1 INTRODUCTION

1.1 Clinical application of cardiovascular patches

Cardiovascular diseases are the most common causes of death and serious morbidity in the world [1-4]. Additionally, congenital heart disease is a considerable problem worldwide, affecting approximately 1% of infants, and is associated with significant morbidity and mortality [5]. Many cardiovascular diseases necessitate operative therapy. The application of patches or grafts in cardiovascular surgery and particularly in pediatric cardiac surgery is a widely accepted surgical technique for repair or reconstruction of cardiovascular structures [6]. Congenital heart defects such as atrial septal defect, ventricular septal defect, double outlet ventricles and hypoplastic left heart syndrome as well as ischemic heart disease are associated with aplastic, defective or necrotic myocardial structures. In many of these instances, patch closure, correction of the defect or revascularization is required [7] (Table 1).

Table 1 Spectrum of congenital heart disorders with tissue defects and/or patch requirement

Disease	**Incidence (% of cases)**
Anomalous connection of the pulmonary veins	1.1
Atrial septal defect (secundum)	6.7
Common atrium	0.5
Defects of the endocardial cushion (primum defect, cleft of mitral valve, atrioventricular canal)	7.0
Ventricular septal defect	28.3
Tetralogy of Fallot	6.8
Transposition of the great vessels (Mustard operation)	2.4

1.1.1 Patch materials for cardiovascular practices

Currently, the patch materials used clinically are limited to prosthetic materials, autologous pericardium and allogenic or xenogenic (glutaraldehyde-fixed) pericardium [8].

1.1.1.1 Prosthetic materials

Polyethylene terephthalate (PET) and polytetrafluoroethylene (PTFE) have been extensively applied in cardiovascular prosthetic implants.

PET was first introduced in 1939. Vascular grafts made from Dacron were first implanted by Julian in 1957 and DeBakey in 1958 [9]. PET is composed of long chains of alternating units of glycol and terephthalic acid. The cardiovascular prosthetic applications of PET are largely in textile forms. Clinically available Dacron materials are fabricated in either woven or knitted forms. The woven Dacron fabrics have low porosity; otherwise the knitted fabrics have high porosity [10]. Dacron has good stability and can persist for more than 10 years after implantation without significant deterioration [11, 12].

PTFE is chemically composed of carbon chains saturated with fluorine. Its medical use began with its application in artificial heart valves in the early 1960s. PTFE for vascular prosthetic applications is known as expanded polytetrafluoroethylene (ePTFE). In 1969, Gore patented expanded ePTFE (Gore-Tex), which is the material used in vascular grafts. The PTFE molecule is biostable, and the implant made from it does not undergo biological deterioration within the body. The surface of PTFE is electronegative, which minimizes its reaction with blood components [13]. These physical aspects of PTFE correlate with some of the biological properties, such as low thrombogenicity.

As widely used cardiovascular prosthetic implant materials, Dacron and PTFE have some common advantages and diasadvantages (Table 2) [14].

Table 2 Advantages and disadvantages of Dacron and PTFE

Advantage	**Disadvantage**
Surface modification	Foreign body reaction
Tissue ingrowth into the prostheses	Pseudointimal hyperplasia at the anastomotic site
Surgically well processable	Calcification
Porosity rate 85%-95%	Thrombosis

1.1.1.2 Biological materials

Autologous pericardial patch

Autologous pericardium is harvested during cardiovascular procedures and has been used in the surgical reconstruction of many different areas, including the vena cava, atrial septum and ventricular septum as well as the right side of the heart in patients with congenital heart defects [15]. Using autologous pericardium as a patch material has multiple advantages such as ready availability, excellent handling characteristics, conformability, nonporosity, and lack of bleeding through needle holes. Moreover, in cases of contaminated environment, pericardium may be more resistant to infection than synthetic materials [16]. In addition, it may also be less likely to cause thrombosis or hemolysis [17, 18]. Vital (fresh) autologous pericardium is a low-cost biomaterial that has advantages for cardiovascular implantation. It is free of donor-derived pathogens, will not provoke an immune response and is easy to access [19-22]. Nevertheless, the clinical use of fresh autologous pericardium in cardiovascular surgery is limited because of uncertain factors such as the onset of tissue shrinkage in the right heart or stretching in the left heart several years after implantation [23]. The implants may become fibrotic and retracted [24, 25], exhibiting progressive thinning with dilatation and aneurysm [26, 27]. Retraction and fibrosis were observed when fresh pericardium was used as a patch or artificial chordae in heart valve reconstruction [24, 25, 28, 29]. The closure of large ventricular defects or patch reconstruction of the right ventricular outflow tract was reported to result in aneurysmal changes [26, 27]. Additionally, pediatric patients often have to undergo multiple reconstructive surgical procedures, so that the application of autologous pericardium for repair or reconstruction of congenital defects is also limited [30].

Allogenic patch

Cryopreserved allograft material is an important component in the repair of many congenital heart defects, particularly to augment luminal diameter of obstructed outflow tracts and large-vessel stenoses [31]. Cryopreserved homograft tissue appears to offer advantages over prosthetic replacements by theoretically providing some viability with a potential ability for growth [32-34]. However, cryopreserved nonvalved allografts exhibit sufficient immunogenicity to induce a marked antibody response that involves both class I and class II anti-HLA antibodies within 3 months after operation in children. This alloantibody response

may represent a form of "rejection", and may play a role in the development of early allograft failure [35]. These allogenic tissues are subject to enhanced calcification after implantation in children, and donor scarcity remains a significant problem for pediatric patients [36-38].

Xenogenic patch

The use of xenogenic pericardium in cardiovascular surgery and specifically as material for valve repair and reconstruction is not new. Commercially available xenograft patches include bovine, porcine, or equine pericardium and dura mater [39]. Xenograft pericardium tissue patches are superior in blood and tissue compatibility, but are inferior in *in vivo* durability, mainly due to calcification. Calcification is the most frequent cause of clinical failure of bioprosthetic tissues [40, 41].

1.1.2 Limitations of currently used patches

Currently available synthetic or bioprosthetic replacements for repairing congenital cardiac defects show certain disadvantages that limit long-term benefits. Each type has limitations, which include their inability to grow, repair, and remodel, which leads to aneurysm formation in patch aortoplasty, inelasticity of the prosthetic materials and an increased risk of hemolysis induced by contact of the blood with the prosthetic materials [42,43]. Prosthetic replacements lack growth potential and can become obstructed by tissue ingrowth or calcification, leading to the need for multiple replacements [44, 45]. All synthetic material is thrombogenic and, after implantation, the risk of thrombembolic and infectious complications potentially increases [46].

Table 3 Limitations of currently used patches

Common disadvantages of currently used patches
Non-living structures
Lack of growth
Aneurysmal dilation
Risk of infection

1.1.3 Criteria of ideal patch

The essential characteristics of patch materials were described by Dwight E. Harken [47] in the 1950s with reference to ideal heart valves as durability, absence of thrombogenicity, resistance to infections, lack of antigenicity, and the potential for growth. To meet these requirements tissue engineering provides a new experimental approach aimed at vital, autologous replacement structures.

1.2 Cardiovascular tissue engineering

Tissue engineering is "an interdisciplinary field that applies the principles and methods of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [48, 49]. It is a new approach in which techniques are being developed to transplant autologous cells onto biodegradable scaffolds to ultimately form new functional autologous tissue [50]. The ultimate goal of tissue engineering is to transplant cells onto a biocompatible, biodegradable scaffold that provides appropriate mechanical strength to induce 3-dimensional tissue growth. Once the cells have become attached to the scaffold, they proliferate, organize, and produce their own cellular and extracellular matrix while the polymer scaffold starts to degrade and resorb. This construct can be implanted in the body and can be integrated with surrounding tissue to form a viable structure and should function, remodel, and grow like native tissue [48, 51]. There should be no foreign body response and, with a functional endothelium, there should be no need for long-term anticoagulation [52].

1.2.1 Key issues in cardiac tissue engineering

As in all tissue engineering endeavors, there are 4 critical areas that need to be carefully considered [53]:

1) the source of cells to be used
2) the nutritive medium or media
3) the matrix to be made available for cell seeding
4) the bioreactor

1.2.2 Cell sources

Cells used in tissue engineering can be derived from numerous sources (Table 4). Ideally, the cells should be highly proliferative and easy to harvest [54]. Autologous vascular cells that can be easily isolated and expanded *in vitro* have been used in many tissue engineering investigations.

Table 4 Cell sources for cardiovascular TE

Potential cell sources for cardiovascular TE
-Vascular cells
-Microvascular endothelial cells derived from
-omentum [55]
-subcutaneous fat [56]
-dermal specimens [57]
-Progenitor cells and stem cells [58, 59]

1.2.3 Scaffold materials

Ideally, the characteristics of tissue regeneration templates incorporate desirable properties of both natural and synthetic materials [60]. Scaffold materials for tissue engineering must be biocompatible and are designed to meet both nutritional and biological needs for the cell population involved in tissue formation. The materials should permit the diffusion of nutrients and metabolic waste necessary for cell growth; enable cell adhesion, migration, proliferation and differentiation; facilitate extracellular matrix formation; and permit endothelialization of the endocardiac surface [61].

Table 5 An overview of frequently used scaffolds

Natural origin materials	**Synthetic biomaterials**
Decellularized biological matrices [62, 63]	Polyglycolic acid (PGA) [64, 65]
Collagen gel [66]	Poly-4-hydroxybutyrate (P4HB) [64]
	Polyhydroxyalkanoate (PHA) [64]
	Polylactic acid (PLA) [67]

1.2.4 Bioreactor

The ideal *in vitro* conditions for the formation of tissue engineered constructs are not known, but it has been widely confirmed that a dynamic tissue environment stimulates extracellular matrix that might lead to appropriate biochemical and biomechanical properties in tissue-engineered constructs prior to potential implantation [68, 69]. To grow cardiaovascular tissue, specific bioreactor attributes should be considered. The bioreactor can mimic the mechanical, physiological environments of tissue and encourage the formation and maintenance of the cardiovascular architecture.

1.2.5 Tissue engineered cardiovascular constructs

Cardiovascular tissue engineering focuses on the development of blood vessels, heart valves and myocardium.

1.2.5.1 Blood vessels

The majority of patients with atherosclerotic vascular diseases need blood vessel substitutes to reestablish vascular continuity. Current surgical therapy for diseased vessels less than 6 mm in diameter involves bypass grafting with autologous arteries or veins [70]. Arterial conduits have restricted dimensions and are limited in supply. Venous conduits lack vasomotor tone and may have degenerative varicose alterations that can lead to aneurysm formation in the higher pressure arterial circulation [66,67]. Synthetic materials are excessively thrombotic when used to bypass arteries less than 6 mm in diameter [70, 72]. As a result of these complications, the need for a tissue-engineered vessel of small diameter composed of biological materials and autologous cells has arisen and has been an area of active investigation for more than 15 years [72]. The ideal blood vessel should be a compliant, functioning substitute with the ability to repair, remodel, and grow. The internal surface should be covered with an intact and functioning endothelial cell lining to prevent thrombosis and to provide vascoactivity. Current approaches in tissue engineering use either acellular (polymer based) or decellularized (xenogenic) matrices and autogologous cellular components to achieve the goal of an ideal blood vessel. Small vessel vascular tissue engineering can loosely be categorized into three groups which include using non-biodegradable grafts seeded with cells, using natural materials as grafts with or without cells, and using biodegradable

polymer matrices seeded with cells. A tissue engineering approach has been successfully used in creating a viable pulmonary artery autograft [65]. Living vascular grafts engineered from autologous cells and biodegradable polymers functioned well in pulmonary circulation in lambs as a pulmonary artery replacement.

Resently, Shinoka and colleagues implanted a tissue-engineered pulmonary artery into a human being. This was the first reported human implantion of a tissue-engineered blood vessel constructed from cells and polymers [73].

1.2.5.2 Heart valves

Approximately 300,000 procedures for repair or replacement of heart valves are performed annually worldwide. Over 95% of these operations concern valves in the systemic circulation. Currently, heart valve replacement with either a nonliving xenograft or a mechanical prosthesis is an effective therapy for valvular heart disease. Nevertheless, both of these approaches have limitations, such as inability to grow, remodel, and repair, limited durability, and susceptibility to infection. The currently available prosthetic heart valves have excellent long-term function but need life-long anticoagulation to prevent clotting and are also susceptible to infections. The bioprostheses (porcine valves or bovine pericardium) provide better fluid dynamics and avoid coagualtions. However, these valves have limited durability. The tissue engineering of heart valves focuses on the development of an identical copy of a healthy normal heart valve. The potential advantages of a tissue engineered heart valve created using autologous cells include the capacity for normal repair and growth, greater durability of a living structure, biocompatibility of the tissue with minimal risk of infection and thromboembolism, and avoidance of anticoagulation therapy.

A tissue-engineered trileaflet valve fabricated from porous PHA and vascular cells has been created and implanted in pulmonary position with an appropriate function for 120 days in lambs. The trileaflet tissue-engineered valve appears promising and represents a potentially important improvement in the field of tissue engineering of cardiovascular structures [50]. But tissue engineering of autologous heart valves is still in the early stages of investigation with many issues that remain to be resolved before clinical application can be considered.

1.2.5.3 Myocardium

Heart transplantation is the only established therapy for end-stage heart failure; however, the shortage of donor organs has become a major limitation. The transplantation waiting lists worldwide are growing longer and patients have to wait longer for a donor heart. For this reason there has been great interest in cell transplantation as an alternative to heart transplantation. Invesigators have taken two basic approaches toward this end. The first, cellular cardiomyoplasty, involves direct cell transplantation into the myocardium as a means of augmentation. The second approach to myocardial tissue engineering involves seeding cells onto a biodegradable scaffold such as PGA and Poly-L-lactic acid. Tissure-engineered constructs have a definitive structure and may be more suitable to produce a significant myocardial augmentation when transplanted as opposed to a cell suspension alone [74]. Leor and colleagues seeded porous alginate scaffolds with fetal cardiac cells and implanted the constructs into myocardial scars in a rat model. They noted extensive neovascularization of the grafts. Control animals developed left ventricular dilatation and deterioration of left ventricular function, but the grafted animals did not [75].