Stem cells, tissue engineering and congenital heart disease

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Congenital heart disease (CHD) affects a considerable number of children and adults worldwide. This implicates not only developmental disorders, high mortality, and reduced quality of life but also high costs for the healthcare systems. CHD refers to a variety of heart and vascular malformations which could be very challenging to reconstruct the malformed region surgically, especially when the patient is an infant or a child. Advanced technology and research have offered a better mechanistic insight on the impact of CHD in the heart and vascular system of infants, children, and adults and identified potential therapeutic solutions. Many artificial materials and devices have been used for cardiovascular surgery. Surgeons and the medical industry created and evolved the ball valves to the carbon-based leaflet valves and introduced bioprosthesis as an alternative. However, with research further progressing, beating cells and contracting tissue have been developed in the lab and tissue engineering (TE) could represent a revolutionary answer for CHD surgery. Development of engineered tissue for cardiac and aortic reconstruction for the developing bodies of infants and children can be very challenging. Nevertheless, the use of acellular scaffolds, allograft, xenografts, and autografts is already very common. Seeding of cells on the surface and within the scaffold is a key factor for the use of the above. The use of different types of stem cells have been investigated and proven suitable for tissue engineering. They are the most promising source of cells for heart reconstruction in a developing body, even for adults. Some stem cell types are more effective than others, with some disadvantages which may be eliminated in the future.

Stem Cells, Tissue Engineering, and Congenital Heart Disease

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Abstract

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Many artificial materials and devices have been used for cardiovascular surgery. Surgeons and the medical industry created and evolved the ball valves to the carbon-based leaflet valves and introduced bioprosthesis as an alternative. However, with research further progressing, beating cells and contracting tissue have been developed in the lab and tissue engineering (TE) could represent a revolutionary answer for CHD surgery. Development of engineered tissue for cardiac and aortic reconstruction for the developing bodies of infants and children can be very challenging. Nevertheless, the use of acellular scaffolds, allograft, xenografts, and autografts is already very common. Seeding of cells on the surface and within the scaffold is a key factor for the use of the above.

The use of different types of stem cells has been investigated and proven suitable for tissue engineering. They are the most promising source of cells for heart reconstruction in a developing body, even for adults. Some stem cell types are more effective than others, with some disadvantages which may be eliminated in the future.

1.0 Introduction

Congenital heart diseases (CHD) refer to the abnormal formation of the infant's heart, great thoracic vessels and heart valves during intra-uterine development [1]. CHD is different from the acquired heart diseases which occur as a result of lifestyle or aging [2]. The abnormalities are structural defects, such as valve defects, intravascular or intracardial stenosis, congenital arrhythmias or cardiomyopathies which greatly affect the early and future life of a CHD patient [1, 3]. People born with CHD need immediate medical care after birth which further continues throughout their lives. In 2010, it was estimated that only in the USA approximately 2.4 million people suffered from CHD and more than half of them were adults[4]. In Europe, for the period of 2000 to 2005, about 36,000 live births per year were diagnosed with CHD[5]. The number grows bigger when including the unborn that were diagnosed with CHD and died either by the termination of pregnancy or by intrauterine death or even neonate death[5]. In the UK, about 8 in every 1,000 live babies born have a heart or circulatory condition [1]. Some estimate those numbers to be higher and, commonly, percentages of each type of CHD change depends on the geographical area of investigation [6, 7]. CHD not only have an effect on the individual's and their family's lives but, also, have a huge financial impact on the healthcare system. According to NHS England for the financial year 2013/14 the total spent on CHD was £175 million [8] and in the US for 2008 the total cost for CHD treatment was approximately $298 billion[9]. In general, the number of children and adults being diagnosed with CDH increases due to improved technology of diagnostic tools[6].

CHD can be diagnosed using transabdominal fetal Doppler echocardiography. Such prognostic protocols are performed in high-risk groups of pregnant women, like those with a family history of CHD[10]. In adults with CHD, the most effective diagnostic practice is transesophageal echocardiography, electrocardiogram, pulse oximetry, X-rays, cardiac catheterization and MRI [11].

The CHDs are managed by surgeries, and the efficiency of this approach is largely dependent on the materials that are used during the surgery. These materials are expected to be close in structure and function to the native cardiac tissue. Several materials and biomaterials have been used in the surgical management of CHDs but the ideal one is yet to be found. Nevertheless, CHD could

present extremely complicated malformations which cannot be spontaneously or by singular surgical procedure reconstructed, hence the dire need for more research into biomaterials for TE. The recent extensive research focuses on possible ways to fabricate a near ideal tissue. So far, TE appears to be the way forward in creating ideal tissue that can probably mimic the native heart tissue both in structure and function.

Many would think that TE is an idea which conceived and developed in a very recent past. However, it has been proven that tissue regeneration and TE is a concept which was born thousands of years ago and it has inspired Greek mythology, history, arts, and religion. In arts, religion inspired the well-known painting of "*Healing of Justinian*" based on the miracle of St. Cosmas and St. Damian, physicians and Christian martyrs who appear to have transplanted the leg of an Ethiopian to the body of a patient. The closest to an artificial replacement of a body part was discovered in Egypt on a mummy which had a wooden replacement of the hallux. However, today, TE involves a combination of creating scaffolds and cell seeding. With regards to the heart, the most commonly used and known artificial parts are the mechanical heart valves and conduits [12, 13]. The first artificial heart valve was placed on live patients only in the last century [12, 14].

In 2003, some evidence were presented to support the regenerative ability of the adult heart [15]. This evidence shows the existence of Lin c-kit^{+} cells which confirm cardiomyocyte proliferation and cardiac regeneration [15]. In contrast to the adult heart, infants' and children's hearts regenerate in a larger capacity because the regenerative ability is proportionally correlated to age [16]. Additionally, there is an insufficient number of heart donors which becomes more challenging because of the heterogeneous relation of recipient-donor and the diverse range of CHD. These points result in high mortality rates and further financial costs to the healthcare systems[17].

However, only a small number of preclinical studies have focused on CHD treatments[18, 20]. Stem cells (SC) have been widely investigated mainly for myocardial infarction (MI), as it is currently the leading cause of morbidity and mortality worldwide [19]. Cell seeding is a fundamental component of TE. Several studies have examined the possibility of direct cell delivery in the damaged area, cardiac patch implantation and engineered heart tissue, with the former being the most popular[18, 20, 21]. All possible types of stem cells are under investigation to identify the most appropriate cell types for tissue engineering using in corrective surgery of CHD. This

115 review looks into the congenital heart diseases, biomaterials and scaffolds, and, types of stem cells
116 used in TE.
117

118 **2.0 Method**

119 This paper was based on review articles and reports in reputable peer-reviewed journals and
120 government websites. The research was conducted using Medline on OvidSP, PubMed, books, e-
121 books, and reports. Other online search engines were also used. The words "congenital heart
122 disease", "tissue engineering", "surgical treatment", "stem cells", "scaffolds", "biomaterials" and
123 a combination of those were used to retrieve literature from the databases.

124

125 **3.0 Congenital Heart Disease: Types, malformations, and surgical intervention**

126 CHD includes a diverse range of conditions which shows a variety of symptoms, indications, and
127 malformations detected during pregnancy or after birth[11].However, these malformations are
128 much influenced by the age of diagnosis [6].The etiology of CHD is unknown, but it is generally
129 accepted that many factors or a combination of them could contribute to CHD, and considered to
130 be caused by multifactorial inheritance. These factors could be genetic, epigenetic or
131 environmental such as alcohol and drugs consumption, as well as viral infections like Rubella
132 [11].The severity of the disease varies, and a number of malformations could be present in each
133 case. Based on the severity of CHD, they are categorized to mild, moderate, and severe CHDs,
134 which the latter is subcategorized to Cyanotic and Acyanotic lesions [17]. Some types of severe
135 CHD are discussed in the following. The most frequent type of CHD is Ventricular Septal Defect
136 (VSD) [22]. VSD could cause myocardial defects which disappear in the first year of the infant's
137 life [22]. Nevertheless, the VSD could also cause some malformations which can be managed only
138 by surgical intervention, i.e. infant pulmonary hypertension[6, 22].The other CHD type is Atrial
139 Septal Defect (ASD) which is usually asymptomatic and in most of the case will only be diagnosed
140 in adulthood[6]. Atrioventricular septal defects (AVSD) is mainly observed in trisomy 21 [6].
141 AVSD is usually characterized by "complete AV-canals with one common AV valve for both
142 ventricles and an interatrial and intraventricular communication" and requires surgical correction.
143 The results of long-term patient follow up after operation has shown very satisfactory survival rate

[23]. Tetralogy of Fallot (ToF) is characterized by VSD, pulmonary stenosis, right ventricular hypertrophy and over-riding of the aorta [24]. Infants who suffer from ToF will require immediate surgical intervention for better survival rates and avoid cyanosis, a result of inadequate pulmonary blood flow[24]. Calcific Aortic Valve (CAV), another type of CHD. is a disease which progresses slowly and results to a mild valve thickening and obstructing blood flow, aortic sclerosis or severe calcification with impaired leaflet motion[25]. CAV presents many similarities with arteriosclerosis in adults which is caused by lifestyle or aging[25].However, CAV is a congenital, progressive disease which could be diagnosed in patients less than one year of age, and those in childhood or even adulthood[26, 27]. Other most frequent types of CHDs are:

- Hypoplastic Left Heart Syndrome (HLHS)
- Isolated partial anomalous pulmonary venous
- Pulmonic stenosis
- Aortic stenosis
- Bicuspid aortic valves
- Coarctation of the aorta
- Mitral incompetence
- Single ventricle
- Ebstein anomalies

Some types of the CHDs are misdiagnosed, undiagnosed, or diagnosed very late in life which could make a successful treatment challenging [29].

The mortality rate among all patients who are waiting for any type of organ transplantation is highest in infants who wait for heart transplant [30]. CHDs patients usually require medication, cardiac catheterization or a series of surgical interventions throughout their lives[11, 30]. This among other risks increases the chances of HLA-sensitization which eventually makes it more difficult to find a cross-match[30]. The main challenge with artificial materials is growing and alterations in the size and function of the heart, which current artificial materials cannot adjust to changes of the heart in a developing body [11]. The need for early intervention is essential for normal physical and cognitive development [28].

The use of bioprosthetics including allografts and xenografts is widely used for the treatment of CHD as they present good survival rates for the patient and fewer interventions throughout their lifetime [29]. The observed symptoms, defects, and complications upon bioprosthetic treatment of CHD patient could differ from case to case [22, 28].

TE refers to the creation of functional three-dimensional tissue using biomaterials and cells for the replacement or restoration of damaged organs and/or parts of them. TE is the most promising approach at the present for CHD, as treatments can be "custom-made" and the engineered tissue could adjust to the developing body of the recipient.

4.0 CARDIAC TISSUE REGENERATION

Unlike other human tissue, cardiac tissue is a more complex tissue considering not only because of its mechanical and structural function but also due to its electrical properties as well. The human heart mainly consists of cardiomyocytes, functions as a blood pump which is regulated by the electrical signal generated by the pacemaker cells in the sinoatrial nodes. This signal is directed and spread through the atrioventricular node to Purkinje fibers, and this is highly important for the direction of blood flow [31]. The diastolic and systolic function of the heart is necessary to be synchronized and also adjusted according to the body needs. The isolation of this signal from the rest of cardiac tissue is as important as this electrical signal by himself. This importance is achieved by the extracellular matrix (ECM) of heart which is also responsible for mechanical support and endurance [31].

Aside the functional aspects which briefly mentioned above, the structural aspects (the cardiomyocyte and its ECM) are another factor for strong consideration in the success of TE. To yield an engineered tissue with the best functionality, this engineered tissue has to be similar in every sense to the native tissue. The ECM of the heart is mainly a complex mesh of structural elements such as cardio fibroblasts and collagen fibrils, and also non-structural elements such as proteoglycans, glycosaminoglycans, and glycoproteins [32, 33] among others components.

Repairing the heart using materials which do not have or do not comply with the above characteristics and also cannot work in harmony with the host-heart, will result in a non-efficient functioning heart accompanied by a series of complications. The concept and act of repairing heart using various engineered techniques have evolved over the years from the use of artificial heart

valves and grafts to bioprosthesis, and currently forward to the use of biomaterials and scaffolds cells.

4.1 Artificial Heart Valve and Grafts

Charles Hufnagel was the first to experiment on animals with an artificial valve which he designed in the 1940's[13]. A few years later, the same type of valve was transplanted into humans[13]. Nonetheless, Hufnagel's valve required changes which were succeeded by Harken-Soroff and later by Starr-Edwards ball valve [13]. Following several improvements, the latest version was made of pure carbon as a lighter, smoother material for blood flow and more durable in comparison to other materials[12]. Due to the position of the valve, there is a high transvascular pressure which leads to 'impact wear' and 'friction wear'[12]. Further complications with the use of valve replacements include inflammation around the prosthesis and calcification of the valve itself[34]. The main disadvantage of artificial valves is the thromboembolic risk which leads to lifetime treatment with anticoagulants. This type of treatment involves various complications and brings different risks to the patient [12].

Similarly to valves, artificial vascular grafts have been used for many years as a surgical treatment for CHD [35]. Nevertheless, they provoke an inflammatory response, and they are much less flexible than the body's natural tissue [28]. Originally porous fabric knitted of Dacron and polytetrafluoroethylene (PTFE) was used for stenosis treatment. Later alterations in the porosity of the fabric were introduced to prevent the material's corruption[28]. The first attempt to combine biomaterials with patient's cells was made by Wesolowki and colleagues by "preclotting the graft with the patient's blood "[28]. However, future attempts to produce successful results created many doubts about the actual function of cells following citation on the clot[28]. Nonetheless, both artificial heart valves and conduits require continuous use of anticoagulants. Remarkably, they do not grow with the patients' heart as patients with CHD are highly likely to require surgical intervention when they are an infant or a child [28, 36].

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234

235 **4.2 Bioprosthetics: Allograft, Xenografts, and Autografts**

236 Allograft heart valves and arterial grafts are collected by deceased humans. In contrast, xenografts
237 are harvested from porcine and bovine animals including heart valves and carotid arteries [12, 14,
238 34]. Allografts and xenografts came into the picture as an alternative to artificial valves and
239 conduits. Their main advantage is that they do not require lifetime treatment with anticoagulants
240 [12, 37, 38].

241 Animal tissue is treated with glutaraldehyde [37]. Glutaraldehyde is a five-carbon bifunctional
242 aldehyde used to stabilize tissue to protect from chemical and enzymatic degradation and maintain
243 "its mechanical integrity and natural compliance"[37]. Also, treatment is necessary to reduce
244 immunogenicity of the xenograft by decellularization and sterilization of the tissue [37]. Similar
245 to xenograft, allografts also need treatment before transplantation and can even be
246 cryopreserved[29]. Despite the great advantage, a number of complications are related to
247 bioprosthetic grafts related to their preparation [37].

248 The risk of cytotoxicity leading to inflammation, as well as the partial loss of mechanical properties
249 of tissue, have been reported [37]. Moreover, calcification is often observed in infants and children
250 with bioprosthetics. Many efforts are being made to find out an alternative treatment for heart
251 bioprosthetics therapy. However, there is a controversy regarding their efficiency [37].

252 In a study contacted by Homann and colleagues, the outcomes of 25 years using allografts and
253 xenografts for reconstruction of the right ventricular outflow tract showed 66% survival at 10-
254 years' follow up. Furthermore, patients with allografts had a mean reoperation-free interval time
255 of 16 years in contrast to the xenograft recipients which this interval time is 10.3 years [29].
256 Allografts may present better outcomes, but they are not in abundance like xenografts. Therefore,
257 many studies are concentrating on the development of tissue valves and vascular grafts created by
258 stem cell-seeded on artificial or natural scaffold [14].

259 A very common surgical practice for CHD is the use of pericardial patches for repairing the septal
260 defect [28]. The autologous pericardium is the best choice for infants as it is free, it does not
261 provoke any immune-response, and it is sterile. Even though it requires some preparation before

application, autologous pericardium creates less fibrotic tissue in comparison to Dacron [28]. Allograft pericardium is available, but quite a few risk factors are associated with its use [28].

The relatively recent "CorMatrix" patch fabricated from the decellularized porcine small intestinal submucosa extracellular matrix (SIS-ECM) mainly composed of collagen, elastin, glycan, and glycoproteins has been introduced into cardiac surgery. SIS-ECM has not only been used in animal models for cardiac surgery[39-41], but also in humans studies, for cardiac and vascular reconstructions such as; augmentation of the tricuspid valve [42], vascular repair of ascending aorta, aortic arch, right ventricular outflow tract, pulmonary artery, valvular reconstruction [43], and closure of septal defects [44]. The study by Witt et al. reported a small risk of stenosis when SIS-ECM is used in the reconstruction of the outflow tracts and great vessels. Interestingly, the SIS-ECM has effectively proved the function in the high-pressure vessels [44]. The pitfall of this study, however, was the short follow up period.

4.3 Biomaterials and Scaffolds

Generally, scaffolds work as a primary base for cells to enhance and produce relevant tissue. The scaffolds should have specific morphological, functional, and mechanical properties to support cells survival and differentiation [21]. Biomaterials used to produce scaffolds should be made of components which will accommodate the above characteristics and create a friendly cell microenvironment [21]. The previously mentioned characteristics apply for all different types of engineered tissue, and the goal is to mimic the host tissue in the best possible way. With regards to artificial and bioprosthetic cardiac correction choices, scaffolds and biomaterials should contain various properties such as s being biodegradable, biocompatible, flexible and durable and also the absence of immunogenicity and calcification. Due to the variety of sizes of patients' heart and defects, designed scaffolds should have various size and ability to be able to grow and adapt to the heart [38]. The biomaterial should allow neo-vascularization for adequate oxygenation of the tissue, create minimal scaring tissue and thrombotic risk, as the latter could lead to life treatment with anticoagulants [45, 46]. Furthermore, these biomaterials scaffolds should be bioactive, meaning they should enhance cellularization *in vitro* and *in vivo*, and also optimize cell efficiency and degrade at a desirable rate [46]. What is more, biomaterials and scaffolds should be in abundance and cost-effective, as high cost could restrict development and use of it in TE as a

routine therapeutic choice [47]. The most common biomaterials for cardiac and vascular TE used today are synthetic, and natural polymers [31], and the electrospinning technique has been proven to be the most efficient way to produce scaffolds with these biomaterials [48].

4.3.1 Synthetic polymers for cardiac scaffolds

The easiest way to have materials in abundance is to manufacture them. The need for suitable biomaterials for cardiac tissue repair has triggered the development of synthetic polymers which are easy to fabricate and manipulate. These polymers can be manipulated with respect to their physical properties, molecular weight, heterogeneity index, and degradation speed [49]. Many synthetic polymers are biocompatible and have excellent mechanical properties which make them a popular choice for sutures and mesh production [31]. Frequently used polymers in cardiac surgery are polyglycolic acid (PGA) and polylactic acid (PLA). These two polymers have been used as a single biomaterial or as 50:50 composite to reconstruct tissue-engineered vascular grafts for treating children with congenital heart disease [50]. Carrier et al. presented acceptable ultrastructural features and metabolic cell ability when cells were cultured on PGA scaffold in a rotating bioreactor [51, 52]. The rotating bioreactor increases cell adherence and decreases cell damage [51]. The highest concern with synthetic polymers is their toxicity. Therefore the use of poly-L-lactic acid (PLLA) has increasingly become more in interest. PLLA has demonstrated very good results when combined with bone marrow mesenchymal SC (BM-MSC) for vascular tissue engineering [53]. PLLA, when degraded in the body, can “be excreted in carbon dioxide and water” [31]. Polyurethane, unless copolymerized, is biocompatible but not biodegradable. Polyurethane has been successfully experimented in combination with other materials for cardiac tissue repair, such as siloxane films [54, 55], cellulose [56], urea [57], PLLA [58]. Poly(Ɛ-caprolactone) in combination with other biomaterials have also been proven to be efficient composite for cardiac tissue repair. They have been used in combination with PLLA alone [59, 60] PLLA and collagen [58], polypyrrole and gelatin [62], polyglycolic acid [63], poly(hydroxymethyl glycolide) [64], chitosan and gelatin [65]. Based on our understanding of the heart as an electroactive tissue, Hitscherich and colleagues have created a piezoelectric scaffold fabricated by electrospinning Polyvinylidene Fluoride-Tetrafluoroethylene (PVDF-TrFE) for cardiac tissue engineering [66]. The combination of synthetic with natural polymers has been

suggested to increase cell adherence. However, pure natural polymers have also been examined as an option for polymers [31].

4.3.2 Natural polymers for cardiac scaffolds

Natural polymers are biodegradable, biocompatible and easily manipulated matrices composed of complex elements which make up the native tissue [67]. These attributes give them an advantage over the synthetic polymers [68]. The natural polymers used so far for cardiac repair include collagen, gelatin, alginate, silk, fibrin, chitosan and hyaluronic acid[31]. Despite their poor mechanical properties, they are good biomaterial for heart TE, as they have high biocompatibility, promote cell-binding, and could biodegrade with no "additional treatment or modifications" [31].

Collagen is the most widely utilized natural polymer which is the most abundant ECM protein. It functions to guide biological processes, provide structural scaffolding, and tensile integrity [65]. Several kinds of literature have reported the use of various collagen types and their modifications in cardiac tissue repair [70-77].

Fibrin can be manipulated to create gels, microbeads, and hydrogels [49]. Likewise, biological molecules like the growth factors can be incorporated [78]. Fibrin glue can be used as a stand-alone therapy in cardiac tissue repair as it possesses intrinsic regenerative properties [69]. The success of fibrin patch seeded with human embryonic stem cell-derived cardiac progenitor cells (hESC-CPC) in non-human primate model [79] has resulted in its translation to the first case report of the use of hESC-CPC in severe heart failure with an encouraging patient functional outcome [80]. Other studies on fibrin have demonstrated its efficacy as a sealant after intramyocardial injection [81]; for myocardial tissue repair when seeded with adult stem cells, neonatal cardiac cell, and mesenchymal stem cells [82-84]; to form aortic valves in tissue engineering [85].

Chitosan has been experimented in several literature as a biomaterial for cardiac regeneration [86-91]. Overall, in cardiomyogenesis, many researchers have agreed on the fact that chitosan seems to be more effective when combined with other factors enhancing integration of stem cells into cardiac tissues [92]. Alginate, when used alone, has proven to have a remarkable effect on the function of heart models with myocardial infarction. Furthermore, seeding alginate with stem cells has proven to be more efficient in the repair of the cardiac tissue [93-96]. The use of hyaluronic

acid has been shown to be largely dependent on its molecular weight, and several kinds of literature have reported its successful use in cardiac tissue repair [97-100].

Evidence of gelatin scaffold placed subcutaneously, and/or on infarcted myocardial in adult rat hearts have shown a good survival of the graft, vessel formation and junctions with recipient rat heart cells [101]. Gelatin was reported to sustain neonatal rat cardiomyocyte tissue *in vitro* for three weeks [102]; supported the growth of human induced pluripotent stem cell (iPSC)-derived cardiomyocytes [102]; its hydrogel seeded with autologous human cardiac-derived stem cell and basic fibroblast growth factor (bFGF) effectively released bFGF for repair of ischemic cardiomyopathy [103]; and several other studies have shown the efficacy of gelatin as a scaffold for cardiomyogenesis when seeded with cells [104-107].

Fibrinogen/Thrombin-based Collagen Fleece (TachoCombo) have been successfully used to secure hemostasis and enhance complete reconstruction of a large pulmonary artery defect in a canine model [108]. Hence, this biomaterial may be used in the reconstruction of the low-pressure pulmonary vessels during a cardiac surgery for a total anomalous pulmonary venous return or the transposition of the great vessels.

Nevertheless, not all of these polymers can tune well for cardiac TE, and the risk of inflammation still exists [109].

4.3.3 Native Extracellular Matrix

Native-specific ECM could be a category itself or part of natural polymers. ECM is collected from animal or donor tissue and processed for culturing cells [110]. Studies have shown that the ratio of native ECM in culture could play a key role in SC enhancement, differentiation, survival, and phenotype [111]. Other studies have shown that contractible engineered heart patches cultured in ECM mixture can vascularize and survive up to 8 weeks *in vivo* [110].

Furthermore, ECM of decellularized and repopulated hearts and other organs are being used for drug testing [112]. An experiment on decellularized mouse hearts which were repopulated with human cells through coronary vessels exhibited myocardium, vessel-like structures and intracellular $Ca2^{+}$ transients contracted spontaneously and responded as expected to various drug interventions [113]. It was concluded that heart "ECM could promote proliferation, specific cell

differentiation and myofilament formation" [113]. The option of ECM for TE could help overcome the challenges faced using synthetic and other natural biomaterials to replace tissue, valves or organs [114].

4.3.4 Electrospinning; a viable technique for fabricating nanofibrous Scaffolds

The way a scaffold is constructed is as crucial as the biomaterial and culturing conditions. It has been proven that decellularized heart ECM promotes differentiation due to the ultrastructure of natural scaffold [113]. Therefore, the ideal scaffold should have the biomimetic architecture of the organ/tissue targeted for regeneration. Electrospinning is a technique using ultrafine polymer fibers to give shape and size to a scaffold with very detailed microstructure [48]. There are different ways of fabrication using this technique which allow defining porous size, shape or additional components which will help to resemble the target tissue. For heart structures, spontaneous contraction of the cardiomyocytes is taken into consideration and scaffolds are fabricated to support and promote such a function [48]. Electrospinning permits the combination of materials which could promote synchronous contracting of the graft *in vitro* and *in vivo* [48].

4.4 Scaffoldless Cell Sheet

Another technique, independent of scaffolds, has been developed and this is based on the cells' ability to connect via cell-to-cell junction proteins and create ECM [52]. The cells are cultured in normal conditions at 37°C in a temperature-responsive polymer. When the culture temperature conditions change, the cells detach from the polymer as one cell sheet [52]. This technique was developed as a way to avoid inflammatory reactions and fibrotic deposits in the area of graft where scaffold was placed following degradation [52]. A study has shown that contractile chick cardiomyocyte sheets could function effectively around rat thoracic aorta when applied on host myocardium [45]. This cell sheet could synchronize within 1 hour of implantation with the host tissue [45]. Similar results have been shown in 3D structures using several cell sheets aiming to create a thick cardiac patch [115]. The number of sheets is limited, as more than three exhibits poor vascularization. However, the combination of endothelial cells and cardiomyocytes is being examined to promote vascularization before implantation [52].

Table 1 summarizes the pros and cons of the various materials and biomaterials used in tissue engineering.

Table 1: Advantages and disadvantages of materials and biomaterials used in TE

5.0 Stem Cells for TE

Equally important point to the factor of choosing suitable biomaterial, scaffold or scaffoldless cell sheet is the factor of choosing the most appropriate cells types for TE. Stem cells (SC) as a known cell source, possess the ability to differentiate toward cardiomyocytes (CMC), smooth muscle cells (SMC) and endothelial cells (EC), and regenerate cardiac tissue. Based on these properties, they play a key role in TE field. The currently used SCs in TE is discussed in the following and summarized in Table 2.

5.1 Embryonic Stem Cells

Embryonic stem cells (ESC) derive from the inner cell mass of the preimplantation blastocyst [116]. They can differentiate into all different cell types of three germ layers. Human ESC (hESC) could be a good candidate for cardiac tissue engineering. In a study conducted by Landry et al., hESC-derived cardiomyocytes (hESC-CMC) showed very good phenotype including myofibril alignment, density, morphology, contractile performance and gene expression profile which, however, was only confirmed after 80-120 days *in vitro* culture [117]. Various groups apart from Landry and colleagues conducted studies to confirm the successful differentiation of ESC to cardiomyocytes as presented in a review by Boheler and colleagues in 2002 [118]. Duan et al. investigated how native cardiac ECM could affect hESC differentiation. This group processed porcine hearts to collect digested cardiac ECM which then mixed with collagen to create a hydrogel for cell cultivation purposes. The cultured hESCs on biomaterials comprised of 75% native cardiac porcine ECM and 25% hydrogel with no additional growth factors have shown a great differentiation with cardiac troponin T expression and contractile behavior, compared to the hydrogel with a smaller ratio of native ECM [111]. Many other examples point out the ESC could

438 be a great option for cardiac TE [110]. Additionally, some factors such as the ethical concerns, the
439 provoked immunogenicity, and the risk of tumorigenesis make the ESCs a very controversial
440 choice of cell source for TE [38].

441

442

443 **5.2 Induced Pluripotent Stem Cells**

444 Another type of SCs which are used in TE is induced pluripotent stem cells (iPSCs). iPSCs are
445 somatic cells which are reprogrammed to behave like ESC and show the same properties. The
446 iPSCs can differentiate into all three germ layers, potentially to any cell types [119, 120].
447 Takahashi and his group were the first groups who were able to reprogram the somatic adult cells
448 like fibroblasts to iPSCs using viral vectors to introduce four key factors OCT4, SOX2, c-Myc,
449 and KFL-4 to fibroblasts [119]. This method was used to reprogram fibroblasts to embryonic-like
450 cells and from this state differentiate them into a relevant type of cells [119]. Ludry et al. have also
451 shown that human iPSC-derived cardiomyocytes (hiPSC-CMCs) present the same characteristics
452 as hESC-derived cardiomyocytes in long-term *in vitro* culture [117]. Lu and colleagues presented
453 the successful repopulation of decellularized cadaveric mouse heart with hiPSC-derived
454 multipotential cardiovascular progenitors [117]. This group demonstrates that the heart ECM
455 promotes proliferation, differentiation and myofilament formation of CMs from the repopulated
456 hiPSC-derived cells. Furthermore, they have checked the electrical coupling of these cells and also
457 examined the constructive ability of the repopulated heart using electrocardiogram which
458 presented arrhythmia. They have also performed histological analysis to identify muscle-like or
459 vessel cavities-like structures. Lu et al. have further examined the effects of pharmacological
460 agents on repopulated heart, and observed remarkable responses [113]. This model is explored as
461 an option to personalized medicine in connection with drug testing [113, 121]. Specifically for
462 CHD which presents such a variety of profiles, individual human models development could help
463 to understand how each patient would respond to existing pharmaceutical treatments. Even though
464 it may not be possible to create actual organ heart models with individual clinical features of the
465 disease. The possibility to examine cell-autonomous genetic disorders could give a better insight
466 into the disease and develop more effective treatments [122].

Nonetheless, similar to ESC, iPSC has demonstrated tumorigenesis [38]. The group of Ieda and colleagues were able to make a direct transdifferentiation of fibroblasts to functional cardiomyocytes using three key factors, Gata4, Mef2c, and Tbx5, within a very short time and suggested that direct reprogramming could reduce the risk of tumorigenesis [123]. Still, using viral vectors for reprogramming procedure is problematic and involves various risks [38]. Therefore, today more different ways for iPSC production are being used and investigated to find out safer and more effective alternatives for this reprogramming procedure [19, 124]. In the event this problem is solved, iPSC could be the safest type of cell sources for TE as they will not provoke any immune-response and also cell harvesting procedure to produce iPSCs is not life-threatening for the patients. Moreover, iPSC raises less ethical concerns in comparison to ESC or fetal SC.

5.3 Prenatal, Perinatal, and Postnatal Stem Cells

Prenatal, perinatal and postnatal SCs include chorionic villi-derived multipotent SCs, amniotic fluid-derived SCs (AFSCs), umbilical cord blood derived-endothelial progenitor cells (UCB-EPCs) and umbilical cord- or cord blood-derived- multipotent SCs [125]. UCB progenitors, like endothelial progenitor cells (EPCs), have distinctive proliferative properties in comparison to other cells sources [126]. This category of SCs is exceptionally important as the child's own SCs could be used for heart TE, for CHD patients who are diagnosed before birth. Immunogenicity or an additional procedure to harvest autologous SCs form the infant or child could be avoided in this procedure. Furthermore, it has been proven that AFSCs do not form teratomas in contrast to ESCs and iPSCs [127]. All categories have been investigated with remarkable results on engineered valves and vascular grafts [125, 128-132]. This type of SCs is not applicable to adults who have been diagnosed later in life with CHD.

5.4 Adult Stem Cells

5.4.1 Bone Marrow-derived Stem cells

Apart from UCB, EPCs can be found in the peripheral blood (PB-EPCs) and bone marrow (BM-EPCs) of adults [133,134]. In 1997, Asahara et al. identified the $CD34^+$ mononuclear hematopoietic progenitor cells in the peripheral blood which *in vitro* presented endothelial-like

characteristics [133]. The EPCs which are originated from BM considered to play a crucial role in endothelial repair, and they have been suggested for ischemia patients and vascular TE with very encouraging results [126,129,134,135]. A successful complete endothelium regeneration of decellularised canine carotid arteries has been reported in animal studies using PB-EPCs [136]. In addition to vascular TE, the EPCs have been assessed for tissue-engineered heart valves [137]. Bone marrow, however, is a richer source of EPCs in comparison to peripheral blood.

Bone marrow EPCs are greatly involved in *de novo* vessel formation and neovascularisation in pathological conditions like ischemia and cancer [133]. Similar applications to PB-EPCs and prenatal EPCs have been recorded for vascular graft in the congenital heart surgery using bone marrow-derived stem cells [138]. Mirensky and colleagues used sheets of non-woven PGA mesh as a scaffold to create vascular graft in combination with human bone marrow mononuclear cells (BM-MNCs). The results were very encouraging as no aneurysm or thrombotic incidence were reported, despite the absence of anticoagulants. This group suggests this method as a suitable vascular treatment for CHD based on their results from 6 weeks follow up after graft implantation, which has shown signs of degradation, and also it was fully accommodated by the host's cells [138]. Nonetheless, the host mice were at full-growth which makes it questionable how successful this application would be in a developing animal model. Interestingly only one week after implantation no human BM-MNCs were detected, which suggests that BM-MNCs play a paracrine role rather than cell replacement [134]. More studies have reported similar results with the same conclusion [38,139]. Furthermore, an investigation on 25 young (under 30 years old) patients who underwent extracardiac total cardiopulmonary connection with BM-MNCs engineered vascular graft has also presented convincing results. A long-term patients follow up has shown zero deaths in relation to the implanted grafts, no thromboembolic, hemorrhagic, or infectious complications, however, 6 developed grafts stenosis which was treated successfully [38]. Despite the encouraging results with BM-MNCs, BM-MSCs still present more advantages. These advantages are e.g. their ability to differentiate into a variety of cell types even progenitor cells; relatively easy procedure for their collection, isolation, storage, and proliferation; presenting a similar phenotype to the valve cells; present anti-thrombogenic properties; and their immunogenicity is manageable [53, 114]. In a comparative study, Vincentelli et al. examined short- and long-term characteristics of the porcine decellularized scaffold which were processed with in-situ injections of BM-MNCs and BM-MSCs, before transplanting in lamb of animal models [140]. Short-term results did not show any

significant differences. However, the 4 months (long-term) follow up has shown a significant decrease of transvalvular and distal gradients, more inflammatory reaction, more structural deterioration as well as calcification, and a thick fibrous pannus around the suture line in the BM-MNCs group. These observations in the BM-MNCs group were significantly different from the BM-MSCs group [140].

Table 2: Scaffolds and SCs used for TE in animal models, bioreactors and humans

5.4.2 Cardiac Progenitor Cells

Cardiac progenitor cells (CPCs) or also known as cardiac resident stem cells are a type of cells which are found in the adult heart and express surface antigen c-kit^{+}, a factor which also identified in the fetal and neonatal myocardium [15]. CPCs are proliferating and have multipotent characteristics. They can also differentiate into all three different cardiogenic cell types which are cardiomyocytes, smooth muscle cells and epithelial cells [15]. CPCs has only been in the spotlight for about 15 years now. Beltrami and colleagues confirmed the existence of CPCs which can differentiate into myocytes, smooth muscle, and endothelial vascular cells [15]. Earlier, the limitations on regenerative ability of the heart turned the attention to alternative sources of cells [141]. Apparently, the number of CPCs is significantly higher in neonates but dramatically decreases after the age of 2 [30, 142]. This information, in combination with a suitable scaffold, could be the answer to treat a number of CHD including HLHS, as CPCs could be collected during palliative surgery or even before that via endomyocardial biopsy [17,30].

The possible therapeutic approaches using the discussed SCs and biomaterials in CHDs are represented in the figure 1 below.

Figure 1: Promising strategies for CHDs treatment.

Figure 1. The schematic diagram represents the potential of SCs and TE for corrective surgical treatment of infants as well as adolescent CHD patients. Various sources for SCs are presented here as alternatives to

harvesting the appropriate SCs which can be used to seed on clinically certified biomaterial scaffolds for reconstructing functional cardiac tissue-engineered grafts. These grafts will be implanted via the corrective surgery into the heart of infants and adolescence CHD patients for definitive correction of cardiac defects. This optimized cardiac tissue engineered grafts should have a potential to grow in parallel with the child growth, while are lacking any tumorigenicity, immunogenicity, thrombogenicity, calcification, and other risk factors.

7.0 STANDPOINT

Due to the high number of patients as well as newborns who are suffering from CHD and also high costs of their implications, it is necessary and vital to making intensive research for finding an effective treatment for CHD patients. Stem cell research has shown remarkable results in all kinds of tissue engineering including skin [146], cartilage [147], vascular [148], ocular [149] and cardiac [46, 51, 143].

The importance of engineered cardiac tissue lays in the fact that synthetic non-degradable materials cannot adjust to the patient's developing body. A number of patients who suffer from CHD are adults, and they are more suitable for this type of therapies. However, many patients with severe CHD are infants and children whose body is constantly developing. Although, various synthetic and natural biodegradable biomaterials have been used so far which have shown with good results, the one with the best degradation rate is yet to be found. There are various complications related to existing surgical treatments and scaffolds which cannot be ignored. Calcification, inflammatory reaction and life-long anticoagulants treatment are the most important known complications for the conventional methods of CHD treatment [12, 34]. The complexity of CHD makes TE possibly the most suitable solution for the treatment of patients with CHD.

8.0 CONCLUSION

The replacement or correction of a malformation in a complex system like the cardiovascular system, could only be successful with tissues which can mimic the native heart and vascular tissues. SCs have opened the door to such treatments. The best SCs candidates and biomaterials are yet to be identified, despite the encouraging results. All different types of SCs which have been

researched so far still present some disadvantages. Extensive research would be required to deeper understand, solve drawbacks, and promote SCs use for tissue engineering in the future.

All the efforts channeled at obtaining proper legal regulation for using SCs, developing new technologies for scaffold production as well as scaffoldless techniques, developing faster and safer methods for producing patient-specific iPSCs, and also research into the effectiveness of SCs in TE for treatment of CHD, predicts a very positive future for patients, researchers and surgeons.

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Table 1(on next page)

Advantages and disadvantages of materials and biomaterials used in TE

	Artificial Prosthesis	Biological Prosthesis	Biomaterial Scaffolds	Scaffoldless Tissue
Advantages	• Available in abundance • Many different sizes • Long term results available	• Available in abundance • No requirement for life-long treatment with anticoagulants	• Good mechanical properties • Ultrastructural features • Cell adherence • Biocompatibility • Biodegradable • 3D-priting allows any shape and size	• No need for scaffold • Spontaneous and synchronous pulsation • Could create tubular construct • Can grow with host
Disadvantages	• Impact & friction wear • Inflammation • Calcification of valve • Less flexible than natural tissue • Life-long treatment with anticoagulants • Do not grow with the patients' heart	• Risk of cytotoxicity • Inflammation • Loss of mechanical properties • Calcification in infants and children • Immunological reactions	• Some present toxicity • Risk of inflammation • Not all tune well with heart	• Limited number of cell sheets (max 3) • Poor vascularisation in more than 3 cell-sheets

1

Table 2(on next page)

Scaffolds and SCs used for TE in animal models, bioreactors and humans

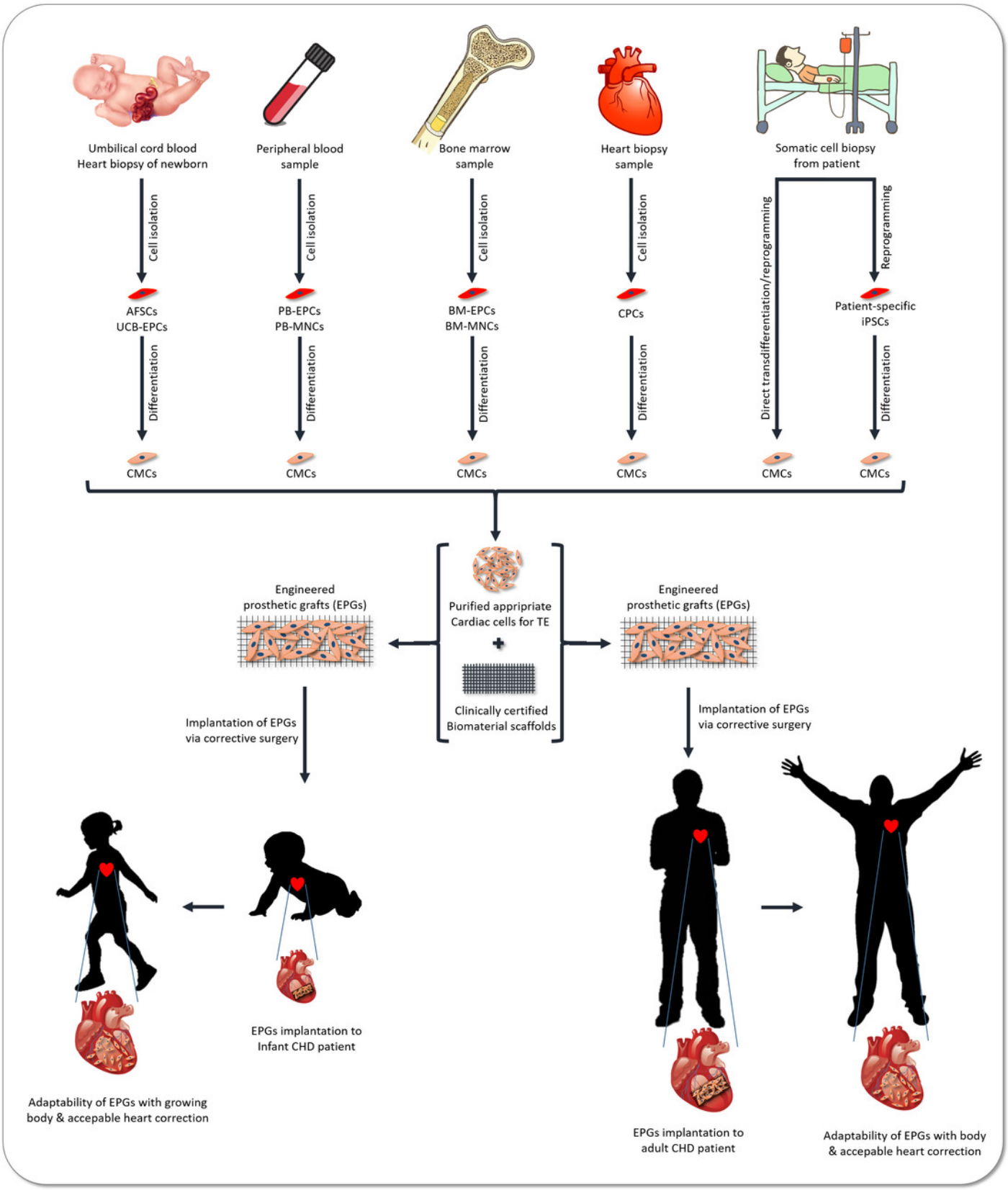
Engineered Tissue	**Scaffold**	**Type of SCs**	**Animal models/ Humans/Bioreactors**	**Reference**
Heart valve	Synthetic biodegradable non-woven PGA mesh	Human Chorionic villi-derived cells & hCB-EPCs	Culture in bioreactor	[128]
Heart valve	Synthetic biodegradable	hAFSCs	Culture in bioreactor	[130]
Vascular grafts	Various synthetic biodegradable	Human Umbilical CB-EPCs	Static conditions and biomimetic flow system	[129]
Vascular grafts	biodegradable non-woven PGA	BM-MNCs	Mice	[138]
Heart valve	porcine decellularised scaffold	BM-MSCs & BM-MSCs	Lambs	[140]
Vascular Grafts	Biodegradable PLA & PGA	BM-MNCs	Humans	[38]

1

Figure 1

Promising strategies for CHDs treatment.

The schematic diagram represents the potential of SCs and TE for corrective surgical treatment of infants as well as adolescent CHD patients. Various sources for SCs are presented here as alternatives to harvesting the appropriate SCs which can be used to seed on clinically certified biomaterial scaffolds for reconstructing functional cardiac tissue-engineered grafts. These grafts will be implanted via the corrective surgery into the heart of infants and adolescence CHD patients for definitive correction of cardiac defects. This optimized cardiac-tissue engineered grafts should have a potential to grow in parallel with the child growth, while are lacking any tumorigenicity, immunogenicity, thrombogenicity, calcification, and other risk factors.


Umbilical cord blood
Heart biopsy of newborn
Peripheral blood sample
Bone marrow sample
Heart biopsy sample
Somatic cell biopsy from patient
Cell isolation
Cell isolation
Cell isolation
Cell isolation
Reprogramming
AFSCs
UCB-EPCs
PB-EPCs
PB-MNCs
BM-EPCs
BM-MNCs
CPCs
Patient-specific iPSCs
Direct transdifferentiation/reprogramming
Differentiation
Differentiation
Differentiation
Differentiation
Differentiation
CMCs
CMCs
CMCs
CMCs
CMCs
CMCs
Engineered prosthetic grafts (EPGs)
Purified appripriate Cardiac cells for TE
Clinically certified Biomaterial scaffolds
Engineered prosthetic grafts (EPGs)
Implantation of EPGs via corrective surgery
Implantation of EPGs via corrective surgery
EPGs implantation to Infant CHD patient
Adaptability of EPGs with growing body & accepable heart correction
EPGs implantation to adult CHD patient
Adaptability of EPGs with body & accepable heart correction