent due to its known ability to denaturate proteins [42], but also SDS has the potential to reduce the biomechanical strength of obtained cell-free scaffold, predisposing the allograft to anevrysm formation once *in vivo*, and to increase the immunogenic potential of the allograft due to denaturation of the extracellular matrix proteins [8]. In addition, complete SDS removal from the tissue is difficult and residual detergents can adversely affect cell adhesion and repopulation [35].

So, SDS seems to be effective for removing cell residues from tissue compared to other detergents, but it is also disruptive to ECM [43].

♦ **SDC** (Sodium Deoxycholate) is an ionic detergent (even it tends to act more like a non-ionic detergent, because of its polar properties it is classified as ionic one) that is useful for disrupting and dissociating many types of protein interactions [44].

♦ **NLS** (N-Lauroyl Sarconsinate) is an effective solubilizer that permits a complete decellularization, additionally it possesses bactericidal properties [45]. In conjunction with a recombinant endonuclease it has been successfully utilized to decellularize pulmonary artery patch grafts [46, 47].

Characteristics of non-ionic detergents

Non-ionic detergents contain unchanged hydrophilic head groups and are suited for breaking lipid-lipid and lipid-protein interactions [42]. Even if **Triton X-100** has proven effective at cell and DNA removal from thicker tissues where enzymatic and osmotic methods are insufficient and appears to be more effective for tissue delipidation than ionic detergents [35, 48], it has demonstrated to lack sufficient strength to decellularize cardiovascular tissue in some hands.

Characteristics of zwitterionic detergents

Zwitteronic detergents offer combined properties of ionic and non-ionic detergents. They do not possess a net charge like non-ionic detergents, but are able to break protein-protein interactions like ionic detergents [42]. For example, **CHAPS** (3-(cholamidopropyl)dimethylammonio)-

1-propansulfonate) is effective for decellularization of thinner tissues and is less effective for cell removal from thicker tissues [49].

To summarize, there are many different detergents that can be used in decellularization protocols, but it is critical to understand how different detergents with distinct chemical properties effect ECM scaffolds in the process of decellularization [42].

c. Osmotic gradient, or osmotic shock, can be used to lyse cells, but it is not efficient at removing the hydrophobic cell membranes and remnants. Thus, it cannot be recommended as the sole decellularization technique [50, 51], but if used in combination with detergents or enzymatic-based methods as an initial step, the required enzyme concentrations and/or exposure time may be reduced [52, 53].

The methodical evaluation of the effect of different agents on the ECM scaffold can be performed by applying the following criteria (safety and effectiveness assessments) [4, 17]:

1. DNA content: < 50 ng ds DNA/mg ECM (dry weight) or < 200 bp DNA fragment lengths.

2. Histological and immunohistochemical assessments:

2.1 Hematoxylin and Eosin (H&E) and 4,6-diamidino-2-phenylindole (DAPI) assess for cellularity and inflammation (lack of visual nuclear material).

2.2 Movat's Pentachrome assesses for extracellular matrix structure.

2.3 Alizarin Reds assesses for the presence of calcification.

2.4 Factor VIII assesses for the presence of endothelia cells.

2.5 Alpha smooth muscle actin assesses for myofibroblasts and smooth muscle cells.

2.6 TUNEL assesses for apoptotic cells and Hsp 27 assesses for this chaperonin protein specifically expressed during the manufacture of collagen Types I and III.

3. Residual assessment:

3.1 Enzyme Residuals may be assessed by ELISA, mass spectroscopy or zymography.

3.2 Detergent Residuals can be assessed by radiolabeling the detergent and conducting a time course experiment or performing a colorimetric assay.

4. Biomechanical assessments:

4.1 Uniaxial tensile.

4.2 Ball burst testing (assesses the biaxial strength of the conduit).

4.3 Fluid mediated burst.

4.4 Hydrodynamic assessment

4.5 Durability testing.

4.6 *In vivo*, durability and functional assessments usually performed in the female juvenile sheep model (according to ANSI/ISO/AAMI 5840 "Cardiovascular Valve Prostheses").

To summarize, decellularization of the tissue to produce extracellular matrix (ECM) scaffold is a complex process that is not standardized even for a specific anatomic source tissue, furthermore it is highly desirable to preserve the complex composition and three-dimensional ultrastructure of the ECM. But it is recognized that all methods of tissue decellularization result in some degree of disruption of the architecture with potential loss of surface structure and composition, that may subsequently impact the host response (such as chronic inflammation, fibrotic encapsulation, and scar tissue formation or a constructive remodeling response with the formation of site-specific functional tissue) [34, 42, 54].

Numerous protocols with applying different agents for decelularization are reported. However, no references exist on how each one may affect the properties of the final ECM scaffold.

C. Characteristics of biological/polymeric starter matricies

The engineered construct with single material and single technique can hardly mimic the whole structure, properties, and function of native valve tissue [5]. Biological/polymeric composite materials are complex structures and have recently been introduced as a further strategy in tissue-engineering. These hybrids may be used for production of heart valves, e. g. fabricated from decellularized porcine aortic valve and enhanced with bioresorbable polymer. Assessments of a novel hybrid heart valve (tensile tests, suture retention strength, pulse duplicator system used for functional testing of the valve under physiological systemic load conditions) demonstrated its feasibility for an application in tissue engineering [12, 55].

Conclusions

Fabricating of a living valve that can grow and functionally integrate to patients' cardiovascular system is the ultimate goal. Heart valve tissue-engineering is a field already almost 20 years old and has advanced considerably since the first published study that galvanized the research. Tissue engineering is a promising approach that may lead novel constructs that will satisfy the need and overcome the limitations of current valve prosthetics.

Some more common and traditional techniques have been improved, including using biopolymers and decellularization. Scaffolds, fabricated from synthetic or biological materials, do not require donor tissue, but have struggled to recreate the macro- and micro valve anatomy and mechanical properties of native valve.

Decellularized cardiovascular grafts have the opportunity to improve patients care by reducing the risk of sensitization to donor antigens, calcification and stenosis and providing with a good graft that will grow (especially important in children). In this way the emotional and financial drain on the patient enduring multiple surgeries may be significantly minimized. Decellularization process typically involves exposure to different agents (chemical, biological, physical ones) that unavoidably cause disruption of the associated ECM. Although some of decellularized valve technology showed promising results, the critical weakness of obtained decellularized TEHV is a somewhat unpredictable rapid graft failure because of immune response and incom-

plete recellularization. As conclusion, the choice of decellularization method can be rational if mechanism of action is contemplated and clearly understood. In addressing to challenges associated with the TEHV, researches must achieve the following goals:

- ♦ Improvement of decellularization technique.
- ♦ Preservation of valve biomechanical properties (equal with valve functional safety).
- ♦ Achieving of the entire valve recellularization *in vivo.*

To summarize, many challenges have been encountered in the pursuit of a TEHV and, probably, it may take another 20 years before many complex challenges are finally solved.

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