Recent advances and challenges on application of tissue engineering for treatment of 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, 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 developing bodies of infants and children can be very challenging. Nevertheless, the use of acellular scaffolds, allograft, xenografts, and autografts are already very common. Seeding of cells on surface and within scaffold is a key challenging factor for use of the above. The use of different types of stem cells has been investigated and proven to be 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.

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ABSTRACT

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.

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The use of different types of stem cells has been investigated and proven to be 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 because 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 instance, 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 depend on the geographical area of investigation [6, 7], table 1 further mentions other CHD frequencies for other countries. CHDs not only have an effect on the individual's and their family's life but also, have a huge financial impact on 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 the total cost for CHD treatment in 2008 was approximately $298 billion [9]. In general, the number of children and adults being diagnosed with CHD 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 surgery, 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 to the native cardiac tissue in both structure and function. In structure, 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 Tissue Engineering (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. TE refers to creation of functional three-dimensional tissue using biomaterials and cells for replacement or restoration of damaged organs and/or parts of them. TE is the most promising approach at the present for CHD, as treatment can be "patient-specific" and the engineered tissue could adjust to the developing body of the recipient. 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 [12]. 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 [13]. 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 [14, 15]. The first artificial heart valve was placed on live patients only in the last century [14, 16]. In 2003, some evidence was presented to support the regenerative ability of the adult heart [17].

This evidence shows the existence of cardiac stem/progenitor cells which can differentiate into new cardiomyocytes and participate in cardiac regeneration [17]. 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 [18]. 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 [19]. However, only a small number of preclinical studies have focused on CHD treatments [20, 21]. Stem cells (SC) have been widely investigated mainly for myocardial infarction (MI), as it is currently the leading cause of morbidity and mortality worldwide [22]. 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[20, 22, 23]. 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 review looks into the congenital heart diseases, biomaterials and scaffolds, and, types of stem cells used in TE.

2.0 METHOD

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

3.0 Congenital Heart Disease: Types, malformations, presentations and interventions

CHD includes a diverse range of conditions which shows a variety of symptoms, indications, and malformations detected during pregnancy or after birth [11].However, these malformations are much influenced by the age of diagnosis [6].The etiology of CHD is unknown, but it is generally accepted that many factors or a combination of them could contribute to CHD and considered to be caused by multifactorial inheritance. These factors could be genetic, epigenetic or environmental factors such as alcohol and drugs consumption, as well as viral infections like Rubella [11]. The severity of the disease varies, and a number of malformations could be present in each case. Based on the severity of CHD, they are categorized to mild, moderate, and severe CHDs, which the latter is subcategorized to Cyanotic and Acyanotic lesions [19]. The most frequent type of severe CHD is Ventricular Septal Defect (VSD) [24]. VSD could cause myocardial defects which disappear in the first year of the infant's life [24]. Nevertheless, the VSD could also cause some malformations which can be managed only by surgical intervention, that is, infant pulmonary hypertension [6, 24].The other CHD type is Atrial Septal Defect (ASD) which is usually asymptomatic and in most of the case will only be diagnosed in adulthood[6]. Atrioventricular septal defects (AVSD) is mainly observed in trisomy 21 [6]. AVSD is usually characterized by "complete AV-canals with one common AV valve for both ventricles and an interatrial and intraventricular communication" and requires surgical correction. The results of

long-term patient follow up after operation have shown very satisfactory survival rate [25]. Another type of CHD, tetralogy of Fallot (ToF), is characterized by VSD, pulmonary stenosis, right ventricular hypertrophy and over-riding of the aorta [26]. Infants who suffer from ToF will require immediate surgical intervention for better survival rates and avoid cyanosis, a result of inadequate pulmonary blood flow [26]. 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 [27]. CAV presents many similarities with arteriosclerosis in adults which is caused by lifestyle or aging [27]. 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 [28, 29]. Table 1 summarizes the frequencies of congenital heart diseases, their presentations and possible management.

Table 1 Frequencies, Presentations, and management of Congenital Heart Diseases

Some types of the CHDs are misdiagnosed, undiagnosed, or diagnosed very late in life which could make a successful treatment challenging [64].

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 [18]. 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. The mortality rate among all patients who are waiting for any type of organ transplantation is highest in infants who wait for heart transplant [65, 66]. CHDs patients usually require medication, cardiac catheterization or a series of surgical interventions throughout their life [11, 66]. This among other risks increases the chances of HLA-sensitization which eventually makes it more difficult to find a cross-match [66]. The main challenge with artificial materials is the alterations in the size and function of the heart from the neonatal period, infanthood, and to adulthood, to which current artificial materials cannot adjust to [11]. The need for early intervention is essential for normal physical and cognitive development [64].

The use of bioprosthetics including allografts and xenografts is widely used for treatment of CHD as they present good survival rates in the patient and fewer interventions throughout their lifetime [65]. The observed symptoms, defects, and complications upon bioprosthetic treatment of CHD patient could differ from case to case [24, 64].

4.0 Heart, a complex organ for tissue engineering

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 [67]. The diastolic and systolic function of the heart is necessary to be synchronized and 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 [67]. 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 must 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 non-structural elements such as proteoglycans, glycosaminoglycans, and glycoproteins [68, 69] among other components. Repairing the heart using materials which do not have or do not comply with the above characteristics and 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 the heart using various engineered techniques has 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 [15]. A few years later, the same type of valve was transplanted into humans [15]. Nonetheless, Hufnagel's valve required changes which were succeeded by Harken-Soroff and later by Starr-Edwards ball valve [15]. Following several improvements, the latest version is made of pure carbon as a lighter, smoother material for blood flow and more durable in comparison to other materials [14]. Due to the position of the valve, there is a high transvascular pressure which leads to 'impact wear' and 'friction wear' [14]. Further complications with the use of valve replacements include inflammation around the prosthesis and calcification of the valve itself [70]. 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 patient [14]. Similarly, to valves, artificial vascular grafts have been used for many years as a surgical treatment for CHD [71]. Nevertheless, they provoke an inflammatory response, and they are much less flexible than the body's natural tissue [64]. Originally porous fabric knitted of Dacron and polytetrafluoroethylene (PTFE) was used for stenosis treatment. Later alterations in the porosity of fabric were introduced to prevent the material's corruption [64]. 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" [64]. However, future attempts to produce successful results created many doubts about the actual function of cells following citation on clot [64]. 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 [64, 72].

4.2 Bioprosthetics: Allograft, Xenografts, and Autografts

Allograft heart valves and arterial grafts are collected from deceased humans. In contrast, xenografts are harvested from porcine and bovine animals including heart valves and carotid arteries [14, 16, 70]. Allografts and xenografts came into the picture as an alternative to artificial valves and conduits. Their main advantage is that they do not require lifetime treatment with anticoagulants [14, 73, 74]. Animal tissue is treated with glutaraldehyde [73]. Glutaraldehyde is a five-carbon bifunctional aldehyde used to stabilize tissue to protect from chemical and enzymatic degradation and maintain "its mechanical integrity and natural compliance" [73]. Also, treatment is necessary to reduce immunogenicity of the xenograft by decellularization and sterilization of the tissue [73]. Like xenograft, allografts also need treatment before transplantation and can even be cryopreserved [65]. Despite the great advantage, a number of complications are related to bioprosthetic grafts related to their preparation [73].

The risk of cytotoxicity leading to inflammation, as well as the partial loss of mechanical properties of tissue, have been reported [73]. Moreover, calcification is often observed in infants and children with bioprosthetics. Many efforts are being made to find out an alternative treatment for heart bioprosthetics therapy. However, there is a controversy regarding their efficiency [73].

In a study carried out by Homann and colleagues, the outcomes of 25 years using allografts and xenografts for reconstruction of the right ventricular outflow tract showed 66% survival at 10-years' follow up. Furthermore, patients with allografts had a mean reoperation-free interval time of 16 years in contrast to the xenograft recipients which this interval time is 10.3 years [65]. Allografts may present better outcomes, but they are not in abundance like xenografts. Therefore,

241 many studies are concentrating on the development of tissue valves and vascular grafts created
242 by stem cell-seeded on artificial or natural scaffold [16].

243 The relatively recent "CorMatrix" patch fabricated from the decellularized porcine small
244 intestinal submucosa extracellular matrix (SIS-ECM) mainly composed of collagen, elastin,
245 glycan, and glycoproteins have been introduced into cardiac surgery. SIS-ECM has not only
246 been used in animal models for cardiac surgery[65-77], but also in humans studies, for
247 cardiac and vascular reconstructions such as; augmentation of the tricuspid valve [78], vascular
248 repair of ascending aorta, aortic arch, right ventricular outflow tract, pulmonary artery, valvular
249 reconstruction [79], and closure of septal defects [80]. The study by Witt et al. reported a small
250 risk of stenosis when SIS-ECM is used in the reconstruction of the outflow tracts and great
251 vessels. Interestingly, the SIS-ECM has effectively proved the function in the high-pressure
252 vessels [80]. The pitfall of this study, however, was the short follow up period.

253 A very common surgical practice for CHD is the use of pericardial patches for repairing the
254 septal defect [64]. The autologous pericardium is the best choice for infants as it is free, it does
255 not provoke any immune-response, and it is sterile. Even though it requires some preparation
256 before application, autologous pericardium creates less fibrotic tissue in comparison to Dacron
257 [64]. Allograft pericardium is available, but quite a few risk factors are associated with its use
258 [64]

259

260 **4.3 Biomaterials and Scaffolds for tissue engineering**

261 Generally, scaffolds work as a primary base for cells to enhance and produce relevant tissue. The
262 scaffolds should have specific morphological, functional, and mechanical properties to support
263 cells survival and differentiation [23]. Biomaterials used to produce scaffolds should be made of
264 components which will accommodate the above characteristics and create a friendly cell
265 microenvironment [23]. The previously mentioned characteristics apply for all different types of
266 engineered tissue, and the goal is to mimic host tissue in the best possible way. With regards to
267 artificial and bioprosthetic cardiac correction choices, scaffolds and biomaterials should contain
268 various properties such as being biodegradable, biocompatible, flexible and durable and absence
269 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 [74]. 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 [81, 82]. Furthermore, these biomaterials scaffolds should be bioactive, meaning they should enhance cellularization in vitro and in vivo, and optimize cell efficiency and degrade at a desirable rate [82]. 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 [83]. The most common biomaterials for cardiac and vascular TE used today are synthetic, and natural polymers [67], and the electrospinning technique has been proven to be the most efficient way to produce scaffolds with these biomaterials [84].

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 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 [85]. Many synthetic polymers are biocompatible and have excellent mechanical properties which make them a popular choice for sutures and mesh production [67]. 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 [86]. Carrier et al. Presented acceptable ultrastructural features and metabolic cell ability when cells were cultured on PGA scaffold in a rotating bioreactor [87, 88]. The rotating bioreactor increases cell adherence and decreases cell damage [87]. The highest concern with synthetic polymers is their toxicity. Therefore, the use of poly-L-lactic acid (PLLA) has increasingly become more of interest. PLLA has demonstrated very good results when combined with bone marrow mesenchymal SC (BM-MSC) for vascular tissue engineering [89]. In vivo studies by Hashi et al showed that nanofibrous scaffolds created with PLLA can remodel in both cellular and ECM content, similar to that of the native artery [89]. Both acellular and were implanted into the common carotid artery of live animal models (rats). The cellular scaffold was cited with MSC and “exhibited very little platelet aggregation on

the luminal surfaces" compared to the acellular grafts. This is due to the antithrombogenic property of MSC [89]. PLLA, when degraded in the body, can "be excreted in carbon dioxide and water" [67]. 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 [90, 91], cellulose [92], urea [93], PLLA [94]. 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 [95, 96] PLLA and collagen [97], polypyrrole and gelatin [98], polyglycolic acid [99], poly (hydroxymethyl glycolide) [100], chitosan and gelatin [101].

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 [102]. 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 [67].

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 [103] The natural polymers used so far for cardiac repair include collagen, gelatin, alginate, silk, fibrin, chitosan and hyaluronic acid[67]. 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" [67].

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 [101]. Several kinds of literature have reported the use of various collagen types and their modifications in cardiac tissue repair [104-111].

Fibrin can be manipulated to create gels, microbeads, and hydrogels [85]. Likewise, biological molecules like the growth factors can be incorporated [112]. Fibrin glue can be used as a stand-alone therapy in cardiac tissue repair as it possesses intrinsic regenerative properties [113]. The success of fibrin patch seeded with human embryonic stem cell-derived cardiac progenitor cells (hESC-CPC) in non-human primate model [114] 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 [115]. Other studies on fibrin have demonstrated its efficacy as a sealant after intramyocardial injection [116]; for myocardial tissue repair when seeded with adult stem cells, neonatal cardiac cell, and mesenchymal stem cells [117-119]; to form aortic valves in tissue engineering [120].

Chitosan has been experimented in several literature as a biomaterial for cardiac regeneration [121, 122]. 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 [123].

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 repair of the cardiac tissue [124-127].

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 [128-131].

Evidence of gelatin scaffold placed subcutaneously, and/or on infarcted myocardium in adult rat hearts have shown a good survival of the graft, vessel formation and junctions with recipient rat heart cells [132]. Gelatin was reported to sustain neonatal rat cardiomyocyte tissue in vitro for three weeks [133]; supported the growth of human induced pluripotent stem cell (iPSC)-derived cardiomyocytes [133]; its hydrogel seeded with autologous human cardiac-derived stem cell and basic fibroblast growth factor (bFGF) effectively released bFGF for repair of ischemic cardiomyopathy [134]; and several other studies have shown the efficacy of gelatin as a scaffold for cardiomyogenesis when seeded with cells [135-138]. 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 [139]. Hence, this biomaterial may be used in reconstruction of the low-pressure pulmonary vessels during a cardiac surgery for a total anomalous pulmonary venous return or transposition of the great vessels.

Nevertheless, not all of these polymers can tune well for cardiac TE, and risk of inflammation still exists [140].

4.3.3 Native Extracellular Matrix as scaffold

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 [141]. Studies have shown that the ratio of native ECM in culture could play a key role in stem cells (SCs) enhancement, differentiation, survival, and phenotype [142]. Other studies have shown that contractible engineered heart patches cultured in ECM mixture and implanted in syngeneic Fischer 344 rats can vascularize, become innervated and survive up to 8 weeks in vivo [141].

Furthermore, ECM of decellularized and repopulated hearts and other organs are being used for drug testing [143]. 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 [144]. It was concluded that heart "ECM could promote proliferation, specific cell differentiation and myofilament formation" [144]. The option of ECM for TE could help overcome the challenges faced using synthetic and other natural biomaterials to replace tissue, valves or organs [145].

4.4 Scaffoldless Cell Sheet

Another technique, which is independent of scaffolds, has been developed based on the cells' ability to connect via cell-to-cell junction proteins and create ECM [88]. The cells are cultured in normal conditions at 37°C in a temperature-responsive polymer cultures dishes. When the

384 culture temperature conditions change, the cells detach from the polymer culture dish as one cell
385 sheet [88]. This technique was developed to avoid inflammatory reactions and fibrotic deposits
386 in the area of graft where scaffold was placed following degradation [88]. A study has shown
387 that contractile chick cardiomyocyte sheets could function effectively around rat thoracic aorta
388 when applied on host myocardium [81]. This cell sheet could synchronize within 1 hour of
389 implantation with the host tissue [81]. Similar results have been shown in 3D structures using
390 several cell sheets aiming to create a thick cardiac patch [146]. The number of sheets is limited,
391 as more than three exhibits poor vascularization. However, the combination of endothelial cells
392 and cardiomyocytes is being examined to promote vascularization before implantation [88].
393 Table 2 summarizes the pros and cons of the various materials and biomaterials used in tissue
394 engineering.

395

396 ***Table 2 Advantages and disadvantages of materials and biomaterials used in TE***

397

398

399 **5.0 Stem Cells for tissue engineering**

400 An equally important point in choosing a suitable biomaterial, scaffold or scaffoldless cell sheet,
401 is the choice of the most appropriate cell types suitable for the TE. Stem cells (SCs) as a known
402 cell source possesses the ability to differentiate toward Cardiac Muscle Cells/cardiomyocytes
403 (CMCs), smooth muscle cells (SMCs) and endothelial cells (ECs), can regenerate cardiac tissue.
404 Based on these properties, they play a key role in TE field. The currently used SCs in TE are
405 summarized in Table 3.

406 **5.1 Embryonic Stem Cells**

407 Embryonic stem cells (ESCs) are one of the cell sources which are used in TE approaches. ESCs
408 are derived from the inner cell mass of preimplantation blastocyst [147]. They can differentiate
409 into all different cell types of three germ layers. Human ESC (hESC) could be a good candidate
410 for cardiac tissue engineering. In a study conducted by Landry et al., hESC-derived
411 cardiomyocytes (hESC-CMC) showed very good phenotype including myofibril alignment,
412 density, morphology, contractile performance and gene expression profile which, however, was
413 only confirmed after 80-120 days in vitro culture [148]. Various groups apart from Landry and
414 colleagues conducted studies to confirm the successful differentiation of ESC to cardiomyocytes
415 as presented in a review by Boheler and colleagues in 2002 [149]. Duan et al. investigated how
416 native cardiac ECM could affect hESC differentiation. This group processed porcine hearts to
417 collect digested cardiac ECM which then mixed with collagen to create a hydrogel for cell
418 cultivation purposes. The cultured hESCs on biomaterials comprised of 75% native cardiac
419 porcine ECM and 25% hydrogel with no additional growth factors have shown a great
420 differentiation with cardiac troponin T expression and contractile behavior, compared to the
421 hydrogel with a smaller ratio of native ECM [142]. Based on various studies, ESCs could be a
422 good option for cardiac TE [141]. Additionally, some factors such as ethical concerns, provoked
423 immunogenicity, and risk of tumorigenesis make the ESCs a very controversial choice of cell
424 source for TE [74].

425

426 **5.2 Induced Pluripotent Stem Cells**

427 Another type of SCs which are used in TE is induced pluripotent stem cells (iPSCs). The iPSCs
428 are somatic cells which are reprogrammed to behave like ESC and show the same properties.
429 The iPSCs can differentiate into all three germ layers [150, 151]. Takahashi and his group were
430 the first groups who were able to reprogram the somatic adult cells like fibroblasts to iPSCs
431 using viral vectors to introduce four key factors OCT4, SOX2, c-Myc, and KFL-4 to fibroblasts
432 [150]. This method was used to reprogram fibroblasts to embryonic-like cells and from this state
433 differentiate them into a relevant type of cells [150]. Ludry et al. have also shown that human
434 iPSC-derived cardiomyocytes (hiPSC-CMCs) present the same characteristics as hESC-derived
435 cardiomyocytes in long-term in vitro culture [148]. Lu and colleagues presented the successful
436 repopulation of decellularized cadaveric mouse heart with hiPSC-derived multipotential
437 cardiovascular progenitors [148]. They also demonstrate that the heart ECM promotes
438 proliferation, differentiation and myofilament formation of CMs from the repopulated hiPSC-
439 derived cells. Furthermore, they have checked the electrical coupling of these cells and also
440 examined the constructive ability of repopulated heart using electrocardiogram which
441 presented arrhythmia. Lu et al. have further examined the effects of pharmacological agents on
442 repopulated heart and observed remarkable responses [141]. This model is explored as an option
443 to personalized medicine concerning drug testing/discovery [141, 152]. Specifically, for CHD
444 which presents such a variety of profiles, individual patient-specific human models development
445 could help to understand how each patient would respond to existing pharmaceutical treatments.
446 Even though it may not be possible to create actual organ heart models with individual clinical
447 features of the disease [153].

448 Nonetheless, like ESC, iPSC has demonstrated tumorigenesis [74]. The group of Leda and
449 colleagues showed a direct transdifferentiation of fibroblasts to functional cardiomyocytes
450 using three key factors, Gata4, Mef2c, and Tbx5, within a very short time and suggested that
451 direct reprogramming could reduce the risk of tumorigenesis [154]. Still, using viral vectors for
452 reprogramming procedure is problematic and involves various risks [74]. Therefore, today more
453 different ways for iPSC production are being used and investigated to find out safer and more
454 effective alternatives for this reprogramming procedure [21, 155]. In the event this problem is

455 solved, iPSC could be the safest type of cell sources for TE as they will not provoke any
456 immune-response and cell harvesting procedure to produce iPSCs is not life-threatening for the
457 patients. Moreover, iPSC raises less ethical concerns in comparison to ESC or fetal SC.

458

459 **5.3 Prenatal, Perinatal, and Postnatal Stem Cells**

460 Prenatal, perinatal and postnatal SCs are other cell sources used in TE, which include chorionic
461 villi derived multipotent SCs, amniotic fluid-derived SCs (AFSCs), umbilical cord blood
462 derived-endothelial progenitor cells (UCB-EPCs) and umbilical cord- or cord blood-derived-
463 multipotent SCs [156]. UCB progenitors, like endothelial progenitor cells (EPCs), have
464 distinctive proliferative properties in comparison to other cells sources [157]. This category of
465 SCs is exceptionally important as the child's own SCs could be used for heart TE, for CHD
466 patients who are diagnosed before birth. Immunogenicity or an additional procedure to harvest
467 autologous SCs from the infant or child could be avoided in this procedure. Furthermore, it has
468 been proven that AFSCs do not form teratomas in contrast to ESCs and iPSCs [158]. All
469 categories of these cells have been investigated with remarkable results on engineered valves and
470 vascular grafts [156, 159-163]. This type of SCs is not applicable to adults who have been
471 diagnosed later in life with CHD.

472

473 **5.4 Adult Stem Cells**

474 **5.4.1 Bone Marrow-derived Stem cells**

475 Apart from UCB, EPCs can be found in the peripheral blood (PB-EPCs) and bone marrow (BM-
476 EPCs) of adults [164,165]. However, bone marrow is a richer source of EPCs in comparison to
477 peripheral blood. In 1997, Asahara et al. identified the CD34+ mononuclear hematopoietic
478 progenitor cells in the peripheral blood which in vitro presented endothelial-like characteristics
479 [164]. The EPCs which are originated from BM considered to play a crucial role in endothelial
480 repair, and they have been suggested for treatment of ischemia patients and vascular TE with
481 very encouraging results [158,160,165,166]. A successful complete endothelium regeneration of

decellularized canine carotid arteries has been reported in animal studies using PB-EPCs [167]. In addition to vascular TE, the EPCs have been assessed for tissue-engineered heart valves [168].

Bone marrow derived EPCs are greatly involved in de novo vessel formation and neovascularization in pathological conditions like ischemia and cancer [164]. 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 [169]. 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 it was fully accommodated by the host's cells [169]. Nonetheless, the host mice were at full-growth which makes it questionable how successful this application could 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 [165]. More studies have reported similar results with the same conclusion [74,170]. Furthermore, an investigation on 25 young patients under 30 years old who underwent extracardiac total cardiopulmonary connection with BM-MNCs engineered vascular graft has also presented convincing results. A long-term patient follow-up has shown zero deaths in relation to the implanted grafts, no thromboembolic, hemorrhagic, or infectious complications, however, 6 of them developed grafts stenosis which was treated successfully [74]. Despite the encouraging results with BM-MNCs, BM-MSCs still present more advantages. These advantages are, for example, 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 [89, 145]. 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 a lamb of animal models [171]. 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

513 the suture line in the BM- MNCs group. These observations in the BM-MNCs group were
514 significantly different from the BM-MSCs group [171].

515

516 **5.4.2 Cardiac Progenitor Cells**

517 Cardiac progenitor cells (CPCs) or also known as cardiac resident stem cells are a type of cells
518 which are found in the adult heart and express surface antigen c-kit+, Sca-1+, PDGFr-alpha+,
519 CD31-, CD34+, CD45-, and tryptase [17]. CPCs are self-renewing and have multipotent
520 characteristics; differentiating into all three different cardiogenic cell types which are
521 cardiomyocytes, smooth muscle cells and endothelial cells [17]. CPCs has only been in the
522 spotlight for about 15 years now. Beltrami and colleagues confirmed the existence of CPCs
523 which can differentiate into myocytes, smooth muscle, and endothelial vascular cells [17].
524 Earlier, the limitations on regenerative ability of the heart turned the attention to alternative
525 sources of cells [172]. Apparently, the number of CPCs is significantly higher in neonates but
526 dramatically decreases after the age of 2 [71, 173]. This information, in combination with a
527 suitable scaffold, could be the answer to treat a number of CHD including HLHS, as CPCs could
528 be collected during palliative surgery or even before that via endomyocardial biopsy [19,64].

529 The potential SCs and biomaterials for TE in CHDs are represented in the figure 1 below. Also,
530 the figure 2 below shows the promising strategies for the treatment of CHDs.

531

532 ***Table 3 Scaffolds and SCs used for TE in some study models***

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541 **Figure 1:** This schema represents the different types of stems that can be used on the biomaterial
542 backbone (depicted as the background characters) for cardiovascular Tissue Engineering (TE).
543 A) Induced pluripotent stem cells (iPSCs) derived from fibroblast. B) Prenatal, Perinatal, and
544 Postnatal Stem cells (PPPSCs) are derived from amniotic fluid, umbilical cord, and chorionic
545 villi. C) Bone Marrow Stem Cells (BMSCs) such endothelial progenitor cells (EPCs) and
546 Mesenchymal stem cells (MSCs) can easily be isolated from the bone marrow. D) Cardiac
547 progenitor cells (CPCs) can be harvested during palliative surgery or endomyocardial biopsy. E)
548 Embryonic stem cells (ESCs) derived from the inner cell mass of the blastocyst

549

Figure 2: Promising strategies for CHDs treatment

Figure 2 : The schematic diagram represents the potential of Stem Cells (SCs) and Tissue Engineering (TE) for corrective surgical treatment of infants as well as adolescent patients with Congenital Heart Disease (CHD). Various sources for Stem Cells (SCs) are presented here as alternatives to harvesting the appropriate Stem Cells (SCs) which can be used to seed on clinically certified biomaterial scaffolds for reconstructing functional cardiac tissue- engineered grafts. These grafts could be implanted via the corrective surgery into the heart of infants and adolescent patients with Congenital Heart Disease (CHD) for definitive correction of cardiac defects. This optimized cardiac-tissue engineered grafts should have potential to grow in parallel with the child, 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 [174], cartilage [175], vascular [176], ocular [177] and cardiac tissues [82, 87, 178, 179].

The importance of engineered cardiac tissue lies 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 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

578 important known complications for the conventional methods of CHD treatment [14, 70]. The
579 complexity of CHD makes TE possibly the most suitable solution for treatment of patients with
580 CHD.

581

582

583 **8.0 CONCLUSION**

584 The replacement or correction of a malformation in a complex system like the cardiovascular
585 system could only be successful with tissues which can mimic the native heart and vascular
586 tissues. SCs have opened the door to such treatments. The best SC candidates and biomaterials
587 are yet to be identified, despite the encouraging results. All different types of SCs which have
588 been investigated so far still present some disadvantages. Extensive research would be required
589 to enable deeper understanding, solve drawbacks, and promote SCs use for tissue engineering in
590 the future. All the efforts channeled at obtaining proper legal regulation for using SCs,
591 developing new technologies for scaffold production as well as scaffoldless techniques,
592 developing faster and safer methods for producing patient-specific iPSCs, and research into the
593 effectiveness of SCs in TE for treatment of CHD, predicts a very positive future for patients,
594 researchers and surgeons.

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596

597

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606

607 **Reference**

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609 1. *National Congenital Heart Disease Audit Report 2012-2015.* (2016) London: National Institute for
610 Cardiovascular Outcomes Research. Retrieved from;
611 https://nicor5.nicor.org.uk/chd/an_paeds.nsf/vwContent/home?Opendocument

612 2. *British Heart Foundation*. (2016). Retrieved from: www.bhf.org.uk: https://www.bhf.org.uk/research/heart-
613 statistics

614 3. Okudo M, Benson LD. (2001) Intravascular and intracardiac stents used in congenital heart disease. *Current*
615 *Opinions in Cardiology*, 16(2): 84-91.

616 4. Gilboa SM, Devine OJ, Kucik JE, Oster ME, Riehle-Colarusso T, Nembhard WN, Xu P, Correa A,
617 Jenkins K, Marelli AJ. (2016) Congenital Heart Defects in the United States: Estimating the
618 Magnitude of the Affected Population in 2010. *Circulation* 134(2):101-9. doi: 608
619 10.1161/CIRCULATIONAHA.115.019307.

620 5. Dolk H, Loane M, Garne E. (2011). Congenital Heart Defects in Europe: Prevalence and Perinatal Mortality
621 2000-2005. *Circulation* 123(8):841-9. doi:10.1161/CIRCULATIONAHA.110.958405

622 6. Hoffman JI, Kaplan S. (2002). The incidence of congenital heart disease. *Journal of the American*
623 *College of Cardiology*, 39 (12): 1890-1900, https://doi.org/10.1016/S0735-1097(02)01886-7

624 7. van der Linde D, Konings EE, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJ, Roos-
625 Hesselink JW. (2011) Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic
626 Review and Meta-Analysis, *Journal of the American College of Cardiology*, 58(21): 2241-
627 2247. https://doi.org/10.1016/j.jacc.2011.08.025

628 8. NHS England. (2015). *Proposed National Standards and Service Specifications for Congenital*
629 *Heart Disease Services: Financial Impact Analysis.* London: NHS England.
630 https://www.england.nhs.uk/wp-content/uploads/2015/06/chd-finance.pdf

631 9. Lundberg, M. (2013) Cardiovascular Tissue Engineering Research Support at the National Heart,
632 Lung, and Blood Institute. *Circulation Research*, 112(8): 1097-1103.
633 doi:10.1161/CIRCRESAHA.112.300638

634 10. Nayak K, Chandra G S N, Shetty R, Narayan PK. (2016) Evaluation of fetal echocardiography as a
635 routine antenatal screening tool for detection of congenital heart disease. *Cardiovascular*
636 *Diagnosis and Therapy*, 6(1): 44-49. doi:10.3978/j.issn.2223-3652.2015.12.01

637 11. Sun R., Liu M., Lu L., Zheng Y., Zhang P. (2015) Congenital Heart Disease: Causes, Diagnosis,
638 Symptoms, and Treatments. *Cell Biochemistry and Biophysics*, 72(3): 857–860.

639

640 12. Durant, M. (2018). *The Ethiopian's Leg : Saint Lucy*. [online] Saint-lucy.com. Available at: https://saint-
641 lucy.com/slider/the-ethiopians-leg/ [Accessed 7 Jul. 2018].

642 13. Finch, J. (2011). The ancient origins of prosthetic medicine. *The Lancet*, 377(9765), pp.548-549.

643 14. Zilla P, Brink J, Human P, Bezuidenhout D. (2008) Prosthetic Heart Valves: Catering for the few. *Biomaterials*,

644 29(4): 385-406.

645 15. Gott VL, Alejo DE, Cameron DE. (2003) Mechanical heart valves: 50 years of evolution. *The Annals of*
646 *Thoracic Surgery*, 76(6): S2230-S2239.

647 16. Sun R, Liu M, Lu L, Zheng Y, Zhang P. (2003) Thoracic Surgery Directors Association Award.
648 Bone marrow as a cell source for tissue engineering heart valves. *The Annals of Thoracic Surgery*,
649 75(3): 761-767.

650 17. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E,
651 Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. (2003) Adult Cardiac Stem Cells Are
652 Multipotent and Support Myocardial Regeneration. *Cell*, 114(6): 763–776.
653 https://doi.org/10.1016/S0092-8674(03)00687-1

654 18. Rupp S, Schranz D. (2015) Cardiac Regeneration in Children. *Pediatric Cardiology*, 36(4): 713-718.

655 19. Saxena, A. K. (2010) Congenital Anomalies of Soft Tissues: Birth Defects Depending on Tissue
656 Engineering Solutions and Present Advances in Regenerative Medicine. *Tissue Engineering*,
657 16(5): 455-466. doi: 10.1089/ten.TEB.2009.0700.

658 20. Ebert AD, Diecke S, Chen IY, Wu JC. (2015) Reprogramming and transdifferentiation for
659 cardiovascular development and regenerative medicine: where do we stand? *Molecular Medicine*,
660 7(9), 1090-1103. doi: 10.15252/emmm.201504395.

661 21. Tarui S, Sano S, and Oh H. (2014) Stem cell therapies in patients with single ventricle physiology.

662 *Methodist Debakey Cardiovascular Journal*, 10(2): 77-81.

663 22. Fakoya AOJ. (2017) New Delivery Systems of Stem Cells for Vascular Regeneration in Ischemia.

664 *Frontiers in Cardiovascular Medicine,* 4:7. doi: 10.3389/fcvm.2017.00007.

665 23. Feric NT, Radisic M. (2016) Strategies and Challenges to Myocardial Replacement Therapy.

666 *Stem Cells Transplantation Medicine*, 17(6): 410-416. doi: 10.3390/ijms17060982.

667 24. Penny DJ, Vick GW (2011) Ventricular Septal Defect. *The Lancet*, 377(9771):1103-12. doi:

668 10.1016/S0140-6736(10)61339-6.

669 25. Boening A, Scheewe J, Heine K, Hedderich J, Regensburger D, Kramer HH, Cremer J. (2002)
670 Long-term results after surgical correction of atrioventricular septal defects. *European Journal of*
671 *Cardio-Thoracic Surgery*, 22(2), p167-73.

672 26. Apitz C, Webb GD, Redington A. (2009) Tetralogy of Fallot, .*The Lancet,* 374(9699), 1462–1471,
673 https://doi.org/10.1016/S0140-6736(09)60657-7

674 27. Freeman R, Otto C. (2005) Spectrum of Calcific Aortic Valve Disease Pathogenesis, Disease
675 Progression, and Treatment Strategies. *Circulation*, 111(24), 3316-3326. doi:
676 10.1161/CIRCULATIONAHA.104.486738

677 28. Tanya Smith, Cha Rajakaruna, Massimo Caputo, and Costanza Emanueli. (2015) MicroRNAs in
678 congenital heart disease. *Annals of Translational Medicine* 21: 333, doi: 10.3978/j.issn.2305-
679 5839.2015.12.25

680 29. *Medscape*. (2015, August 19). Retrieved from http://emedicine.medscape.com/article/894095-

overview.

30. Pedra, C., Haddad, J., Pedra, S., Peirone, A., Pilla, C. and Marin-Neto, J. (2009). Paediatric and congenital heart disease in South America: an overview. *Heart*, 95(17), pp.1385-1392.

31. CDC (2018). *Atrial Septal Defect Facts | Congenital Heart Defects | NCBDDD | CDC*. [online] Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/ncbddd/heartdefects/atrialseptaldefect.html [Accessed 29 May 2018].

32. MayoClinic (2018). *Atrial septal defect (ASD) - Diagnosis and treatment - Mayo Clinic*. [online] Mayoclinic.org. Available at: https://www.mayoclinic.org/diseases-conditions/atrial-septal-defect/diagnosis-treatment/drc-20369720 [Accessed 25 Jan. 2018].

33. Krasuski, R. and Bashore, T. (2016). Congenital Heart Disease Epidemiology in the United States. *Circulation*, 134(2), pp.110-113.

34. van der Linde, D., Konings, E., Slager, M., Witsenburg, M., Helbing, W., Takkenberg, J. and Roos-Hesselink, J. (2011). Birth Prevalence of Congenital Heart Disease Worldwide. *Journal of the American College of Cardiology*, 58(21), pp.2241-2247.

35. NHS (2018). *Symptoms*. [online] nhs.uk. Available at: https://www.nhs.uk/conditions/congenital-heart-disease/symptoms/ [Accessed 5 Jun. 2015].

36. Calderón-Colmenero, J., Cervantes-Salazar, J., Curi-Curi, P. and Ramírez-Marroquín, S. (2013). Congenital Heart Disease in Mexico. *World Journal for Pediatric and Congenital Heart Surgery*, 4(2), pp.165-171.

37. HeartKids (2018). *Congenital Heart Disease - Heartkids site*. [online] Heartkids.org.au. Available at: https://www.heartkids.org.au/congenital-heart-disease [Accessed 5 Jun. 2011].

38. Postoev, V., Talykova, L. and Vaktskjold, A. (2014). Epidemiology of cardiovascular malformations among newborns in Monchegorsk (north-west Russia): a register-based study. *Journal of Public Health Research*, 3(2):270.

39. Patnana, S. and Turner, D. (2018). *Pediatric Hypoplastic Left Heart Syndrome: Background,Pathophysiology, Etiology*. [online] Emedicine.medscape.com. Available at: https://emedicine.medscape.com/article/890196-overview [Accessed 7 Jan. 2017].

40. van der Linde, D., Konings, E., Slager, M., Witsenburg, M., Helbing, W., Takkenberg, J. and Roos-Hesselink, J. (2011). Birth Prevalence of Congenital Heart Disease Worldwide. *Journal of the American College of Cardiology*, 58(21), pp.2241-2247.

41. Zühlke, L., Mirabel, M. and Marijon, E. (2013). Congenital heart disease and rheumatic heart disease in Africa: recent advances and current priorities. *Heart*, 99(21), pp.1554-1561.

42. Canadian Heart Alliance (2018). *Facts and issues*. [online] Canadian Congenital Heart Alliance. Available at: https://www.cchaforlife.org/facts-issues [Accessed 6 Jun. 2018].

43. CDC (2018). *Hypoplastic Left Heart Syndrome Facts | Congenital Heart Defects*. [online] Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/ncbddd/heartdefects/hlhs.html [Accessed 8 Nov. 2016].

44. AHA (2018). *Atrial Septal Defect (ASD)*. [online] Heart.org. Available at: http://www.heart.org/HEARTORG/Conditions/CongenitalHeartDefects/AboutCongenitalHeartDefects/Atrial-Septal-Defect-ASD_UCM_307021_Article.jsp [Accessed 19 Jan. 2018].

45. Mai, C., Riehle-Colarusso, T., O'Halloran, A., Cragan, J., Olney, R., Lin, A., Feldkamp, M., Botto, L., Rickard, R., Anderka, M., Ethen, M., Stanton, C., Ehrhardt, J. and Canfield, M. (2012). Selected birth defects data from population-based birth defects surveillance programs in the United States, 2005-2009: Featuring critical congenital heart defects targeted for pulse oximetry screening. *Birth Defects Research Part A: Clinical and Molecular Teratology*, 94(12), pp.970-983.

46. MayoClinic (2018). *Tricuspid atresia - Symptoms and causes*. [online] Mayo Clinic. Available at: https://www.mayoclinic.org/diseases-conditions/tricuspid-atresia/symptoms-causes/syc-20368392 [Accessed 8 Mar. 2018].

47. MayoClinic (2018). *Tetralogy of Fallot - Symptoms and causes*. [online] Mayo Clinic. Available at: https://www.mayoclinic.org/diseases-conditions/tetralogy-of-fallot/symptoms-causes/syc-20353477 [Accessed 9 Mar. 2018].

48. Baffa, J. (2018). *Tetralogy of Fallot - Pediatrics - MSD Manual Professional Edition*. [online] MSD Manual Professional Edition. Available at: https://www.merckmanuals.com/professional/pediatrics/congenital-cardiovascular-anomalies/tetralogy-of-fallot [Accessed 22 Nov. 2016].

49. Northwestern Medicine (2018). *Bicuspid Aortic Valve Symptoms*. [online] Northwestern Medicine. Available at: https://www.nm.org/conditions-and-care-areas/cardiovascular-care/center-for-heart-valve-disease/bicuspid-aortic-valve/symptoms [Accessed 7 Jun. 2018].

50. MayoClinic (2018). *Bicuspid aortic valve - Overview - Mayo Clinic*. [online] Mayoclinic.org. Available at: https://www.mayoclinic.org/diseases-conditions/bicuspid-aortic-valve/cdc-20385577 [Accessed 7 Jun. 2018].

51. MayoClinic (2018). *Patent ductus arteriosus (PDA) - Symptoms and causes*. [online] Mayo Clinic. Available at: https://www.mayoclinic.org/diseases-conditions/patent-ductus-arteriosus/symptoms-causes/syc-20376145 [Accessed 25 Jan. 2018].

52. Cleveland Clinic (2018). *Patent Ductus Arteriosus (PDA) Treatment Options | Cleveland Clinic*. [online] Cleveland Clinic. Available at: https://my.clevelandclinic.org/health/diseases/17325-patent-ductus-arteriosus-pda/treatment-options [Accessed 7 Jun. 2018].

53. MayoClinic (2018). *Pulmonary valve stenosis - Symptoms and causes*. [online] Mayo Clinic. Available at: https://www.mayoclinic.org/diseases-conditions/pulmonary-valve-stenosis/symptoms-causes/syc-20377034 [Accessed 8 Jun. 2018].

54. Spicer, D., Hsu, H., Co-Vu, J., Anderson, R. and Fricker, F. (2014). Ventricular septal defect. *Orphanet Journal of Rare Diseases*, 9(144),pp1-16.

55. Carminati, M., Butera, G., Chessa, M., De Giovanni, J., Fisher, G., Gewillig, M., Peuster, M., Piechaud, J., Santoro, G., Sievert, H., Spadoni, I. and Walsh, K. (2007). Transcatheter closure of congenital ventricular septal defects: results of the European Registry. *European Heart Journal*, 28(19), pp.2361-2368.

56. Minette, M. and Sahn, D. (2006). Ventricular Septal Defects. *Circulation*, 114(20), pp.2190-2197.

57. NIH (2018). *Hirschsprung Disease | NIDDK*. [online] National Institute of Diabetes and Digestive and Kidney Diseases. Available at: https://www.niddk.nih.gov/health-information/digestive-diseases/hirschsprung-disease [Accessed 7 Jun. 2018].

58. Martins, P. and Castela, E. (2018). *Transposition of the great arteries*. [online] BMC. Available at: https://ojrd.biomedcentral.com/articles/10.1186/1750-1172-3-27 [Accessed 8 Jun. 2018].

59. Cincinnati Children Health (2018). *Hirschsprung Disease in Infants & Children | Diagnosis & Treatment*. [online] Cincinnatichildrens.org. Available at: https://www.cincinnatichildrens.org/health/h/hirschsprung [Accessed 5 Jun. 2018].

60. Khan, A., Ramsey, K., Ballard, C., Armstrong, E., Burchill, L., Menashe, V., Pantely, G. and Broberg, C. (2018). Limited Accuracy of Administrative Data for the Identification and Classification of Adult Congenital Heart Disease. *Journal of the American Heart Association*, 7(2), p.e007378.

61. Safi, L., Liberthson, R. and Bhatt, A. (2016). Current Management of Ebstein's Anomaly in the Adult. *Current Treatment Options in Cardiovascular Medicine*, 18(9).

62. Skybchyk, V. and Melen, Y. (2017). Calcific Aortic Valve Stenosis: Current Approaches to the Diagnosis, Medical and Surgical Treatment. *HYPERTENSION*, 0(6.50), pp.74-80.

63. Johnson MC, Swanson PE, Johnson DC, Schuessler RB, Cox JL. (1998), An experimental model of small intestinal submucosa as a growing vascular graft. *Journal of Thoracic and Cardiovascular Surgery*, 116(5), 805–811.

64. Torok, R. (2015). Coarctation of the aorta: Management from infancy to adulthood. *World Journal of Cardiology*, 7(11), p.765.

65. Dodson, R. (2014). *Comprehensive surgical management of congenital heart disease*. 2nd ed. Florida: CRC press.

66. Homann M, Haehnel JC, Mendler N, Paek SU, Holper K, Meisner H, Lange R. (2000) Reconstruction of the RVOT with valved biological conduits: 25 years' experience with allografts and xenografts. *European Journal of Cardio-Thoracic Surgery*, 17(6), 624-630. https://doi.org/10.1016/S1010-7940(00)00414-0

67. Files MD, Boucek RJ. (2012). 'Shovel-Ready' applications of stem cell advances for pediatric heart disease. *Current Opinion In Pediatric*, 24(5), 577–583, doi: 10.1097/MOP.0b013e328357a4cf.

68. Kaiser N, Coulombe K. (2016) Physiological inspired cardiac scaffolds for tailored in vivo function and heart regeneration. *Biomedical Materials*, 10(3): 034003. doi:10.1088/1748-6041/10/3/034003

69. Rienks M, Papageorgiou A, Frangogiannis N, Heymans S. (2014) Myocardial Extracellular Matrix: An Ever-Changing and Diverse Entity. *Circulation Research*. 114(5):872-888. https://doi.org/10.1161/CIRCRESAHA.114.302533

70. Brown L. (2005) Cardiac extracellular matrix: a dynamic entity. *AJP: Heart and Circulatory Physiology*. 289(3), H973-H974. https://doi.org/10.1152/ajpheart.00443.2005

71. Chaikof EL. (2007), The Development of Prosthetic Heart Valve: Lessons in Form and Function. *The New England Journal of Medicine*, 357:1368-1371. doi: 10.1056/NEJMp078175

72. Ratner, B., Hoffman, A., Schoen, F. and Lemons, J. (2004). *Biomaterials science*. 2nd ed. Amsterdam: Elsevier Academic Press.

73. Hibino N, McGillicuddy E, Matsumura G, Ichihara Y, Naito Y, Breuer C, Shinoka T. (2010) Late-term results of tissue-engineered vascular grafts in humans. *The Journal of Thoracic and Cardiovascular Surgery*, 139(2), 431-436.

74. Schmidt CE, Baier JM. (2010) Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials*, 21 (22), 2215-2231.

75. Kurobe H, Maxfield MW, Breuer CK, Shinoka T. (2012) Concise review: tissue-engineered vascular grafts for cardiac surgery: past, present, and future. *Stem Cells Translational Medicine*, 1(7):566-71. doi: 10.5966/sctm.2012-0044.

76. Johnson MC, Swanson PE, Johnson DC, Schuessler RB, Cox JL. (1998), An experimental model of small intestinal submucosa as a growing vascular graft. *Journal of Thoracic and Cardiovascular Surgery*, 116(5), 805–811.

77. Ruiz CE, Iemura M, Medie S, Varga P, Van Alstine WG, Mack S, Deligio A, Fearnot N, Beier UH, Pavcnik D, Hijazi ZM, Kiupel M. (2005) Transcatheter placement of a low-profile 4biodegradable pulmonary valve made of small intestinal submucosa: a long-term study in a swine model. *Journal of Thoracic and Cardiovascular Surgery*. 130(2), 477–484. 706https://doi.org/10.1016/j.jtcvs.2005.04.008

78. Wainwright JM, Hashizume R, Fujimoto KL, Remlinger NT, Pesyna C, Wagner WR, Tobita K, Gilbert TW, Badylak SF. (2012) Right ventricular outflow tract repair with a cardiac biologic scaffold. *Cells Tissues Organs 195(1-2)*. 159–70. https://doi.org/10.1159/000331400

79. Scholl FB, Boucek MM, Chan K-C, Valdes-Cruz L, Perryman R. (2010) Preliminary experience with cardiac reconstruction using decellularized porcine extracellular matrix scaffold: human applications in congenital heart disease. *World Journal of Pediatric and Congenital Heart Surgery*, 1(1) 713 132-136.

80. Quarti A, Nardone S, Colaneri M, Santoro G, Pozzi M. (2011) Preliminary experience in the use of an extracellular matrix to repair congenital heart diseases. *Interactive CardioVascular and Thoracic Surgery* 13(6), 569–572, https://doi.org/10.1510/icvts.2011.280016.

81. Witt RG, Raff G, Van Gundy J, Rodgers-Ohlau M, Si MS. (2013) Short-term experience of porcine small intestinal submucosa patches in paediatric cardiovascular surgery. European Journal of Cardiothoracic Surgery 44(1), 72–76, https://doi.org/10.1093/ejcts/ezs638

82. Miyagawa S, Roth M, Saito A, Sawa Y, Kostin S. (2011) Tissue-Engineered Cardiac Constructs for Cardiac Repair. *The Annals of Thoracic Surgery*, 91(1), 320-329. https://doi.org/10.1016/j.athoracsur.2010.09.080

83. Avolio, E., Caputo, M., Madeddu, P. (2015) Stem cell therapy and tissue engineering for correction of congenital heart disease. *Frontiers in Cell and Developmental Biology 3(39)*, 1-17. https://doi.org/10.3389/fcell.2015.00039

84. Williams, D. (2004) Benefit and risk in tissue engineering. *Materials Today*, 7(5), 24–29. doi: 10.1016/S1369-7021(04)00232-9

85. Zhao G, Zhang X, Lu TJ, Xu F. (2015) Recent Advances in Electrospun Nanofibrous Scaffolds for Cardiac Tissue Engineering. *Advanced Functional Materials*. 9(8): 5726–5738. doi: 10.3390/ma9080614

86. Maeda K, Tiwari-Pandey R, Ruel M, Suuronen E. (2015) Matrix Therapies for Cell Support and Cardiac Repair. In: Suuronen E, Ruel M, ed. by. *Biomaterials for Cardiac Regeneration*. London: Springer; p. 117-146.

87. Sugiura T, Matsumura G, Miyamoto S, Miyachi H, Breuer CK, Shinoka T. (2018) Tissue-engineered vascular in children with congenital heart disease: Intermediate term followup. *Seminars in Thoracic and Cardiovascular surgery*. doi: 10.1053/j.semtcvs.2018.02.002.

88. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R. (1999) Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnology and Bioengineering*, 5;64(5), 580-9.

89. Masuda S, Shimizu T, Yamato M, Okano T. (2008) Cell sheet engineering for heart tissue repair. *Advanced Drug Delivery Reviews*, 60(2), 277-285.

90. Hashi CK, Zhu Y, Yang G, Young WL, Hsiao BS, Wang K, Chu B, Li S. (2007) Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts. *Proceedings of the National Academy of Sciences of the United States of America*, 104 (29) p11915-11920. https://doi.org/10.1073/pnas.0704581104.

91. Baheiraei N, Gharibi R, Yeganeh H, Miragoli M, Salvarani N, Di Pasquale E, Condorelli G. (2015) Electroactive polyurethane/siloxane derived from castor oil as a versatile cardiac patch, part I: Synthesis, characterization, and myoblast proliferation and differentiation. *Journal of Biomedical Materials Research Part A*., 104(3):775-787.

92. Baheiraei N, Gharibi R, Yeganeh H, Miragoli M, Salvarani N, Di Pasquale E, Condorelli G. (2016) Electroactive polyurethane/siloxane derived from castor oil as a versatile cardiac patch, part II: HL-1 cytocompatibility and electrical characterizations. *Journal of Biomedical Materials Research Part A*. 104(6):1398-1407.

93. Su W, Ho C, Shih T, Wang C, Yeh C. (2016) Exceptional biocompatibility of 3D fibrous scaffold for cardiac tissue engineering fabricated from biodegradable polyurethane blended with cellulose. International Journal of Polymeric Materials and Polymeric *Biomaterials* , 65(14):703-711.

94. Hernández-Córdova R, Mathew D, Balint R, Carrillo-Escalante H, Cervantes-Uc J, Hidalgo-Bastida L, Hernández-Sánchez F. (2016) Indirect three-dimensional printing: A method for fabricating polyurethane-urea based cardiac scaffolds. *Journal of Biomedical Materials Research Part A*., 104(8):1912-1921.

95. Tomecka E, Wojasinski M, Jastrzebska E, Chudy M, Ciach T, Brzozka Z. (2017) Poly(l-lactic acid) and polyurethane nanofibers fabricated by solution blow spinning as potential substrates for cardiac cell culture. *Materials Science and Engineering: C*., 75, 305-316

96. Sharifpanah F, Reinhardt M, Schönleben J, Meyer C, Richter M, Schnabelrauch M, Rode C, Wartenberg A, Bekhite M, Sauer H, Wartenberg M. (2017) Embryonic stem cells for tissue

biocompatibility, angiogenesis, and inflammation testing. *Cells Tissues Organs*, 204: 1-12. https://doi.org/10.1159/000471794

97. Centola M, Rainer A, Spadaccio C, De Porcellinis S, Genovese JA, Trombetta M. (2010) Combining electrospinning and fused deposition modelling for the fabrication of a hybrid vascular graft. *Biofabrication*. 2(1):014102.; doi: 0.1088/1758-5082/2/1/014102.

98. Mukherjee S, Reddy Venugopal J, Ravichandran R, Ramakrishna S, Raghunath M. (2011) Evaluation of the Biocompatibility of PLACL/Collagen Nanostructured Matrices with Cardiomyocytes as a Model for the Regeneration of Infarcted Myocardium. *Advanced Functional Materials*., 21(12):2291-2300.

99. Kai D, Prabhakaran M, Jin G, Ramakrishna S. (2011) Polypyrrole-contained electrospun conductive nanofibrous membranes for cardiac tissue engineering. *Journal of Biomedical Materials Research* Part A., 99A(3):376-385.

100. Sugiura T, Hibino N, Breuer C, Shinoka T. (2016) Tissue-engineered cardiac patch seeded with human induced pluripotent stem cell derived cardiomyocytes promoted the regeneration of host cardiomyocytes in a rat model. *Journal of Cardiothoracic Surgery*., 11(1). doi: 10.1186/s13019-016-0559-z

101. Castilho M, Feyen D, Flandes-Iparraguirre M, Hochleitner G, Groll J, Doevendans P, Vermonden T, Ito K, Sluijter JPG, Malda J. (2017) Melt Electrospinning Writing of Poly-Hydroxymethylglycolide-co-ε-Caprolactone-Based Scaffolds for Cardiac Tissue Engineering. *Advanced Healthcare Materials*., 6(18):1700311

102. Pok S, Myers JD, Madihally SV, Jacot JG. (2013) A multi-layered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering. *Acta Biomater*., 9: 5630-5642.

103. Hitscherich P, Wu S, Gordan R, Xie L-H, Arinzeh T, Lee EJ. (2016) The effect of PVDF-TrFE scaffolds on stem cell derived cardiovascular cells. *Biotechnol Bioeng*., 113:1577–85. doi:10.1002/bit.25918

104. Nelson D, Ma Z, Fujimoto K, Hashizume R, Wagner W. (2011) Intra-myocardial biomaterial injection therapy in the treatment of heart failure: Materials, outcomes and challenges. *Acta Biomaterialia*. 7(1):1-15.

105. Herpel E, Pritsch M, Koch A, Dengler T, Schirmacher P, Schnabel P. (2006) Interstitial fibrosis in the heart: differences in extracellular matrix proteins and matrix metalloproteinases in end-stage dilated, ischaemic and valvular cardiomyopathy. *Histopathology.* 48 (6), 736-747.

106. Serpooshan V, Zhao M, Metzler S, Wei K, Shah P, Wang A, Mahmoudi M, Malkovskiy AV, Rajadas J, Butte MJ, Bernstein D, Ruiz-Lozano P. (2016) The effect of bioengineered acellular collagen patch on cardiac remodeling and ventricular function post myocardial infarction. *Biomaterials.* 34(36), 9048-9055.

877 107. Dawson J, Schussler O, Al-Madhoun A, Menard C, Ruel M, Skerjanc I. (2011) Collagen scaffolds
878 with or without the addition of RGD peptides support cardiomyogenesis after aggregation of mouse
879 embryonic stem cells. *In Vitro Cellular & Developmental Biology – Animal*, 47(9):653-664.

880 108. Yu H, Zhao H, Huang C, Du Y. (2017) Mechanically and Electrically Enhanced CNT–Collagen
881 Hydrogels As Potential Scaffolds for Engineered Cardiac Constructs. *ACS Biomaterials Science &*
882 *Engineering*, 3(11), 3017–3021 doi: 10.1021/acsbiomaterials.6b0062

883 109. Sun H, Zhou J, Huang Z, Qu L, Lin N, Liang C, Dai R, Tang L, Tian F. (2017) Carbon nanotube-
884 incorporated collagen hydrogels improve cell alignment and the performance of cardiac constructs.
885 *International Journal of Nanomedicine,* 12:3109-3120.

886 110. Frederick J, Fitzpatrick J, McCormick R, Harris D, Kim A, Muenzer J, Marotta N, Smith MJ, Cohen JE,
887 Hiesinger W, Atluri P, Woo YJ. (2010) Stromal Cell-Derived Factor-1 Activation of Tissue-
888 Engineered Endothelial Progenitor Cell Matrix Enhances Ventricular Function After Myocardial
889 Infarction by Inducing Neovasculogenesis. *Circulation.* 122(11 suppl: 1):S107-S117.

890 111. Hsieh SC, Chen HJ, Hsu SH, Yang YC, Tang CM, Chu MY, Lin PY, Fu RH, Kung ML, Chen
891 YW, Yeh BW, Hung HS. (2016) Prominent Vascularization Capacity of Mesenchymal Stem Cells in
892 Collagen–Gold Nanocomposites. *ACS Applied Materials & Interfaces*. 8(42):28982-29000.

893 112. Nie SP, Wang X, Qiao SB, Zeng QT, Jiang JQ, Liu XQ, Zhu XM, Cao GX, Ma CS. (2010)
894 Improved myocardial perfusion and cardiac function by controlled-release basic fibroblast growth
895 factor using fibrin glue in a canine infarct model. *Journal of Zhejiang University Science* B.,
896 11(12):895-904.

897 113. Menasché P, Vanneaux V, Fabreguettes JR, Bel A, Tosca L, Garcia S, Bellamy V, Farouz Y, Pouly J,
898 Damour O, Périer MC, Desnos M, Hagège A, Agbulut O, Bruneval P, Tachdjian G, Trouvin JH,
899 Larghero J. (2014) Towards a clinical use of human embryonic stem cell-derived cardiac progenitors: a
900 translational experience. *European Heart Journal,* 36(12):743-750.

901 114. Gil-Cayuela C, Roselló-LLetí E, Ortega A, Tarazón E, Triviño J, Martínez-Dolz L, González-
902 Juanatey JR, Lago F, Portolés M, Rivera M. (2016) New Altered Non-Fibrillar Collagens in Human
903 Dilated Cardiomyopathy: Role in the Remodeling Process. *PLOS ONE*., 11(12):e0168130.

904 115. Menasché P, Vanneaux V, Hagège A, Bel A, Cholley B, Cacciapuoti I, Parouchev A,
905 Benhamouda N, Tachdjian G, Tosca L, Trouvin JH, Fabreguettes JR, Bellamy V, Guillemain
906 R, SuberbielleBoissel C, Tartour E, Desnos M, Larghero J. (2015) Human embryonic stem cell-
907 derived cardiac progenitors for severe heart failure treatment: first clinical case report. *European*
908 *Heart Journal*, 36(30):2011-7.
909

910 116. Terrovitis J, Lautamäki R, Bonios M, Fox J, Engles JM, Yu J, Leppo MK, Pomper MG, Wahl
911 RL, Seidel J, Tsui BM, Bengel FM, Abraham MR, Marbán E. (2009). Noninvasive Quantification and
912 Optimization of Acute Cell Retention by In Vivo Positron Emission Tomography After
913 Intramyocardial Cardiac-Derived Stem Cell Delivery. *Journal of the American College of*
914 *Cardiology*, 54(17):1619-1626.

915 117. Llucià-Valldeperas A, Soler-Botija C, Gálvez-Montón C, Roura S, Prat-Vidal C, Perea-Gil I,
916 Sanchez B, Bragos R, Vunjak-Novakovic G, Bayes-Genis A. (2016) Electromechanical
917 Conditioning of Adult Progenitor Cells Improves Recovery of Cardiac Function After
918 Myocardial Infarction. *STEM CELLS Translational Medicine.* 6(3):970-981.

118. Tao Z, Mohamed M, Hogan M, Gutierrez L, Birla R. (2014) Optimizing a spontaneously contracting heart tissue patch with rat neonatal cardiac cells on fibrin gel. *Journal of Tissue Engineering and Regenerative Medicine*. 11(1):153-163.

119. Ichihara Y, Tano N, Fields L, Murugesu N, Shintani Y, Ishida M, Sato N, Suzuki K. Abstract 14735: (2017) Epicardial Placement of Instantly Produced Fibrin Glue- Incorporating Mesenchymal Stromal Cells for the Treatment of Heart Failure [Internet]. *Circulation*. from: http://circ.ahajournals.org/content/134/Suppl_1/A14735

120. Moreira R, Neusser C, Kruse M, Mulderrig S, Wolf F, Spillner J, Schmitz-Rode T, Jockenhoevel S, Mela P. (2016) Tissue-Engineered Fibrin-Based Heart Valve with Bio-Inspired Textile Reinforcement. *Advanced Healthcare Materials*. 5(16):2113-2121.

121. Camci-Unal G, Annabi N, Dokmeci M, Liao R, Khademhosseini A. (2014) Hydrogels for cardiac tissue engineering. *NPG Asia Materials*., 6(5):e99.

122. Wang H, Shi J, Wang Y, Yin Y, Wang L, Liu J, Liu Z, Duan C, Zhu P, Wang C. (2014) Promotion of cardiac differentiation of brown adipose derived stem cells by chitosan hydrogel for repair after myocardial infarction. *Biomaterials*. 35(13):3986-3998.

123. Chopra S, Mahdi S, Kaur J, Iqbal Z, Talegaonkar S, Ahmad F. (2006) Advances and potential applications of chitosan derivatives as mucoadhesive biomaterials in modern drug delivery. *Journal of Pharmacy and Pharmacology*. 58(8):1021-1032

124. Wang L, Shansky J, Borselli C, Mooney D, Vandenburgh H. (2012) Design and Fabrication of a Biodegradable, Covalently Crosslinked Shape-Memory Alginate Scaffold for Cell and Growth Factor Delivery. *Tissue Engineering Part A,* 18(19-20):2000-2007.

125. Landa N, Miller L, Feinberg M, Holbova R, Shachar M, Freeman I, Ci. (2008) Effect of Injectable Alginate Implant on Cardiac Remodeling and Function After Recent and Old Infarcts in Rat. *Circulation*. 117(11):1388-1396.

126. Leor J, Tuvia S, Guetta V, Manczur F, Castel D, Willenz U, Petneházy O, Landa N, Feinberg MS, Konen E, Goitein O, Tsur-Gang O, Shaul M, Klapper L, Cohen S. (2009) Intracoronary Injection of In Situ Forming Alginate Hydrogel Reverses Left Ventricular Remodeling After Myocardial Infarction in Swine. *Journal of the American College of Cardiology*. 54(11):1014-1023.

127. Sabbah H, Wang M, Gupta R, Rastogi S, Ilsar I, Sabbah M, Kohli S, Helgerson S, Lee RJ. (2013) Augmentation of Left Ventricular Wall Thickness With Alginate Hydrogel Implants Improves Left Ventricular Function and Prevents Progressive Remodeling in Dogs With Chronic Heart Failure. *JACC: Heart Failure*. 1(3):252-258.

128. Bonafè F, Govoni M, Giordano E, Caldarera C, Guarnieri C, Muscari C. (2014) Hyaluronan and cardiac regeneration. *Journal of Biomedical Science*. 21: 100. doi: 10.1186/s12929-014-0100-4

129. Yoon SJ, Fang YH, Lim CH, Kim BS, Son HS, Park Y, Sun K. (2009) Regeneration of ischemic heart using hyaluronic acid-based injectable hydrogel. Journal of Biomedical Materials Research Part B*:* *Applied Biomaterials*. 91B (1):163-171.

130. Yang MC, Chi NH, Chou NK, Huang YY, Chung TW, Chang YL, Liu HC, Shieh MJ, Wang SS. (2010) The influence of rat mesenchymal stem cell CD44 surface markers on cell growth, fibronectin expression, and cardiomyogenic differentiation on silk fibroin – Hyaluronic acid cardiac patches. *Biomaterials*. 31(5):854-862.

131. Göv E, Kenar H, Halbutoğullari Z, Arğa K, Karaöz E. (2016) Cardiomyogenic differentiation potential of human lipoaspirate-derivedstem cells on hyaluronic acid/gelatin plasma gels. *Turkish Journal of Biology*. 40:369-379.

132. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. (1999) Survival and function of bioengineered cardiac grafts. *Circulation 100(19)*, 1163-1169.

133. McCain M, Agarwal A, Nesmith H, Nesmith A, Parker K. (2014) Micromolded gelatin hydrogels for extended culture of engineered cardiac tissues. *Biomaterials*. 35(21):5462-5471.

134. Yacoub M, Terrovitis J. (2013) CADUCEUS, SCIPIO, ALCADIA: Cell therapy trials using cardiac-derived cells for patients with post myocardial infarction LV dysfunction, still evolving. *Global Cardiology Science and Practice*. (1):3: 6-8

135. Takehara N, Tsutsumi Y, Tateishi K, Ogata T, Tanaka H, Ueyama T, Takahashi T, Takamatsu T, Fukushima M, Komeda M, Yamagishi M, Yaku H, Tabata Y, Matsubara H, Oh H. (2008) Controlled Delivery of Basic Fibroblast Growth Factor Promotes Human Cardiosphere-Derived Cell Engraftment to Enhance Cardiac Repair for Chronic Myocardial Infarction. *Journal of the American College of Cardiology*. 52(23):1858-1865.

136. Navaei A, Saini H, Christenson W, Sullivan R, Ros R, Nikkhah M. (2016) Gold nanorod-incorporated gelatin-based conductive hydrogels for engineering cardiac tissue constructs. *ActaBiomaterialia.* 41:133-146.

137. Kudová J, Procházková J, Vašiček O, Perečko T, Sedláčková M, Pešl M, Pacherník J, Kubala L. (2016) HIF-1alpha Deficiency Attenuates the Cardiomyogenesis of Mouse Embryonic Stem Cells. *PLOS ONE*. 11(6):e0158358.

138. Cristallini C, Cibrario Rocchietti E, Gagliardi M, Mortati L, Saviozzi S, Bellotti E, Turinetto V, Sassi MP, Barbani N, Giachino C. (2016). Micro- and Macrostructured PLGA/Gelatin Scaffolds Promote Early Cardiogenic Commitment of Human Mesenchymal Stem Cells In Vitro. *Stem Cells International*. 2016:1-16.

139. Okada M, Yamashita C, Okada M, Okada K. (1995). Role of Endothelin-1 in Beagles With Dehydromonocrotaline-Induced Pulmonary Hypertension. *Circulation.* 92(1):114-119.

140. Sakai T, Li RK, Weisel RD, Mickle DA, Kim ET, Jia ZQ, Yau TM. (2001). The fate of a tissue-engineered cardiac graft in the right ventricular outflow tract of the rat. *Journal of Thoracic and Cardiovascular Surgery 121(5)*, 932-942.

141. Zimmermann, W., Melnychenko, I., Eschenhagen, T. (2004). Engineered heart tissue for regeneration of diseased hearts. *Biomaterials 25(9)*, 1639-1647.

142. Duan Y, Liu Z, O'Neill J, Wan LQ, Freytes DO, Vunjak-Novakovic G. (2011). Hybrid gel composed of native heart matrix and collagen induces cardiac differentiation of human embryonic stem

cells without supplemental growth factors. *Journal of Cardiovascular Translational Research 4(5)*, 605-615.

143. Lewis, T. (2016). *The Scientist*. Retrieved from the-scientist.com: http://mobile.the-scientist.com/article/46588/will-organs-in-a-dish-ever-replace-animal-models.

144. Lu TY, Lin B, Kim J, Sullivan M, Tobita K, Salama G, Yang L. (2013). Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. *Nature Communications*. 4:2307. doi: 10.1038/ncomms3307

145. Iop L, Renier V, Naso F, Piccoli M, Bonetti A, Gandaglia A, Pozzobon M, Paolin A, Ortolani F, Marchini M, Spina M, De Coppi P, Sartore S, Gerosa G. (2009). The influence of heart valve leaflet matrix characteristics on the interaction between human mesenchymal stem cells and decellularized scaffolds. *Biomaterials 30(25)*, 4104–4116.

146. Yamato, M., & Okano, T. (2004). Cell sheet engineering. *Materials Today*. Volume 7, Issue 5, Pages 42-47. https://doi.org/10.1016/S1369-7021(04)00234-2

147. Boroviak T., Loos R., Bertone P., Smit A., Nichols J. (2014). The ability of inner cell mass cells to self-renew as embryonic stem cells is acquired upon epiblast specification. *Nature Cell Biology 16*, 513–525.

148. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. (2013). Structural and Functional Maturation of Cardiomyocytes Derived from Human Pluripotent Stem Cells. *Stem Cells and Development*. Jul 15;22(14):1991-2002. doi: 10.1089/scd.2012.0490.

149. Boheler KR, Czyz J, Tweedie D, Yang HT, Anisimov SV, Wobus AM. (2002). Differentiation of Pluripotent Embryonic Stem Cells Into Cardiomyocytes. *Circulation Research*. Aug 9;91(3):189-201.

150. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. Nov 30;131(5):861-72.

151. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science*. Dec 21;318(5858):1917-20

152. Bosman A, Edel MJ, Blue G, Dilley RJ, Harvey RP, Winlaw DS. (2015). Bioengineering and Stem Cell Technology in the Treatment of Congenital Heart Disease. *Journal of Clinical Medicine*. Apr 22;4(4):768-81. doi: 10.3390/jcm4040768.

153. Caputo M, Saif J, Rajakaruna C, Brooks M, Angelini GD, Emanueli C. (2015). MicroRNAs in vascular tissue engineering and post-ischemic neovascularization. *Advanced Drug Delivery Reviews*. Jul 1;88:78-91. doi: 10.1016/j.addr.2015.05.003.

154. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. (2010). Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. *Cell*. Aug 6;142(3):375-86. doi: 10.1016/j.cell.2010.07.002.

155. Lüningschrör P, Hauser S, Kaltschmidt B, Kaltschmidt C. (2013). MicroRNAs in pluripotency, reprogramming, and cell fate induction. *BiochimicaetBiophysicaActa (BBA) - Molecular Cell Research*. Aug;1833(8):1894-903. doi: 10.1016/j.bbamcr.2013.03.025.

156. Webera, B., Zeisbergera, S., Hoerstrup, S. (2011). Prenatally harvested cells for cardiovascular tissue engineering: Fabrication of autologous implants prior to birth. *Placenta*. Oct;32 Suppl 4:S316-9. doi: 10.1016/j.placenta.2011.04.001.

157. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki Ki, Eguchi H, Onitsuka I, Matsui K, Imaizumi T. (2000). Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. *The Journal of Clinical Investigation*. Jun;105(11):1527-36.

158. De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. (2007). Isolation of amniotic stem cell lines with potential for therapy. *Nature Biotechnology*. Jan;25(1):100-6.

159. Schmidt D, Mol A, Breymann C, Achermann J, Odermatt B, Gössi M, Neuenschwander S, Prêtre R, Genoni M, Zund G, Hoerstrup SP. (2006). Living autologous heart valves engineered from human prenatally harvested progenitors. *Circulation*. Jul 4;114(1 Suppl):I125-31.

160. Schmidt D, Breymann C, Weber A, Guenter CI, Neuenschwander S, Zund G, Turina M, Hoerstrup SP. (2004). Umbilical Cord Blood Derived Endothelial Progenitor Cells for Tissue Engineering of Vascular Grafts. *The Annal of Thoracic Surgery*. Dec;78(6):2094-8

161. Schmidt D, Achermann J, Odermatt B, Breymann C, Mol A, Genoni M, Zund G, Hoerstrup SP. (2007). Prenatally fabricated autologous human living heart valves based on amniotic fluid derived progenitor cells as single cell source. *Circulation*. 11;116(11 Suppl):I64-70.

162. Yao, C. (2016). Combination of Endothelial Progenitor Cells, Extracellular Matrix and Aligned Eletrospun Polyhydroxyalkanoate Scaffold for Vascular Tissue Engineering. *Cytotherapy*. 18(6) Supplement, S75. https://doi.org/10.1016/j.jcyt.2016.03.149

163. Petsche Connell J, Camci-Unal G, Khademhosseini A, Jacot JG. (2013). Amniotic Fluid-Derived Stem Cells for Cardiovascular Tissue Engineering Applications. *Tissue Engineering*. Aug;19(4):368-79. doi: 10.1089/ten.TEB.2012.0561

164. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM.(1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* Feb 14;275(5302):964-7.

165. Zisch, A. (2004). Tissue engineering of angiogenesis with autologous endothelial progenitor cells. *Current Opinion in Biotechnology*. 15(5):424-9.

166. Olausson M, Kuna VK, Travnikova G, Bäckdahl H, Patil PB, Saalman R, Borg H, Jeppsson A, Sumitran-Holgersson S. (2014). In Vivo Application of Tissue-Engineered Veins Using Autologous Peripheral Whole Blood: A Proof of Concept Study. *Ebiomedicine*. Sep22;1(1):72-9. doi: 10.1016/j.ebiom.2014.09.001.

167. Zhou M, Liu Z, Liu C, Jiang X, Wei Z, Qiao W, Ran F, Wang W, Qiao T, Liu C. (2012). Tissue engineering of small-diameter vascular grafts by endothelial progenitor cells seeding heparin-coated decellularized scaffolds. *Journal of Biomedical Materials Research*. 100(1):111-20. doi: 10.1002/jbm.b.31928.

168. Sales VL, Mettler BA, Engelmayr GC Jr, Aikawa E, Bischoff J, Martin DP, Exarhopoulos A, Moses MA, Schoen FJ, Sacks MS, Mayer JE Jr. (2011). Endothelial Progenitor Cells as a Sole Source for Ex Vivo Seeding of Tissue-Engineered Heart Valves. *Tissue Engineering*. 16(1):257-67. doi: 10.1089/ten.TEA.2009.0424.

169. Mirensky TL, Hibino N, Sawh-Martinez RF, Yi T, Villalona G, Shinoka T, and Breuer CK. (2010). Tissue-engineered vascular grafts: does cell seeding matter? *Journal of Pediatric Surgery*. 45(6): 1299–1305. doi: 10.1016/j.jpedsurg.2010.02.102.

170. Fernandes S, Chong JJH, Paige SL, Iwata M, Torok-Storb B, Keller G, Reinecke H, Murry CE. (2015). Comparison of Human Embryonic Stem Cell-Derived Cardiomyocytes, Cardiovascular Progenitors, and Bone Marrow Mononuclear Cells for Cardiac Repair. *Stem Cell Reports* 5(5): 753–762. doi: 10.1016/j.stemcr.2015.09.011.

171. Vincentelli A, Wautot F, Juthier F, Fouquet O, Corseaux D, Marechaux S, Le Tourneau T, Fabre O, Susen S, Van Belle E, Mouquet F, Decoene C, Prat A, Jude B. (2007). In vivo autologous recellularization of a tissue-engineered. *Surgery*. Aug;134(2):424-32.

172. Garbern, J., Lee, R. (2013). Cardiac Stem Cell Therapy and the Promise of Heart Regeneration. *Cell Stem Cell*. Jun 6; 12(6): 689–698.

173. Bosman A, Edel MJ, Blue G, Dilley RJ, Harvey RP, Winlaw DS. (2011). Bioengineering and Stem Cell Technology in the Treatment of Congenital Heart Disease. *Circulation*. Apr 22;4(4):768-81. doi: 10.3390/jcm4040768.

174. Alessandri, G., Emanueli, C., & Madedu, P. (2004). Genetically Engineered Stem Cell Therapy for Tissue Regeneration. *Annals of the New York Academy of Science*. May;1015:271-84. DOI:10.1196/annals.1302.023.

175. Metcalfe AD and Ferguson MWJ. (2007). Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells, and regeneration. *The Royal Society Interface*. Jun 22; 4(14): 413–437. doi: 10.1098/rsif.2006.0179

176. Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA (2015). Repair and tissue engineering techniques for articular cartilage. *Nature Reviews Reumatology*. 11(1): 21–34. doi: 10.1038/nrrheum.2014.157

177. Chlupác, J., Filová, E., Bacáková, E. (2009) Blood vessel replacement: 50 years of development and tissue engineering paradigms in vascular surgery. *Physiological Research* ;58 Suppl 2:S119-39.

178. Karamichos, D. (2015) Ocular Tissue Engineering: Current and Future Directions. *Journal of Functioning Biomaterials*. Feb 17;6(1):77-80. doi: 10.3390/jfb6010077.

179. Kane NM, Thrasher AJ, Angelini GD, Emanueli C. (2014). Concise Review: MicroRNAs as Modulators of Stem Cells and Angiogenesis. *Stem Cell*. May;32(5):1059-66. doi: 10.1002/stem.1629.

Table 1(on next page)

Frequencies, Presentations, and management of Congenital Heart Diseases

1

2 Table 1.1 *Frequencies of CHD in some regions*

Frequencies of CHD in some regions	
United State	Affects 1% of live births [33]
South America	Colombia: 1.2 per 1000 live births Brazil (Minas Gerais): 9.58 in 1000 live births Brazil (Londrina): 5.49 in 1000 live births [30]
Mexico	Affects 6-8 per 1,000 newborns. Drawing to the conclusion that there about 12,000 or 16,000 babies living with CHD [36]
Asia	Affects 9.3 per 1,000 live births [34]
Europe	Affects 8.2 per 100 live births [40]
United kingdom	Affects about 9 in every 1,000 babies [35]
Russia	Affects 2.7-3.8 per 1,000 newborns estimating as 86 newborns per year being affected with CHD [38]
Australia	Affects 8-10 cases per 1,000 live births. Resulting in 2,400-3,000 newborns with CHD each year. About 65,000 adults are living with CHD [37]
Africa	Mozambique: 2.3 in 1000 live births Northern Nigeria: 9.3% (122 of 1312 patients) [41]
Canada	Affects 1 in 80-100 live births [42]

3

4

5 Table 1.2 *Types, presentations and management of CHDs*

Types of CHD	Presentations of CHD	Medical & Surgical Therapeutic Approach to CHD
	➢ This defect manifests as a hole in the wall (septum) that separates the chambers above (atria) from those below (ventricles). ➢ The volume of blood that flows through the lungs is increased over time due to the hole caused by the atrial defect resulting in damage to the	➢ **Medical monitoring:** the patient is monitored to see if the atrial septal defect would close on its own. ➢ **Medications:** beta blockers (to maintain a regular heartbeat) or anticoagulants (to help reduce blood clots).

Atrial Septal Defects	blood vessels in the lungs. ➢ Frequent respiratory or lung infections ➢ Difficulty breathing ➢ Tiring when feeding (infants) ➢ Shortness of breath when being active or exercising ➢ Skipped heartbeats or a sense of feeling the heartbeat ➢ A heart murmur, or a whooshing sound that can be heard with a stethoscope ➢ Stroke ➢ Swelling of legs, feet, or stomach area [31].	➢ **Surgery:** can be done through Cardiac catheterization or Open-heart surgery. ➢ **Follow-up care** [31, 32]
Hypoplastic Left Heart Syndrome **Hypoplastic Left Heart Syndrome** *(continued)*	➢ This defect affects the normal blood flow through the heart. The left side of the heart does not form correctly and as such it is considered a critical congenital heart defect. ➢ The following structures on the left side of the heart is affected: a. The left ventricle is underdeveloped. b. The mitral valves are not formed. c. The aortic valve is not formed. d. The ascending aorta is underdeveloped. ➢ The left side of the heart cannot pump oxygen-rich blood. ➢ Rapid, difficult breathing ➢ Pounding heart ➢ Weak pulse ➢ Poor feeding ➢ Being unusually drowsy or inactive ➢ Ashen or bluish color ➢ Dilated pupils ➢ Lackluster eyes that seem to stare [43, 44].	➢ **Medication:** inpatient medications include prostaglandin E1, Dopamine and Potassium Chloride and outpatient medications are Furosemide, Digoxin and Captopril [39]. ➢ **Nutrition:** feeding tube or special high-calorie formula. ➢ **Surgery:** a. Norwood Procedure: performed on the infant within 2 weeks of a baby's life. b. Bi-directional Glenn Shunt Procedure: done on an infant around 4 to 6 months of age. c. Fontan Procedure: performed on an infant around 18 months to 3 years of age [43]
	➢ Occurs in which the tricuspid valve is not formed leading to the underdevelopment of the	➢ **Medications:** prostaglandins like Alprostadil IV to keep

Tricuspid Atresia	right ventricle. ➢ The right side of the heart can't pump sufficient blood to the lungs. ➢ Problems breathing ➢ Ashen or bluish skin color (cyanosis) ➢ Poor feeding ➢ Extreme sleepiness ➢ Slow growth and poor weight gain ➢ Edema of the abdomen, legs, ankles and feet [45, 46].	open the ductus arteriosus. ➢ **Nutrition:** feeding tube ➢ **Surgery:** a. Atrial Septostomy: performed in the first few days or weeks of a baby's life b. Banding c. Shunt Procedure: done within the first 2 weeks of a baby's life. d. Bi-directional Glenn Procedure: performed around 4 to 6 months of the baby's life. **e.** Fontan Procedure: done around 2 years of age [45].
Tetraogy of Fallot **Tetraogy of Fallot** *(continued)*	➢ Has a combination of four heart defects. This defect is a combination of pulmonary stenosis, ventricular septal defect, overriding aorta and right ventricular hypertrophy. ➢ Cyanosis ➢ Shortness of breath ➢ Rapid breathing especially during feeding or exercise ➢ Fainting ➢ Clubbing of fingers and toes ➢ Poor weight gain ➢ Fatigue during play or exercise ➢ Prolonged crying ➢ Irritability ➢ Heart murmur due to pulmonary stenosis [47, 48].	➢ **Medication:** Prostaglandin E_1 infusion. ➢ **Surgery:** a. Temporary surgery (palliative surgery): improve blood flow to the lungs. b. Intra-cardiac repair: done during the first year after birth [47].
Bicuspid Aortic Valve	➢ Has only two (bicuspid) cusps instead of three. ➢ A bicuspid aortic valve may result in the heart's aortic valve to narrow (aortic valve stenosis) which prevents the valve from opening completely, which reduces or	➢ **Surgery:** a. Aortic valve replacement b. Balloon valvuloplasty c. Aortic valve repair d. Aortic root and ascending aorta surgery [50]

	blocks blood flow from the heart to the body. ➢ Trouble breathing ➢ Chest pain or pressure ➢ Fatigue ➢ Heart racing ➢ Light-headedness ➢ Fainting [49].	
Patent Ductus Arteriosus	➢ A persistent opening between the two major blood vessels leading from the heart. ➢ Large patent arteriosus can cause poorly oxygenated blood to flow in the wrong direction. ➢ Poor eating leads to poor growth. ➢ Sweating with crying or eating ➢ Persistent fast breathing or breathlessness ➢ Easy tiring ➢ Rapid heart taste [51]**.**	➢ **Medications:** NSAIDS (Advil, Infant's Motrin), or indomethacin (Indocin) [51] ➢ **Surgery:** Video-assisted thoracic surgical (VATS) repair ➢ **Catheter procedure:** Trans-catheter occlusion [52] ➢ **Watchful waiting**
Pulmonic Valve Stenosis **Pulmonic Valve Stenosis** *(continued)*	➢ This defect affects the pulmonic valve in which a deformity on or near the valve causes it to be smaller and as such slows the blood flow. ➢ The narrowing is due to the underdevelopment of the valve during fetal growth. The cusps maybe defective or too thick or may not separate from each other well. ➢ Heart murmur ➢ Fatigue ➢ Shortness of breath, especially during exertion ➢ Chest pain ➢ Fainting [53]	➢ **Surgery:** a. Balloon valvulplasty b. Open-heart surgery [53]
	➢ A fissure connecting the two ventricles of the heart. Size varies with each patient [54] ➢ It can occur isolated or in association with other CHDs. ➢ There are three kinds: muscular, periventricular and	➢ Smaller holes resolve themselves with time. ➢ **Surgery:** Usually done on larger fissures. It is indicated where patients express symptoms of heart failure, left heart

Ventricular Septal Defect **Ventricular Septal Defect** *(continued)*	supra-crystal. These are based on location within the septum [55] ➢ **Blood shunting:** Depending on the size of the hole, blood flows from the left ventricle to the right. ➢ **Pulmonary hypertension**: The shunting of blood flow leads to increased ventricular output to the pulmonary artery. With time, this can lead to pulmonary hypertension. ➢ **Eisenmenger's Syndrome:** Rise in pulmonary vascular resistance leads to increase in right ventricular pressure. This can lead to reverse shunting of blood from the right to left. This leads to cyanosis [54] ➢ Patients could also display clubbing [55] ➢ Growth retardation: Increased blood flow to the lungs results in an increase in lung compliance. This increases the energy demand for respiration. Thus, an energy deficit is created where the infant does not consume as much calories as is burned. This impedes growth. ➢ **Airway Obstruction:** a. Increased pulmonary blood flow increases the size of the pulmonary arteries. This can cause the physical obstruction of large and small airways. b. There is also the possibility of the incidence of pulmonary edema due to increased blood flow. The combination of these events can lead to respiratory distress. Thus, symptoms	overload and history of endocarditis. ➢ **Percutaneous techniques:** a. These do not require opening the patient up. b. Trans catheter approach: A catheter is threaded from an artery in the legs, or groin into the heart. A device is then placed to obstruct the hole in the ventricle [54, 55].

	such as wheezing, and tachypnea can be observed [54]. c. Holosystolic/pansystolic murmur on auscultation [56]	
Total Anomalous Pulmonary Venous Condition	➢ The pulmonary veins are attached to the right atrium instead of the left. ➢ Usually associated with atrial septal defect. ➢ **Cyanosis:** Oxygenated blood from the lungs is pumped into the right atrium. It mixes with deoxygenated blood and passes through the atrial septal defect into the left atrium. This decreases oxygen supply to the body leading to cyanosis. ➢ **Pulmonary hypertension:** Some patients have constricted pulmonary veins that lead to pulmonary hypertension. This leads to pulmonary effusion. ➢ **Hypovolemia**: Some patients manifest with a narrow or restrictive atrial septal defect. This significantly reduces the blood flow to the body leading to hypovolemia [57].	➢ **Surgery:** The pulmonary veins are surgically reattached to the left atrium. ➢ **Cardiac catheterization:** For the patients with a restricted atrial septal defect, a balloon pump is used to widen the fissure until corrective surgery can be carried out [57]
Trans-Position of the Great Arteries **Trans-Position of the Great Arteries** *(continued)*	➢ A condition whereby the aorta and the pulmonary arteries are transposed. The aorta arises from the right ventricle and leads to the lungs. The pulmonary artery arises from the left ventricle and leads to the body. ➢ It is comorbid with ventricular septal defect and patent ductus arteriosus. ➢ **Cyanosis:**Mixing of blood leads to supply of poorly oxygenated blood to the body	➢ **Medications:** Prostaglandin E1 is administered to keep the ductus arteriosus open. ➢ **Surgery:** a. Balloon atrial septostomy: A catheter is threaded through the foramen ovale. A balloon is inflated to rip a fissure in the atrium [58]

	[58]	
Truncus Arterious	➢ A condition where the truncus arteriosus of a fetus does not differentiate into an aorta and pulmonary vein. Thus, the patient only has one vessel exiting the heart ➢ **Cyanosis:** This leads to mixing of oxygenated and deoxygenated blood. Thus, the oxygen supply to the body is decreased ➢ **Congestive heart failure:** the excess of volume of blood flow to the heart increases pressure in the lungs. This would eventually lead to cardiac failure. ➢ Usually comorbid with ventricular septal defect [59]	➢ **Surgery:** The truncus arteriosus must be separated into two vessels. This would allow separate blood flow channels to the heart and body [59]
Ebstein's Anomaly **Ebstein's Anomaly** *(continued)*	➢ A congenital malformation of the tricuspid valve ➢ The posterior and septal leaflets are displaced downwards. This leads to a downward enlargement of the right atrium. ➢ The walls of the right atrium become thin. ➢ It can be comorbid with patent foramen ovale or atrial septal defect. ➢ Patients can be asymptomatic but could also present with symptoms. ➢ **Cyanosis:** Shunting of the blood between patent foramen ovale and atrial septal defect leads to blood mixing between the left and right sides of the heart. This can lead to cyanosis. ➢ **Conduction irregularities:** Some patients present with arrhythmias [60]	➢ **Surgery:** a. Only required where patient manifests severe symptoms. b. Cone procedure: Where the anterior septal leaflet is maneuvered and sewn to the true annulus. This attachment causes it to be conical in shape. c. Valve replacement d. Where the defective valves can be surgically replaced [60]
	➢ Characterized by a restriction	➢ **Surgery:**

Pulmonary Atresia	to blood flow from the right ventricle to the pulmonary artery. It could be due to malformation of the pulmonary valve or of the pulmonary artery itself. ➢ It can manifest with a ventricular septal defect where there are collateral arteries supplying the lungs. ➢ It could also manifest without a ventricular septal defect. Here the right ventricle is usually hypoplastic. It would usually be comorbid with a patent ductus arteriosus [61].	a. A shunt must be created between the pulmonary artery and the aorta. This can be done by administration of prostaglandin E to keep the ductus arteriosus open. It could also be doe surgically. b. Fontan's procedure: Done for patients with a hypoplastic right ventricle. The right atrium is surgically connected to the pulmonary artery [61].
Aortic Stenosis	➢ Defect of the aortic valve that restricts its opening. ➢ It can lead to ventricular hypertrophy which can eventually lead to heart failure. ➢ There is also a possibility of development of atrial fibrillation. ➢ Presence of systolic murmur [62]	➢ **Valve replacement :** There are two approaches: a. Surgically b. Trans-catheter approach [62].
Coarctation of the Aorta	➢ It is the constriction of the proximal end of the aorta leading to restriction to blood flow. ➢ Patients can present with acidosis, cardiac failure, as well as shock after ductus arteriosus closes [63]	➢ **Surgery:** a. Coarcted potion can be resected. And the ends of the artery can be re-anastomosed. b. A patch can also be used to surgically dilate the artery. ➢ **Balloon Angioplasty:** A catheter is threaded in to the aorta. A balloon is inflated to enlarge the aorta.

Table 2(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	• No need for scaffold • Spontaneous and synchronous pulsation • Could create tubular construct • Can grow with
Disadvantages	• Impact & friction wear • Inflammation • Calcification of valve • Less flexible than natural tissue • Life-long treatment with anticoagulants	• 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 vascularization in more than 3 cell-sheets

1

Table 3(on next page)

Scaffolds and SCs used for TE in some study models

1

Engineered tissue	Scaffold	Type of SCs	Study models	Reference
Heart valve	Synthetic biodegradable non-woven PGA mesh	Human Chorionic villi-derived cells & hCB- EPCs	Culture in bioreactor	164
	Synthetic biodegradable	hAFSCs	Culture in bioreactor	166
	porcine decellularized scaffold	BM-MSCs & BM-MSCs	Lambs	176
Vascular graft	Various synthetic biodegradable	Human Umbilical CB-EPCs	Static conditions & biomimetic flow system	165
	biodegradable non-woven PGA	BM-MNCs	Mice	174
	Biodegradable PLA & PGA	BM-MNCs	Human	79

2

Figure 1

Schematic of the different types of stems that can be used on the biomaterial backbone for cardiovascular Tissue Engineering (TE)

This schema represents the different types of stems that can be used on the biomaterial backbone (depicted as the background characters) for cardiovascular Tissue Engineering (TE). A) Induced pluripotent stem cells (iPSCs) derived from fibroblast. B) Prenatal, Perinatal, and Postnatal Stem cells (PPPSCs) are derived from amniotic fluid, umbilical cord, and chorionic villi. C) Bone Marrow Stem Cells (BMSCs) such endothelial progenitor cells (EPCs) and Mesenchymal stem cells (MSCs) can easily be isolated from the bone marrow. D) Cardiac progenitor cells (CPCs) can be harvested during palliative surgery or endomyocardial biopsy. E) Embryonic stem cells (ESCs) derived from the inner cell mass of the blastocyst

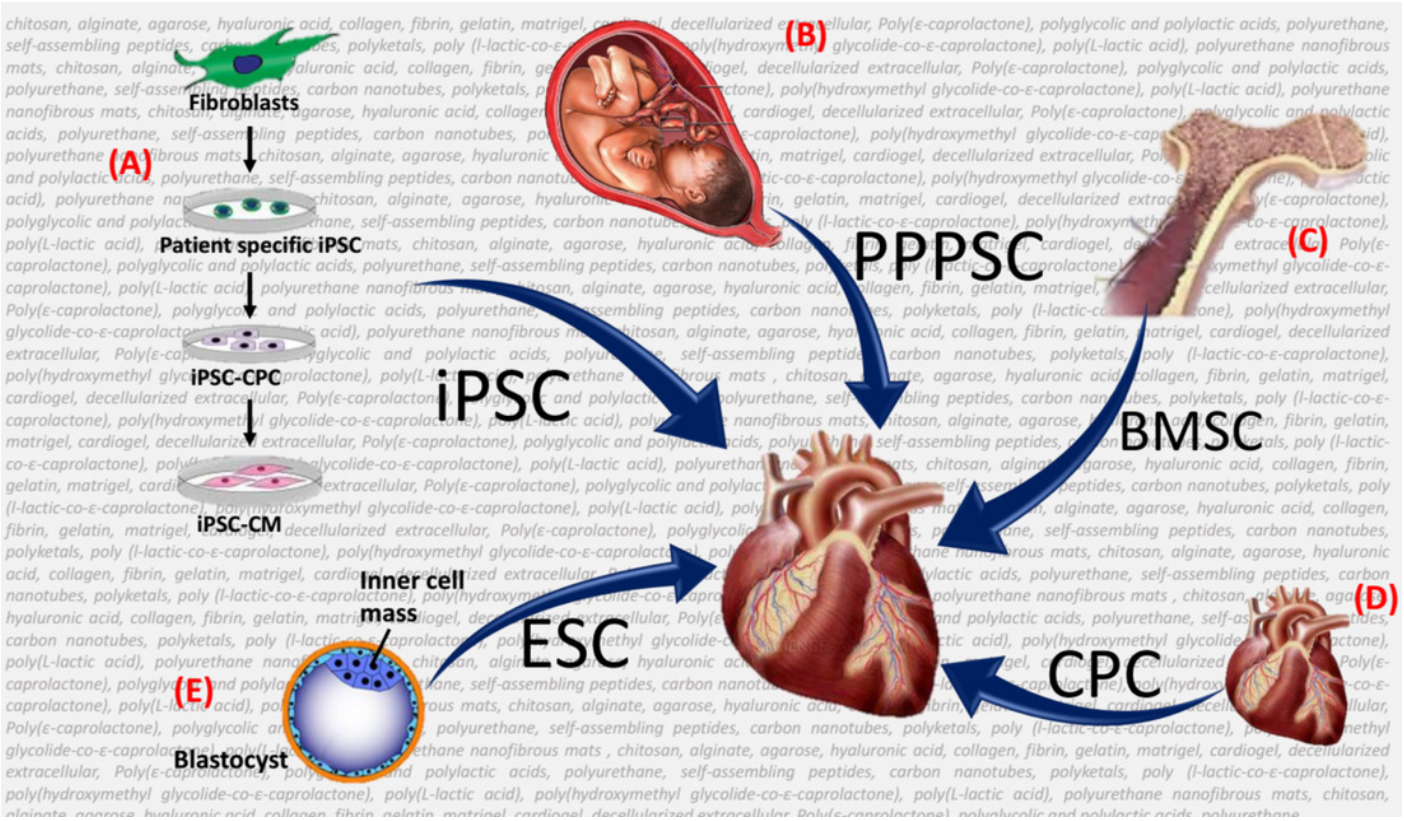
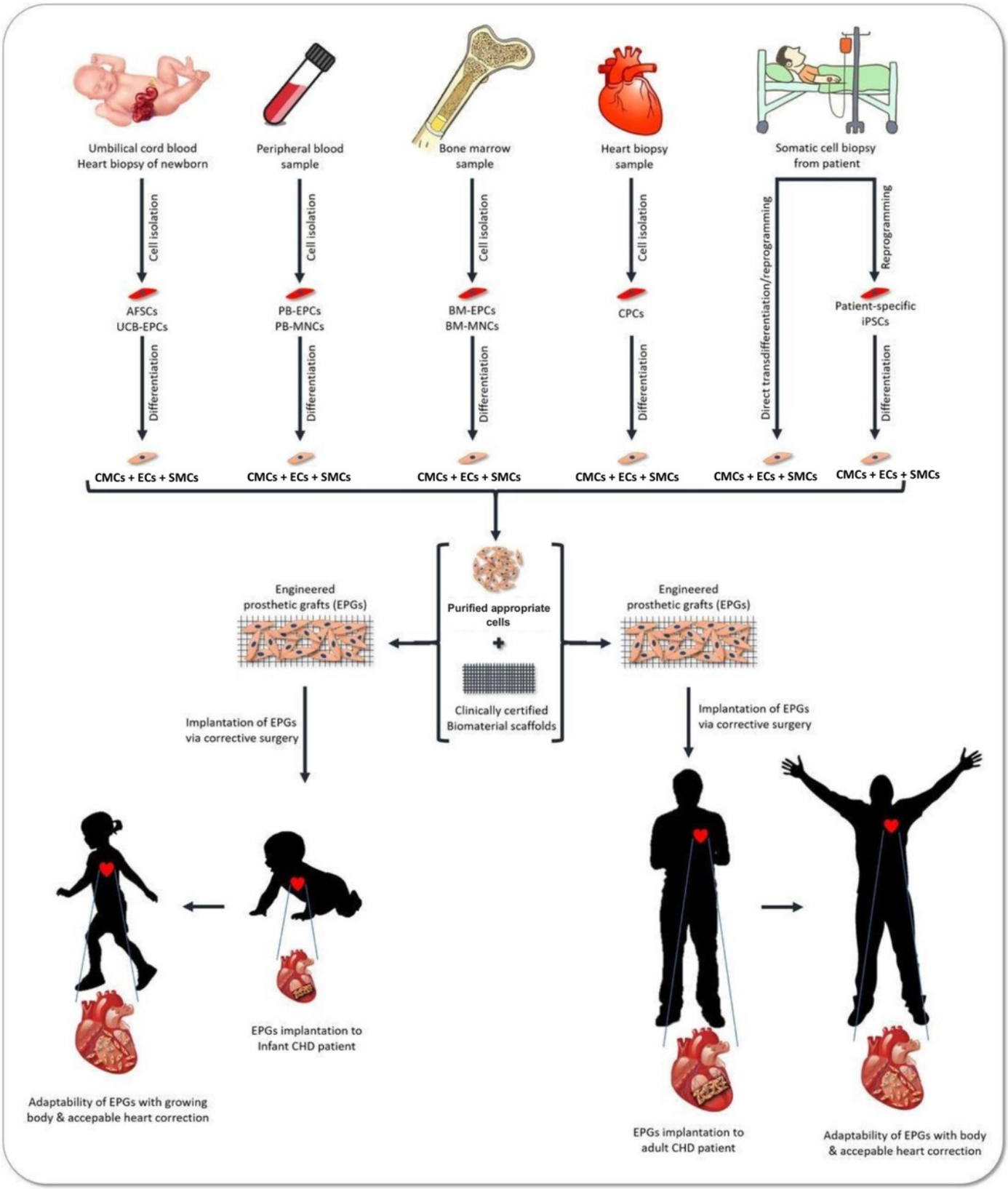


Figure 2

Promising strategies for CHDs treatment

The schematic diagram represents the potential of Stem Cells (SCs) and Tissue Engineering (TE) for corrective surgical treatment of infants as well as adolescent patients with Congenital Heart Disease (CHD). Various sources for Stem Cells (SCs) are presented here as alternatives to harvesting the appropriate Stem Cells (SCs) which can be used to seed on clinically certified biomaterial scaffolds for reconstructing functional cardiac tissue-engineered grafts. These grafts could be implanted via the corrective surgery into the heart of infants and adolescent patients with Congenital Heart Disease (CHD) for definitive correction of cardiac defects. These optimized cardiac-tissue engineered grafts should have the potential to grow in parallel with the child, 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
Direct transdifferentiation/reprogramming
Reprogramming
AFSCs
UCB-EPCs
PB-EPCs
PB-MNCs
BM-EPCs
BM-MNCs
CPCs
Patient-specific iPSCs
Differentiation
Differentiation
Differentiation
Differentiation
Differentiation
CMCs + ECs + SMCs
CMCs + ECs + SMCs
CMCs + ECs + SMCs
CMCs + ECs + SMCs
CMCs + ECs + SMCs
CMCs + ECs + SMCs
Engineered prosthetic grafts (EPGs)
Purified appropriate cells
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