

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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25 **ABSTRACT**

26 Congenital heart disease (CHD) affects a considerable number of children and adults worldwide.
27 This implicates not only developmental disorders, high mortality, and reduced quality of life but
28 also, high costs for the healthcare systems. CHD refers to a variety of heart and vascular
29 malformations which could be very challenging to reconstruct the malformed region surgically,
30 especially when the patient is an infant or a child. Advanced technology and research have offered
31 a better mechanistic insight on the impact of CHD in the heart and vascular system of infants,
32 children, and adults and identified potential therapeutic solutions.

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

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

45

46 1.0 INTRODUCTION

47

48 Congenital heart diseases (CHD) refer to the abnormal formation of the infant's heart, great
49 thoracic vessels and heart valves during intra-uterine development [1]. CHD is different from the
50 acquired heart diseases which occur because of lifestyle or aging [2]. The abnormalities are
51 structural defects, such as valve defects, intravascular or intracardial stenosis, congenital
52 arrhythmias or cardiomyopathies which greatly affect the early and future life of a CHD patient
53 [1, 3]. People born with CHD need immediate medical care after birth which further continues
54 throughout their lives. In 2010, it was estimated that only in the USA approximately 2.4 million
55 people suffered from CHD and more than half of them were adults [4]. In Europe, for instance, for
56 the period of 2000 to 2005, about 36,000 live births per year were diagnosed with CHD [5]. The
57 number grows bigger when including the unborn that were diagnosed with CHD and died either
58 by the termination of pregnancy or by intrauterine death or even neonate death[5]. In the UK,
59 about 8 in every 1,000 live babies born have a heart or circulatory condition [1]. Some estimate
60 those numbers to be higher and, commonly, percentages of each type of CHD change depend on
61 the geographical area of investigation [6, 7], table 1 further mentions other CHD frequencies for
62 other countries. CHDs not only have an effect on the individual's and their family's life but also,
63 have a huge financial impact on healthcare system. According to NHS England for the financial
64 year 2013/14 the total spent on CHD was £175 million [8] and in the US the total cost for CHD
65 treatment in 2008 was approximately \$298 billion [9]. In general, the number of children and adults
66 being diagnosed with CHD increases due to improved technology of diagnostic tools [6].

67 CHD can be diagnosed using transabdominal fetal Doppler echocardiography. Such prognostic
68 protocols are performed in high-risk groups of pregnant women, like those with a family history
69 of CHD [10]. In adults with CHD, the most effective diagnostic practice is transesophageal
70 echocardiography, electrocardiogram, pulse oximetry, X-rays, cardiac catheterization and MRI
71 [11]. The CHDs are managed by surgery, and the efficiency of this approach is largely dependent
72 on the materials that are used during the surgery. These materials are expected to be close to the
73 native cardiac tissue in both structure and function. In structure, CHD could present extremely
74 complicated malformations which cannot be spontaneously or by singular surgical procedure
75 reconstructed, hence the dire need for more research into biomaterials for Tissue Engineering (TE).

76 The recent extensive research focuses on possible ways to fabricate a near ideal tissue. So far, TE
77 appears to be the way forward in creating ideal tissue that can probably mimic the native heart
78 tissue both in structure and function. TE refers to creation of functional three-dimensional tissue
79 using biomaterials and cells for replacement or restoration of damaged organs and/or parts of them.
80 TE is the most promising approach at the present for CHD, as treatment can be “patient-specific”
81 and the engineered tissue could adjust to the developing body of the recipient. Many would think
82 that TE is an idea which conceived and developed in a very recent past. However, it has been
83 proven that tissue regeneration and TE is a concept which was born thousands of years ago, and it
84 has inspired Greek mythology, history, arts, and religion. In arts, religion inspired the well-known
85 painting of “Healing of Justinian” based on the miracle of St. Cosmas and St. Damian, physicians
86 and Christian martyrs who appear to have transplanted the leg of an Ethiopian to the body of a
87 patient [12]. The closest to an artificial replacement of a body part was discovered in Egypt on a
88 mummy which had a wooden replacement of the hallux [13]. However, today, TE involves a
89 combination of creating scaffolds and cell seeding. With regards to the heart, the most commonly
90 used and known artificial parts are the mechanical heart valves and conduits [14, 15]. The first
91 artificial heart valve was placed on live patients only in the last century [14, 16]. In 2003, some
92 evidence was presented to support the regenerative ability of the adult heart [17].

93 This evidence shows the existence of cardiac stem/progenitor cells which can differentiate into
94 new cardiomyocytes and participate in cardiac regeneration [17]. In contrast to the adult heart,
95 infants’ and children’s hearts regenerate in a larger capacity because the regenerative ability is
96 proportionally correlated to age [18]. Additionally, there is an insufficient number of heart donors
97 which becomes more challenging because of the heterogeneous relation of recipient-donor and the
98 diverse range of CHD. These points result in high mortality rates and further financial costs to the
99 healthcare systems [19]. However, only a small number of preclinical studies have focused on
100 CHD treatments [20, 21]. Stem cells (SC) have been widely investigated mainly for myocardial
101 infarction (MI), as it is currently the leading cause of morbidity and mortality worldwide [22]. Cell
102 seeding is a fundamental component of TE. Several studies have examined the possibility of direct
103 cell delivery in the damaged area, cardiac patch implantation and engineered heart tissue, with the
104 former being the most popular [20, 22, 23]. All possible types of stem cells are under investigation
105 to identify the most appropriate cell types for tissue engineering using in corrective surgery of

106 CHD. This review looks into the congenital heart diseases, biomaterials and scaffolds, and, types
107 of stem cells used in TE.

108

109 **2.0 METHOD**

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

115

116 **3.0 Congenital Heart Disease: Types, malformations, presentations and interventions**

117 CHD includes a diverse range of conditions which shows a variety of symptoms, indications, and
118 malformations detected during pregnancy or after birth [11]. However, these malformations are
119 much influenced by the age of diagnosis [6]. The etiology of CHD is unknown, but it is generally
120 accepted that many factors or a combination of them could contribute to CHD and considered to
121 be caused by multifactorial inheritance. These factors could be genetic, epigenetic or
122 environmental factors such as alcohol and drugs consumption, as well as viral infections like
123 Rubella [11]. The severity of the disease varies, and a number of malformations could be present
124 in each case. Based on the severity of CHD, they are categorized to mild, moderate, and severe
125 CHDs, which the latter is subcategorized to Cyanotic and Acyanotic lesions [19]. The most
126 frequent type of severe CHD is Ventricular Septal Defect (VSD) [24]. VSD could cause
127 myocardial defects which disappear in the first year of the infant’s life [24]. Nevertheless, the VSD
128 could also cause some malformations which can be managed only by surgical intervention, that is,
129 infant pulmonary hypertension [6, 24]. The other CHD type is Atrial Septal Defect (ASD) which
130 is usually asymptomatic and in most of the case will only be diagnosed in adulthood [6].
131 Atrioventricular septal defects (AVSD) is mainly observed in trisomy 21 [6]. AVSD is usually
132 characterized by “complete AV-canals with one common AV valve for both ventricles and an
133 interatrial and intraventricular communication” and requires surgical correction. The results of
134 long-term patient follow up after operation have shown very satisfactory survival rate [25].

135 Another type of CHD, tetralogy of Fallot (ToF), is characterized by VSD, pulmonary stenosis,
136 right ventricular hypertrophy and over-riding of the aorta [26]. Infants who suffer from ToF will
137 require immediate surgical intervention for better survival rates and avoid cyanosis, a result of
138 inadequate pulmonary blood flow [26]. Calcific Aortic Valve (CAV), another type of CHD, is a
139 disease which progresses slowly and results to a mild valve thickening and obstructing blood flow,
140 aortic sclerosis or severe calcification with impaired leaflet motion [27]. CAV presents many
141 similarities with arteriosclerosis in adults which is caused by lifestyle or aging [27]. However,
142 CAV is a congenital, progressive disease which could be diagnosed in patients less than one year
143 of age, and those in childhood or even adulthood [28, 29]. Table 1 summarizes the frequencies of
144 congenital heart diseases, while Table 2 summarizes the types of CHDs, presentations and possible
145 management.

146

147 *Table 1: Frequencies of CHD in some regions*

148

149 *Table 2: Types, presentations and management of CHDs*

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

156 In contrast to the adult heart, infants' and children's hearts regenerate in a larger capacity because
157 the regenerative ability is proportionally correlated to age [18]. Additionally, there is an
158 insufficient number of heart donors which becomes more challenging because of the
159 heterogeneous relation of recipient-donor and the diverse range of CHD. The mortality rate among
160 all patients who are waiting for any type of organ transplantation is highest in infants who wait for
161 heart transplant [65, 66]. CHDs patients usually require medication, cardiac catheterization or a
162 series of surgical interventions throughout their life [11, 66]. This among other risks increases the
163 chances of HLA-sensitization which eventually makes it more difficult to find a cross-match [66].
164 The main challenge with artificial materials is the alterations in the size and function of the heart
165 from the neonatal period, infancy, and to adulthood, to which current artificial materials cannot
166 adjust to [11]. The need for early intervention is essential for normal physical and cognitive
167 development [64].

168 The use of bioprosthetics including allografts and xenografts is widely used for treatment of CHD
169 as they present good survival rates in the patient and fewer interventions throughout their lifetime
170 [65]. The observed symptoms, defects, and complications upon bioprosthetic treatment of CHD
171 patient could differ from case to case [24, 64].

172

173 **4.0 Heart, a complex organ for tissue engineering**

174 Unlike other human tissue, cardiac tissue is a more complex tissue considering not only because
175 of its mechanical and structural function but also due to its electrical properties as well. The human
176 heart mainly consists of cardiomyocytes, functions as a blood pump which is regulated by the
177 electrical signal generated by the pacemaker cells in the sinoatrial nodes. This signal is directed
178 and spread through the atrioventricular node to Purkinje fibers, and this is highly important for the
179 direction of blood flow [67]. The diastolic and systolic function of the heart is necessary to be
180 synchronized and adjusted according to the body needs. The isolation of this signal from the rest
181 of cardiac tissue is as important as this electrical signal by himself. This importance is achieved
182 by the extracellular matrix (ECM) of heart which is also responsible for mechanical support and

183 endurance [67]. Aside the functional aspects which briefly mentioned above, the structural aspects
184 (the cardiomyocyte and its ECM) are another factor for strong consideration in the success of TE.
185 To yield an engineered tissue with the best functionality, this engineered tissue must be similar in
186 every sense to the native tissue. The ECM of the heart is mainly a complex mesh of structural
187 elements such as cardio fibroblasts and collagen fibrils, and non-structural elements such as
188 proteoglycans, glycosaminoglycans, and glycoproteins [68, 69] among other components.
189 Repairing the heart using materials which do not have or do not comply with the above
190 characteristics and cannot work in harmony with the host-heart, will result in a non-efficient
191 functioning heart accompanied by a series of complications. The concept and act of repairing the
192 heart using various engineered techniques has evolved over the years from the use of artificial
193 heart valves and grafts to bioprosthesis, and currently forward to the use of biomaterials and
194 scaffolds cells.

195

196 **4.1 Artificial Heart Valve and Grafts**

197 Charles Hufnagel was the first to experiment on animals with an artificial valve which he designed
198 in the 1940's [15]. A few years later, the same type of valve was transplanted into humans [15].
199 Nonetheless, Hufnagel's valve required changes which were succeeded by Harken-Soroff and later
200 by Starr-Edwards ball valve [15]. Following several improvements, the latest version is made of
201 pure carbon as a lighter, smoother material for blood flow and more durable in comparison to other
202 materials [14]. Due to the position of the valve, there is a high transvascular pressure which leads
203 to 'impact wear' and 'friction wear' [14]. Further complications with the use of valve replacements
204 include inflammation around the prosthesis and calcification of the valve itself [70]. The main
205 disadvantage of artificial valves is the thromboembolic risk which leads to lifetime treatment with
206 anticoagulants. This type of treatment involves various complications and brings different risks to
207 patient [14]. Similarly, to valves, artificial vascular grafts have been used for many years as a
208 surgical treatment for CHD [71]. Nevertheless, they provoke an inflammatory response, and they
209 are much less flexible than the body's natural tissue [64]. Originally porous fabric knitted of
210 Dacron and polytetrafluoroethylene (PTFE) was used for stenosis treatment. Later alterations in
211 the porosity of fabric were introduced to prevent the material's corruption [64]. The first attempt
212 to combine biomaterials with patient's cells was made by Wesolowki and colleagues by

213 “preclotting the graft with the patient’s blood” [64]. However, future attempts to produce
214 successful results created many doubts about the actual function of cells following citation on clot
215 [64]. Nonetheless, both artificial heart valves and conduits require continuous use of
216 anticoagulants. Remarkably, they do not grow with the patients’ heart as patients with CHD are
217 highly likely to require surgical intervention when they are an infant or a child [64, 72].

218

219

220 **4.2 Bioprosthetics: Allograft, Xenografts, and Autografts**

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

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

236 In a study carried out by Homann and colleagues, the outcomes of 25 years using allografts and
237 xenografts for reconstruction of the right ventricular outflow tract showed 66% survival at 10-
238 years’ follow up. Furthermore, patients with allografts had a mean reoperation-free interval time
239 of 16 years in contrast to the xenograft recipients which this interval time is 10.3 years [65].
240 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 by
242 stem cell-seeded on artificial or natural scaffold [16].

243 The relatively recent “CorMatrix” patch fabricated from the decellularized porcine small intestinal
244 submucosa extracellular matrix (SIS-ECM) mainly composed of collagen, elastin, glycan, and
245 glycoproteins have been introduced into cardiac surgery. SIS-ECM has not only been used in
246 animal models for cardiac surgery [65-77], but also in humans studies, for cardiac and vascular
247 reconstructions such as; augmentation of the tricuspid valve [78], vascular repair of ascending
248 aorta, aortic arch, right ventricular outflow tract, pulmonary artery, valvular reconstruction [79],
249 and closure of septal defects [80]. The study by Witt et al. reported a small risk of stenosis when
250 SIS-ECM is used in the reconstruction of the outflow tracts and great vessels. Interestingly, the
251 SIS-ECM has effectively proved the function in the high-pressure vessels [80]. The pitfall of this
252 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 septal
254 defect [64]. The autologous pericardium is the best choice for infants as it is free, it does not
255 provoke any immune-response, and it is sterile. Even though it requires some preparation before
256 application, autologous pericardium creates less fibrotic tissue in comparison to Dacron [64].
257 Allograft pericardium is available, but quite a few risk factors are associated with its use [64]

258

259 **4.3 Biomaterials and Scaffolds for tissue engineering**

260 Generally, scaffolds work as a primary base for cells to enhance and produce relevant tissue. The
261 scaffolds should have specific morphological, functional, and mechanical properties to support
262 cells survival and differentiation [23]. Biomaterials used to produce scaffolds should be made of
263 components which will accommodate the above characteristics and create a friendly cell
264 microenvironment [23]. The previously mentioned characteristics apply for all different types of
265 engineered tissue, and the goal is to mimic host tissue in the best possible way. With regards to
266 artificial and bioprosthetic cardiac correction choices, scaffolds and biomaterials should contain
267 various properties such as being biodegradable, biocompatible, flexible and durable and absence
268 of immunogenicity and calcification. Due to the variety of sizes of patients’ heart and defects,
269 designed scaffolds should have various size and ability to be able to grow and adapt to the heart

270 [74]. The biomaterial should allow neo-vascularization for adequate oxygenation of the tissue,
271 create minimal scaring tissue and thrombotic risk, as the latter could lead to life treatment with
272 anticoagulants [81, 82]. Furthermore, these biomaterials scaffolds should be bioactive, meaning
273 they should enhance cellularization in vitro and in vivo, and optimize cell efficiency and degrade
274 at a desirable rate [82]. What is more, biomaterials and scaffolds should be in abundance and cost-
275 effective, as high cost could restrict development and use of it in TE as a routine therapeutic choice
276 [83]. The most common biomaterials for cardiac and vascular TE used today are synthetic, and
277 natural polymers [67], and the electrospinning technique has been proven to be the most efficient
278 way to produce scaffolds with these biomaterials [84].

279

280 **4.3.1 Synthetic polymers for cardiac scaffolds**

281 The easiest way to have materials in abundance is to manufacture them. The need for suitable
282 biomaterials for cardiac tissue repair has triggered development of synthetic polymers which are
283 easy to fabricate and manipulate. These polymers can be manipulated with respect to their physical
284 properties, molecular weight, heterogeneity index, and degradation speed [85]. Many synthetic
285 polymers are biocompatible and have excellent mechanical properties which make them a popular
286 choice for sutures and mesh production [67]. Frequently used polymers in cardiac surgery are
287 polyglycolic acid (PGA) and polylactic acid (PLA). These two polymers have been used as a single
288 biomaterial or as 50:50 composite to reconstruct tissue-engineered vascular grafts for treating
289 children with congenital heart disease [86]. Carrier et al. Presented acceptable ultrastructural
290 features and metabolic cell ability when cells were cultured on PGA scaffold in a rotating
291 bioreactor [87, 88]. The rotating bioreactor increases cell adherence and decreases cell damage
292 [87]. The highest concern with synthetic polymers is their toxicity. Therefore, the use of poly-L-
293 lactic acid (PLLA) has increasingly become more of interest. PLLA has demonstrated very good
294 results when combined with bone marrow mesenchymal SC (BM-MSC) for vascular tissue
295 engineering [89]. In vivo studies by Hashi et al showed that nanofibrous scaffolds created with
296 PLLA can remodel in both cellular and ECM content, similar to that of the native artery [89]. Both
297 acellular and were implanted into the common carotid artery of live animal models (rats). The
298 cellular scaffold was cited with MSC and “exhibited very little platelet aggregation on the luminal
299 surfaces” compared to the acellular grafts. This is due to the antithrombogenic property of MSC

300 [89]. PLLA, when degraded in the body, can “be excreted in carbon dioxide and water” [67].
301 Polyurethane, unless copolymerized, is biocompatible but not biodegradable. Polyurethane has
302 been successfully experimented in combination with other materials for cardiac tissue repair, such
303 as siloxane films [90, 91], cellulose [92], urea [93], PLLA [94]. Poly (ϵ - caprolactone) in
304 combination with other biomaterials have also been proven to be efficient composite for cardiac
305 tissue repair. They have been used in combination with PLLA alone [95, 96] PLLA and collagen
306 [97], polypyrrole and gelatin [98], polyglycolic acid [99], poly (hydroxymethyl glycolide)
307 [100], chitosan and gelatin [101].

308

309 Based on our understanding of the heart as an electroactive tissue, Hitscherich and colleagues have
310 created a piezoelectric scaffold fabricated by electrospinning Polyvinylidene Fluoride-
311 Tetrafluoroethylene (PVDF-TrFE) for cardiac tissue engineering [102]. The combination of
312 synthetic with natural polymers has been suggested to increase cell adherence. However, pure
313 natural polymers have also been examined as an option for polymers [67].

314

315 **4.3.2 Natural polymers for cardiac scaffolds**

316 Natural polymers are biodegradable, biocompatible and easily manipulated matrices composed of
317 complex elements which make up the native tissue [103] The natural polymers used so far for
318 cardiac repair include collagen, gelatin, alginate, silk, fibrin, chitosan and hyaluronic acid [67].
319 Despite their poor mechanical properties, they are good biomaterial for heart TE, as they have high
320 biocompatibility, promote cell-binding and could biodegrade with no “additional treatment or
321 modifications” [67].

322 Collagen is the most widely utilized natural polymer which is the most abundant ECM protein. It
323 functions to guide biological processes, provide structural scaffolding, and tensile integrity [101].
324 Several kinds of literature have reported the use of various collagen types and their modifications
325 in cardiac tissue repair [104-111].

326 Fibrin can be manipulated to create gels, microbeads, and hydrogels [85]. Likewise, biological
327 molecules like the growth factors can be incorporated [112]. Fibrin glue can be used as a stand-

328 alone therapy in cardiac tissue repair as it possesses intrinsic regenerative properties [113]. The
329 success of fibrin patch seeded with human embryonic stem cell-derived cardiac progenitor cells
330 (hESC-CPC) in non-human primate model [114] has resulted in its translation to the first case
331 report of the use of hESC-CPC in severe heart failure with an encouraging patient functional
332 outcome [115]. Other studies on fibrin have demonstrated its efficacy as a sealant after
333 intramyocardial injection [116]; for myocardial tissue repair when seeded with adult stem cells,
334 neonatal cardiac cell, and mesenchymal stem cells [117-119]; to form aortic valves in tissue
335 engineering [120].

336

337 Chitosan has been experimented in several literature as a biomaterial for cardiac regeneration [121,
338 122]. Overall, in cardiomyogenesis, many researchers have agreed on the fact that chitosan seems
339 to be more effective when combined with other factors enhancing integration of stem cells into
340 cardiac tissues [123].

341

342 Alginate, when used alone, has proven to have a remarkable effect on the function of heart models
343 with myocardial infarction. Furthermore, seeding alginate with stem cells has proven to be more
344 efficient in repair of the cardiac tissue [124-127].

345 The use of hyaluronic acid has been shown to be largely dependent on its molecular weight, and
346 several kinds of literature have reported its successful use in cardiac tissue repair [128-131].

347 Evidence of gelatin scaffold placed subcutaneously, and/or on infarcted myocardium in adult rat
348 hearts have shown a good survival of the graft, vessel formation and junctions with recipient rat
349 heart cells [132]. Gelatin was reported to sustain neonatal rat cardiomyocyte tissue in vitro for
350 three weeks [133]; supported the growth of human induced pluripotent stem cell (iPSC)-derived
351 cardiomyocytes [133]; its hydrogel seeded with autologous human cardiac-derived stem cell and
352 basic fibroblast growth factor (bFGF) effectively released bFGF for repair of ischemic
353 cardiomyopathy [134]; and several other studies have shown the efficacy of gelatin as a scaffold
354 for cardiomyogenesis when seeded with cells [135-138]. Fibrinogen/Thrombin-based Collagen
355 Fleece (TachoCombo) have been successfully used to secure hemostasis and enhance complete
356 reconstruction of a large pulmonary artery defect in a canine model [139]. Hence, this biomaterial

357 may be used in reconstruction of the low-pressure pulmonary vessels during a cardiac surgery for
358 a total anomalous pulmonary venous return or transposition of the great vessels.

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

361

362 **4.3.3 Native Extracellular Matrix as scaffold**

363 Native-specific ECM could be a category itself or part of natural polymers. ECM is collected from
364 animal or donor tissue and processed for culturing cells [141]. Studies have shown that the ratio
365 of native ECM in culture could play a key role in stem cells (SCs) enhancement, differentiation,
366 survival, and phenotype [142]. Other studies have shown that contractible engineered heart patches
367 cultured in ECM mixture and implanted in syngeneic Fischer 344 rats can vascularize, become
368 innervated and survive up to 8 weeks in vivo [141].

369 Furthermore, ECM of decellularized and repopulated hearts and other organs are being used for
370 drug testing [143]. An experiment on decellularized mouse hearts which were repopulated with
371 human cells through coronary vessels exhibited myocardium, vessel-like structures and
372 intracellular Ca²⁺ transients contracted spontaneously and responded as expected to various drug
373 interventions [144]. It was concluded that heart “ECM could promote proliferation, specific cell
374 differentiation and myofilament formation” [144]. The option of ECM for TE could help overcome
375 the challenges faced using synthetic and other natural biomaterials to replace tissue, valves or
376 organs [145].

377

378 **4.4 Scaffoldless Cell Sheet**

379 Another technique, which is independent of scaffolds, has been developed based on the cells’
380 ability to connect via cell-to-cell junction proteins and create ECM [88]. The cells are cultured in
381 normal conditions at 37°C in a temperature-responsive polymer cultures dish. When the culture
382 temperature conditions change, the cells detach from the polymer culture dish as one cell sheet
383 [88]. This technique was developed to avoid inflammatory reactions and fibrotic deposits in the
384 area of graft where scaffold was placed following degradation [88]. A study has shown that

385 contractile chick cardiomyocyte sheets could function effectively around rat thoracic aorta when
386 applied on host myocardium [81]. This cell sheet could synchronize within 1 hour of implantation
387 with the host tissue [81]. Similar results have been shown in 3D structures using several cell sheets
388 aiming to create a thick cardiac patch [146]. The number of sheets is limited, as more than three
389 exhibits poor vascularization. However, the combination of endothelial cells and cardiomyocytes
390 is being examined to promote vascularization before implantation [88]. Table 3 summarizes the
391 pros and cons of the various materials and biomaterials used in tissue engineering.

392

393 *Table 3 Advantages and disadvantages of materials and biomaterials used in TE*

394

395 **5.0 Stem Cells for tissue engineering**

396 An equally important point in choosing a suitable biomaterial, scaffold or scaffoldless cell sheet,
397 is the choice of the most appropriate cell types suitable for the TE. Stem cells (SCs) as a known
398 cell source possesses the ability to differentiate toward Cardiac Muscle Cells/cardiomyocytes
399 (CMCs), smooth muscle cells (SMCs) and endothelial cells (ECs), can regenerate cardiac tissue.
400 Based on these properties, they play a key role in TE field. The currently used SCs in TE are
401 summarized in Table 4.

402 **5.1 Embryonic Stem Cells**

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

420

421 5.2 Induced Pluripotent Stem Cells

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

443 Nonetheless, like ESC, iPSC has demonstrated tumorigenesis [74]. The group of Leda and
444 colleagues showed a direct transdifferentiation of fibroblasts to functional cardiomyocytes using
445 three key factors, Gata4, Mef2c, and Tbx5, within a very short time and suggested that direct
446 reprogramming could reduce the risk of tumorigenesis [154]. Still, using viral vectors for
447 reprogramming procedure is problematic and involves various risks [74]. Therefore, today more
448 different ways for iPSC production are being used and investigated to find out safer and more
449 effective alternatives for this reprogramming procedure [21, 155]. In the event this problem is
450 solved, iPSC could be the safest type of cell sources for TE as they will not provoke any immune-

451 response and cell harvesting procedure to produce iPSCs is not life-threatening for the patients.
452 Moreover, iPSC raises less ethical concerns in comparison to ESC or fetal SC.

453

454 **5.3 Prenatal, Perinatal, and Postnatal Stem Cells**

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

466

467 **5.4 Adult Stem Cells**

468 **5.4.1 Bone Marrow-derived Stem cells**

469 Apart from UCB, EPCs can be found in the peripheral blood (PB-EPCs) and bone marrow (BM-
470 EPCs) of adults [164,165]. However, bone marrow is a richer source of EPCs in comparison to
471 peripheral blood. In 1997, Asahara et al. identified the CD34+ mononuclear hematopoietic
472 progenitor cells in the peripheral blood which in vitro presented endothelial-like characteristics
473 [164]. The EPCs which are originated from BM considered to play a crucial role in endothelial
474 repair, and they have been suggested for treatment of ischemia patients and vascular TE with very
475 encouraging results [158,160,165,166]. A successful complete endothelium regeneration of
476 decellularized canine carotid arteries has been reported in animal studies using PB-EPCs [167]. In
477 addition to vascular TE, the EPCs have been assessed for tissue-engineered heart valves [168].

478 Bone marrow derived EPCs are greatly involved in de novo vessel formation and
479 neovascularization in pathological conditions like ischemia and cancer [164]. Similar applications
480 to PB-EPCs and prenatal EPCs have been recorded for vascular graft in the congenital heart
481 surgery using bone marrow-derived stem cells [169]. Mirensky and colleagues used sheets of non-
482 woven PGA mesh as a scaffold to create vascular graft in combination with human bone marrow
483 mononuclear cells (BM-MNCs). The results were very encouraging as no aneurysm or thrombotic
484 incidence were reported, despite the absence of anticoagulants. This group suggests this method
485 as a suitable vascular treatment for CHD based on their results from 6 weeks follow up after graft
486 implantation, which has shown signs of degradation, and it was fully accommodated by the host's
487 cells [169]. Nonetheless, the host mice were at full-growth which makes it questionable how
488 successful this application could be in a developing animal model. Interestingly only one week
489 after implantation no human BM-MNCs were detected, which suggests that BM-MNCs play a
490 paracrine role rather than cell replacement [165]. More studies have reported similar results with
491 the same conclusion [74,170]. Furthermore, an investigation on 25 young patients under 30 years
492 old who underwent extracardiac total cardiopulmonary connection with BM-MNCs engineered
493 vascular graft has also presented convincing results. A long-term patient follow-up has shown zero
494 deaths in relation to the implanted grafts, no thromboembolic, hemorrhagic, or infectious
495 complications, however, 6 of them developed grafts stenosis which was treated successfully [74].
496 Despite the encouraging results with BM-MNCs, BM-MSCs still present more advantages. These
497 advantages are, for example, their ability to differentiate into a variety of cell types even progenitor
498 cells; relatively easy procedure for their collection, isolation, storage, and proliferation; presenting
499 a similar phenotype to the valve cells; present anti-thrombogenic properties; and their
500 immunogenicity is manageable [89, 145]. In a comparative study, Vincentelli et al. examined
501 short- and long-term characteristics of the porcine decellularized scaffold which were processed
502 with in-situ injections of BM-MNCs and BM-MSCs, before transplanting in a lamb of animal
503 models [171]. Short-term results did not show any significant differences. However, the 4 months
504 (long-term) follow up has shown a significant decrease of transvalvular and distal gradients, more
505 inflammatory reaction, more structural deterioration as well as calcification, and a thick fibrous
506 pannus around the suture line in the BM- MNCs group. These observations in the BM-MNCs
507 group were significantly different from the BM-MSCs group [171].

508

509 5.4.2 Cardiac Progenitor Cells

510 Cardiac progenitor cells (CPCs) or also known as Cardiac Stem Cells (CSCs) are a type of cells
511 which are found in the adult heart and express stem cell factor receptor kinase c-kit⁺, also shown
512 to be negative for the markers of blood/endothelial cell lineage [17]. CPCs have only been in the
513 spotlight for about 15 years now with the credit given to Beltrami and colleagues as they
514 demonstrated the self-renewing and multipotent characteristics of these cells; differentiating into
515 all three different cardiogenic cell types which are cardiomyocytes, smooth muscle cells and
516 endothelial cells [17]. More recently, a study carried out by Vicinanza and colleagues helped to
517 lay to rest the controversies about c-kit⁺ cardiac cells by demonstrating that only a small fraction
518 of the c-kit⁺ adult cardiac stem cells possess the tissue-specific progenitor properties [172]. The
519 study was carried out in adult Wistar rats in which an experimental acute myocardial infarction
520 (MI) was induced and the border of the infarcts were injected with GFP+c-kit⁺ for the first group,
521 “GFP-expressing CD45-c-kit⁺ CSCs (CSC^{GFP})” for the second group, and the placebo group were
522 injected with PBS. The study showed that CSC^{GFP} only significantly reduced apoptosis and
523 hypertrophy of the cardiomyocytes, it also significantly reduced scarring and improved ventricular
524 functions [172]. The study further demonstrated that about 10% of the overall c-kit⁺ cardiac cells
525 are CD45-c-kit⁺, and only about 10% of these are clonogenic and multipotent. Therefore, it was
526 inferred that only about 1-2% of the total c-kit⁺ myocardial cells have a demonstrable multipotent
527 CSC phenotype [172]. These c-kit⁺CD45⁻ (deletion of which also renders the cells CD31⁻, CD34⁻)
528 CPCs were also shown to express to some degree Sca-1, Abcg2, CD105, CD166, PDGFR- α , Flk-
529 1, ROR2, CD13, and CD90 [172]. The number of CPCs have however been shown to be
530 significantly higher in neonates but dramatically decreases after the age of 2 [71, 173]. Thus, these
531 CPCs in combination with a suitable scaffold, could be the answer to treat a number of CHD, as
532 CPCs could be collected during palliative surgery or via endomyocardial biopsy [19,64].

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

535

536 *Table 4 Scaffolds and SCs used for TE in some study models*

537

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

547

548

549

550 **Figure 2 :** The schematic diagram represents the potential of Stem Cells (SCs) and Tissue
551 Engineering (TE) for corrective surgical treatment of infants as well as adolescent patients with
552 Congenital Heart Disease (CHD). Various sources for Stem Cells (SCs) are presented here as
553 alternatives to harvesting the appropriate Stem Cells (SCs) which can be used to seed on clinically
554 certified biomaterial scaffolds for reconstructing functional cardiac tissue- engineered grafts.
555 These grafts could be implanted via the corrective surgery into the heart of infants and adolescent
556 patients with Congenital Heart Disease (CHD) for definitive correction of cardiac defects. This
557 optimized cardiac-tissue engineered grafts should have potential to grow in parallel with the child,
558 while are lacking any tumorigenicity, immunogenicity, thrombogenicity, calcification, and other
559 risk factors.

560

561 **7.0 STANDPOINT**

562 Due to the high number of patients as well as newborns who are suffering from CHD and also high
563 costs of their implications, it is necessary and vital to making intensive research for finding an
564 effective treatment for CHD patients. Stem cell research has shown remarkable results in all kinds
565 of tissue engineering including skin [174], cartilage [175], vascular [176], ocular [177] and cardiac
566 tissues [82, 87, 178, 179].

567

568 The importance of engineered cardiac tissue lies in the fact that synthetic non-degradable materials
569 cannot adjust to the patient's developing body. A number of patients who suffer from CHD are
570 adults, and they are more suitable for this type of therapies. However, many patients with severe
571 CHD are infants and children whose body is constantly developing. Although, various synthetic
572 and natural biodegradable biomaterials have been used so far which have shown good results, the
573 one with the best degradation rate is yet to be found. There are various complications related to
574 existing surgical treatments and scaffolds which cannot be ignored. Calcification, inflammatory
575 reaction and life-long anticoagulants treatment are the most important known complications for
576 the conventional methods of CHD treatment [14, 70]. The complexity of CHD makes TE possibly
577 the most suitable solution for treatment of patients with CHD.

578

579

580 **8.0 CONCLUSION**

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

591

592

593

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601

602

603 **Reference**

604

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Table 1 (on next page)

Frequencies of CHDs in some regions

1

2

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

Table 2 (on next page)

Types, presentations and management of CHDs

1

Types of CHD	Presentations of CHD	Medical & Surgical Therapeutic Approach to CHD
Atrial Septal Defects	<ul style="list-style-type: none"> ➤ 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 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]. 	<ul style="list-style-type: none"> ➤ 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). ➤ Surgery: can be done through Cardiac catheterization or Open-heart surgery. ➤ Follow-up care [31, 32]
Hypoplastic Left Heart	<ul style="list-style-type: none"> ➤ 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: <ol style="list-style-type: none"> 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 	<ul style="list-style-type: none"> ➤ 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: <ol style="list-style-type: none"> a. Norwood Procedure: performed on the infant within 2 weeks of a

	ankles and feet [45, 46].	<p>d. Bi-directional Glenn Procedure: performed around 4 to 6 months of the baby's life.</p> <p>e. Fontan Procedure: done around 2 years of age [45].</p>
Tetraogy of Fallot	<ul style="list-style-type: none"> ➤ 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]. 	<ul style="list-style-type: none"> ➤ Medication: Prostaglandin E₁ infusion. ➤ Surgery: <ul style="list-style-type: none"> a. Temporary surgery (palliative surgery): improve blood flow to the lungs. b. Intra-cardiac repair: done during the first year after birth [47].
Tetraogy of Fallot <i>(continued)</i>	<ul style="list-style-type: none"> ➤ 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 	<ul style="list-style-type: none"> ➤ Surgery: <ul style="list-style-type: none"> a. Aortic valve replacement b. Balloon valvuloplasty c. Aortic valve repair d. Aortic root and

<p>Bicuspid Aortic Valve</p>	<p>valve from opening completely, which reduces or blocks blood flow from the heart to the body.</p> <ul style="list-style-type: none"> ➤ Trouble breathing ➤ Chest pain or pressure ➤ Fatigue ➤ Heart racing ➤ Light-headedness ➤ Fainting [49]. 	<p>ascending aorta surgery [50]</p>
<p>Patent Ductus Arteriosus</p>	<ul style="list-style-type: none"> ➤ 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 rate [51]. 	<ul style="list-style-type: none"> ➤ 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
<p>Pulmonic Valve Stenosis</p>	<ul style="list-style-type: none"> ➤ 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] 	<ul style="list-style-type: none"> ➤ Surgery: <ol style="list-style-type: none"> a. Balloon valvuloplasty b. Open-heart surgery [53]

<p>Pulmonic Valve Stenosis <i>(continued)</i></p>		
	<ul style="list-style-type: none"> ➤ 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 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 	<ul style="list-style-type: none"> ➤ Smaller holes resolve themselves with time. ➤ Surgery: Usually done on larger fissures. It is indicated where patients express symptoms of heart failure, left heart overload and history of endocarditis. ➤ Percutaneous techniques: <ol style="list-style-type: none"> 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].

<p>Ventricular Septal Defect</p>	<p>as is burned. This impedes growth.</p> <p>➤ Airway Obstruction:</p> <ol style="list-style-type: none">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 such as wheezing, and tachypnea can be observed [54].c. Holosystolic/pansystolic murmur on auscultation [56]	
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<p style="text-align: center;">Ventricular Septal Defect</p> <p style="text-align: center;"><i>(continued)</i></p>		
<p style="text-align: center;">Total Anomalous Pulmonary Venous Condition</p>	<ul style="list-style-type: none"> ➤ 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]. 	<ul style="list-style-type: none"> ➤ 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]
	<ul style="list-style-type: none"> ➤ A condition whereby the aorta and the pulmonary arteries are transposed. The aorta arises from the right ventricle and 	<ul style="list-style-type: none"> ➤ Medications: Prostaglandin E1 is administered to keep the ductus arteriosus open.

<p>Trans-Position of the Great Arteries</p> <p>Trans-Position of the Great Arteries <i>(continued)</i></p>	<p>leads to the lungs. The pulmonary artery arises from the left ventricle and leads to the body.</p> <ul style="list-style-type: none"> ➤ 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 [58] 	<ul style="list-style-type: none"> ➤ Surgery: <ol style="list-style-type: none"> a. Balloon atrial septostomy: A catheter is threaded through the foramen ovale. A balloon is inflated to rip a fissure in the atrium [58]
<p>Truncus Arteriosus</p>	<ul style="list-style-type: none"> ➤ 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] 	<ul style="list-style-type: none"> ➤ Surgery: The truncus arteriosus must be separated into two vessels. This would allow separate blood flow channels to the heart and body [59]
	<ul style="list-style-type: none"> ➤ 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 	<ul style="list-style-type: none"> ➤ Surgery: <ol style="list-style-type: none"> a. Only required where patient manifests severe symptoms. b. Cone procedure: Where the anterior septal leaflet is maneuvered and

<p>Ebstein's Anomaly</p>	<p>become thin.</p> <ul style="list-style-type: none"> ➤ 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] 	<p>sewn to the true annulus. This attachment causes it to be conical in shape.</p> <ul style="list-style-type: none"> c. Valve replacement d. Where the defective valves can be surgically replaced [60]
<p>Ebstein's Anomaly <i>(continued)</i></p>	<ul style="list-style-type: none"> ➤ Characterized by a restriction 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 	<ul style="list-style-type: none"> ➤ Surgery: <ul style="list-style-type: none"> 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

<p>Pulmonary Atresia</p>	<p>where there are collateral arteries supplying the lungs.</p> <ul style="list-style-type: none"> ➤ 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]. 	<p>arteriosus open. It could also be done surgically.</p> <ul style="list-style-type: none"> b. Fontan's procedure: Done for patients with a hypoplastic right ventricle. The right atrium is surgically connected to the pulmonary artery [61].
<p>Aortic Stenosis</p>	<ul style="list-style-type: none"> ➤ 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] 	<ul style="list-style-type: none"> ➤ Valve replacement : There are two approaches: <ul style="list-style-type: none"> a. Surgically b. Trans-catheter approach [62].
<p>Coarctation of the Aorta</p>	<ul style="list-style-type: none"> ➤ 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] 	<ul style="list-style-type: none"> ➤ Surgery: <ul style="list-style-type: none"> a. Coarcted portion 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.

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Table 3 (on next page)

Advantages and disadvantages of materials and biomaterials used in TE

	Artificial Prosthesis	Biological Prosthesis	Biomaterial Scaffolds	Scaffoldless Tissue
Advantages	<ul style="list-style-type: none"> • Available in abundance • Many different sizes • Long term results available 	<ul style="list-style-type: none"> • Available in abundance • No requirement for life-long treatment with anticoagulants 	<ul style="list-style-type: none"> • Good mechanical properties • Ultrastructural features • Cell adherence • Biocompatibility • Biodegradable 	<ul style="list-style-type: none"> • No need for scaffold • Spontaneous and synchronous pulsation • Could create tubular construct • Can grow with
Disadvantages	<ul style="list-style-type: none"> • Impact & friction wear • Inflammation • Calcification of valve • Less flexible than natural tissue • Life-long treatment with anticoagulants 	<ul style="list-style-type: none"> • Risk of cytotoxicity • Inflammation • Loss of mechanical properties • Calcification in infants and children • Immunological reactions 	<ul style="list-style-type: none"> • Some present toxicity • Risk of inflammation • Not all tune well with heart 	<ul style="list-style-type: none"> • Limited number of cell sheets (max 3) • Poor vascularization in more than 3 cell-sheets

Table 4 (on next page)

Scaffolds and SCs used for TE in some study models

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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

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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.

