



Review

Stem cell-based approaches in cardiac tissue engineering: controlling the microenvironment for autologous cells

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ABSTRACT

Cardiovascular disease is one of the leading causes of mortality worldwide. Cardiac tissue engineering strategies focusing on biomaterial scaffolds incorporating cells and growth factors are emerging as highly promising for cardiac repair and regeneration. The use of stem cells within cardiac microengineered tissue constructs present an inherent ability to differentiate into cell types of the human heart. Stem cells derived from various tissues including bone marrow, dental pulp, adipose tissue and umbilical cord can be used for this purpose. Approaches ranging from stem cell injections, stem cell spheroids, cell encapsulation in a suitable hydrogel, use of pre-fabricated scaffold and bioprinting technology are at the forefront in the field of cardiac tissue engineering. The stem cell microenvironment plays a key role in the maintenance of stemness and/or differentiation into cardiac specific lineages. This review provides a detailed overview of the recent advances in microengineering of autologous stem cell-based tissue engineering platforms for the repair of damaged cardiac tissue. A particular emphasis is given to the roles played by the extracellular matrix (ECM) in regulating the physiological response of stem cells within cardiac tissue engineering platforms.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide accounting for over 30% of deaths [1]. Despite currently available pharmacological approaches for the management of CVD, the quality of life and prolonged survival remain a challenge in the vast majority of patients. Cell-based therapies represent a potential promising approach to promote cardiac regeneration following myocardial infarction (MI), heart failure, heart valve damage, and in congenital heart diseases [2,3]. Over the past several decades, life-saving organ transplantations and the use of biomaterial-based implants have been employed extensively in people with organ damage or failure [4].

However, such interventions are associated with limitations including the lack of available or suitable organs and the need for continuous use of immunosuppressive drugs post organ transplantation, often associated with severe side effects [5]. Recent technological developments in tissue engineering including 3D bioprinting [6] and utilization of pluripotent and multipotent stem cells for organ or tissue regeneration, have shown promising potential [7]. One of the key factors in tissue engineering is the ability to have access to permissive biomaterials for optimal cell viability and function [8]. For instance, porous substrates and biodegradable materials are used to generate scaffolds and facilitate cell adhesion and proliferation [9,10]. Scaffolds based on biodegradable polymers (polylactic acid, polycaprolactone, collagen) or bioresorbable

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materials (hydroxyapatite, calcium phosphate, bioglass) are extensively used in various tissue engineering applications due to the cost effectiveness, biocompatibility and the ability to support cell proliferation [11]. Cells are then added to adhere and proliferate within these scaffolds, hence generating a cellular environment similar to that of the human extracellular matrix (ECM) [12]. Several types of cells including adult cells (e.g. keratinocytes, endothelial cells, fibroblasts) [13] and stem cells [e.g. mesenchymal stem cells (MSCs), dental pulp stem cells, induced pluripotent stem cells (iPSCs)] [14] are being utilized for these purposes.

Stem cells are undifferentiated and pluripotent cells capable of indefinite self-renewal as well as differentiation into a number of cell lineages [15]. Stem cells may be isolated from sources including bone marrow, placenta, dental pulp and adipose tissue [16]. Under physiological conditions, stem cells proliferate and repair the damaged tissues within bone marrow and skin whereas within the pancreas or the heart tissue, specific pathophysiological conditions are required for this process to happen [17]. Under specific experimental conditions, stem cells can be induced to differentiate into fibroblasts, smooth muscle cells [18], cardiomyocytes [19] or endothelial cells [20]. This is generally achieved under the influence of specific biological signals generated by growth factors [21], redox environment [22] and magneto-electrical stimulation [23,24]. The appropriate selection of the source of stem cells is one of the key factors that contributes to the effectiveness and viability of microengineered tissue [25]. Autologous stem cells have a number of advantages over allogeneic stem cells including immunocompatibility [26]. Another approach to generate autologous stem cells is to isolate differentiated adult cells, e.g. skin fibroblasts, and treat these ex vivo with stem cell transcription factors to produce iPSCs [27]. iPSCs are often considered the gold standard source of stem cells for tissue engineering due to their plasticity and pluripotency [28].

In this review, we provide a comprehensive and critical overview of recent advances in the use of autologous stem cells for the generation of cardiac tissue microengineered platforms. We focus on the importance and mechanisms of microenvironmental factors for the maintenance and differentiation of stem cells, as well as challenges and future trends of stem cell-based cardiac microengineered tissues for a number of clinical applications.

2. The importance of stem cells microenvironment in tissue engineering

Stem cell subpopulations residing within the stem-cell niche are important regulators for the maintenance of stemness and tissue regeneration following injury [29]. The stem-cell niche comprises of surrounding cells (both stem and stromal cells), signaling molecules, matrix architecture, physical forces, oxygen tension, and other environmental factors. This highly complex and specialized environment supports stem cell self-renewal and function by providing the necessary biochemical and biophysical signals required for cell proliferation, apoptosis and differentiation [30]. Since Schofield first proposed the principle of stem cell 'niche' in 1978 [31], the concept has gained significant traction, particularly in recent years. Improved understanding of the function and mechanisms of stem cell niche provided an insight into the optimal local microenvironment that supports endogenous stem cells or exogenously applied stem cells, hence facilitating their integration and differentiation at the implantation site [32]. Under physiological conditions, stem-cell niches maintain adult stem cells in a non-differentiating or quiescent state. However, following tissue injury stem cells are triggered by the signals coming from their niche to promote self-renewal and/or differentiation that leads to tissue repair. These signals/factors regulate stem cell niche by promoting the following: (1) cell-cell interactions (between stem cells, and stem cells and neighboring differentiated cells), (2) cell-molecule interactions between stem cells and adhesion molecules/ECM components/the oxygen tension/growth factors/cytokines, and (3) the physicochemical nature

of the environment including the pH, ionic strength (e.g. Ca^{2+} concentration) and metabolites, such as ATP [7]. A number of studies have investigated the mechanisms of interaction between stem cells and their niche, with attempts to replicate these in vitro.

Although challenging, developing novel synthetic stem cell-niche that can recapitulate physiological microenvironment may represent a paradigm shifting for tissue engineering platforms and regenerative therapies [33]. The ultimate success of stem cell-based tissue engineering platforms is reliant upon how reliably synthetic microenvironment can deliver suitable micro-environmental cues to support proliferation, self-renewal and differentiation [34,35]. While the stem cell niche or microenvironment is key in maintaining stem cells, it also protects the host tissue from overstimulated stem cell proliferation that can lead to the development of teratomas [36,37]. Significant factors of stem cell niche are described in Fig. 1.

2.1. Engineering an appropriate ECM microenvironment

Under physiological conditions, ECM acts as a scaffold that provides structural support and topographical cues for cell adhesion and proliferation. ECM in combination with interstitial fluid can resist tensile (via the fibrils) and compressive (via the hydrated network) stresses generated from surrounding tissues [38]. The ECM delivers topographical (via the organization of collagen and glycosaminoglycans) and biological (through integrins, proteoglycans and harboring of growth factors) signals that control cell behavior and function in tissue development and maintenance [39,40]. Fiber-like morphology of ECM components facilitates cell adhesion by ligand clustering, which has been demonstrated to alter cell behavior [41]. Within an engineered microenvironment, the optimal scaffold should mimic the structural and mechano-chemical properties of the physiological ECM [42]. Structural features of scaffolds including fibrils and pores are often a limiting factor for cell adhesion onto tissue engineering scaffolds. Porosity, pore spacing and fiber diameter greatly affect cell migration, and may influence the proliferation and subsequent differentiation of stem cells within the scaffolds [43,44]. Other important biophysical factors of ECM for adequate cell integration include substratum rigidity, topography, shear stress, oxygen concentration, biochemical gradients, cell positioning and pH [45–47]. Advances in microfluidics and micro-nanoengineering technologies have been successful in recapitulating native ECM microenvironment facilitating optimal conditions for required cell functions [48]. These highly regulated microenvironmental parameters (both physico-mechanical and biological) are crucial for generating the desired stem cell fate, whether the goal is to maintain the stemness or to differentiate stem cells into mature lineages [49]. A microenvironment capable of supporting stem cell proliferation and differentiation into cardiomyocytes, is reliant on the following factors: (a) textural and topographic properties, (b) biological signals and (c) stem cell sources [50]. These desirable features of the microenvironment are achieved by utilizing suitable ECM proteins, cellular components (niche cells, immune cells), secreted factors (chemokines, hormones), metabolic factors (oxygen, calcium, lactate, lipids) and electromechanical cues [51,52].

2.2. Textural and topographic microenvironment considerations

Alterations in the substrate surface properties including topography can lead to variations in cell-ECM interactions and their subsequent differentiation into specific cell phenotypes [53]. Some studies also showed that human embryonic stem cells cultured over surfaces with nanoscale roughness can spontaneously differentiate [54]. In fact, changes in the ECM composition can also result in the variation in terminal differentiation [55]. For instance, when the hydrogel composition was changed from alginate to collagen, the fate of stem cells was switched from stem cell to mesodermal lineage [49]. This indicates that the differentiation of stem cells into mature lineages requires the use of engineered micro-environment cues such as substrate stiffness and

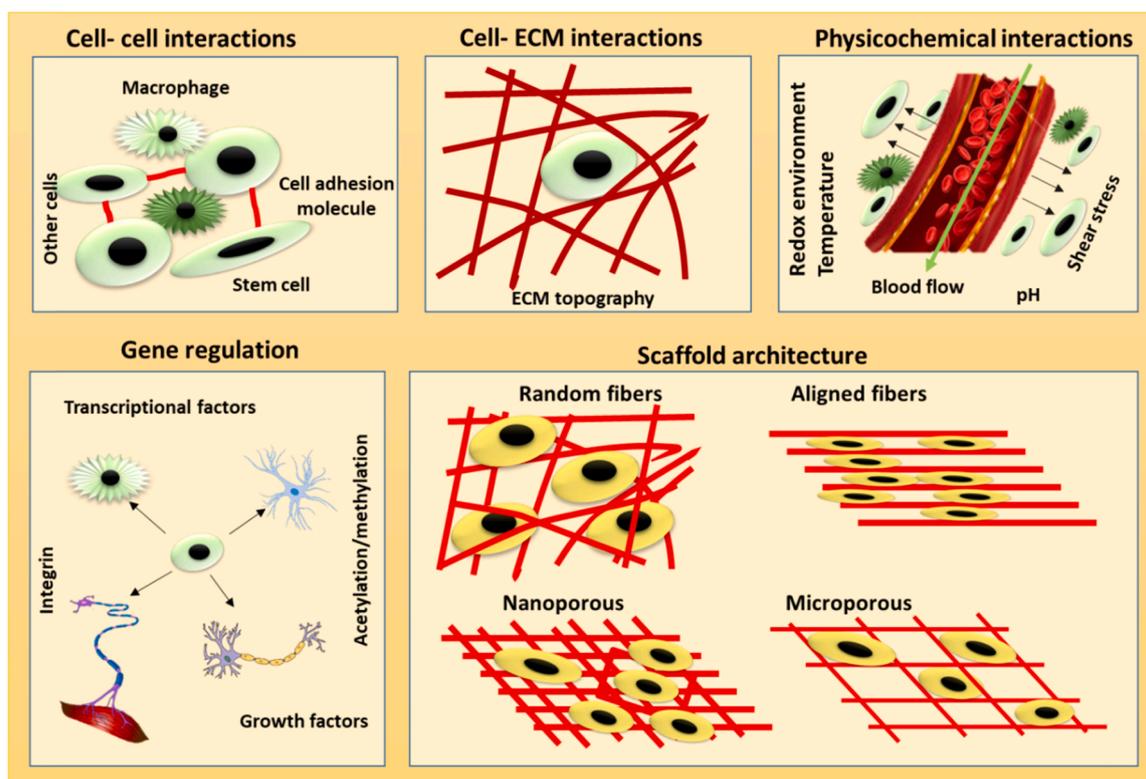


Fig. 1. Schematic representation of the factors that influence stem cell microenvironment in tissue engineering including cell-cell and cell-ECM interactions, physicochemical properties, gene regulation and scaffold architecture.

specific constituents (especially the Gly-Pro-X sequence) present in collagen [56]. However, in the vast majority of cases stem cells cultured on smooth surfaces remained undifferentiated, except when mouse embryonic stem cells are used [57].

In order to make precise nanotopographical patterns within tissue engineered scaffolds by controlling the stiffness, high energy radiation can be used [58]. For this purpose, electron beam irradiation is beneficial for changing the physical properties of the hydrogel scaffolds. This results in the weakening of hydrogel stiffness, faster hydrogel degradation and adipogenic differentiation of MSCs [59]. Similarly, random nanoscale structures generated on glass surfaces by reactive-ion etching (RIE) can create a reproducible biophysical microenvironment for stem cell differentiation [60]. Stem cells are sensitive to nanotopographical cues and tend to undergo differentiation on this patterned surface. This suggests that coexisting biophysical signals as part of the synthetic microenvironment play an important role for stem cells differentiation [60].

2.3. Biological mechanisms important for stem cell function within tissues

In addition to physico-mechanical factors, several biomolecules play key roles in the generation and maintenance of stem cell niche. Kshitiz et al., [48] reported that a number of soluble and biophysical factors regulate cell-cell and cell-ECM interactions closely as well as stem cell fate. For example, Wnt/ β -catenin signaling pathway is important mechanism facilitating self-renewal of stem cells [61]. Purified Wnt ligands and protein immobilization methods can be employed with isolated Wnt-responsive stem cells and a suitable 3D scaffold to produce 3D engineered tissues [30]. These systems can maintain the stemness within the scaffolds. In contrast, many signaling molecules such as the notch ligands, members of the transforming growth factor beta (TGF β) superfamily including bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs) and fibronectin within the microenvironment play essential roles in driving stem cells towards differentiation,

self-renewal and migration [62,63]. Maintaining the viability of cells within microengineered tissue platforms upon implantation into ischemic cardiac tissue is a critical step but also challenging [64–68]. Stromal cell-derived factor 1 α (SDF-1 α) plays a key role in stem cell homing and tissue regeneration after MI [69,70]. SDF-1 α was shown to elicit promising beneficial effects in protecting human myocardium from hypoxia-reoxygenation injury [71]. Secretion of SDF-1 α by MSCs was also shown to modulate endothelial function in patients with dilated cardiomyopathy [72]. Similarly, other growth factors including insulin-like growth factor and vascular endothelial growth factor were also studied in clinical trials but failed to improve left ventricular ejection function [73,74]. An interesting study indicated that myofibroblasts overexpressing SDF-1 α or treated with SDF-1 α can precondition the cells against oxidative or anoxic damage [75]. Development and maintenance of a completely functional, contractile cardiac tissue is also a challenging task. Approaches such as coupling of arginylglycylaspartic acid (RGD) peptides to scaffolds could improve the contractile function of engineered cardiac tissue [76]. A combinatorial approach to manipulate the physico-chemical properties of the scaffolds by integrating physical stiffness and biochemical cues can direct stem cells towards lineage specific differentiation [77,78].

2.4. The importance of the stem cells origin in tissue engineering

Stem cells within tissue engineering constructs through the expression of stemness factors, can undergo self-renewal, proliferation, and differentiation [49]. The origin and properties of stem cells should be closely considered when utilizing these cells within a synthetic niche of a tissue microengineered platform [79]. For instance, stem cells isolated from tissues with a high turnover and regenerative rates including the gut, blood and skin undergo rapid differentiation whereas stem cells from the liver and skeletal muscles show high stem cell recruitment mechanisms from other tissues to facilitate regeneration [79]. On the other hand, brain and heart tissues show low turnover and poor

regenerative capacity [79]. Manipulating biochemical and biophysical properties of the biomaterial scaffold surface has been explored to accelerate stem cell expansion.

2.5. Chemically-induced stem cell differentiation

Small molecules (alone or in combination) control stem cell differentiation and behavior *in vitro* and *in vivo*. When used in combination, these agents are generally safe, stable, and cost-effective. For instance, Williams et al. [80] showed that neurodazine alone is able to control the transdifferentiation of myoblast cells into neurogenic cells. Similarly, fully functional hepatocyte-like cells (HLCs) have also been generated from mouse fibroblasts using a combination of small molecules (CHIR99021 [C], RepSox [R], VPA [V], Parnate [P], TTNPB [T], Forskolin [F], and Dznep [D]; abbreviated as CRVPTD) [81]. In another study, functional hepatocytes were generated from human-iPSCs (hiPSCs) using another combination of small molecules [82]. Interestingly, Forskolin (a cyclic AMP agonist) together with I-BET151 (a BET family bromodomain inhibitor) and CHIR99021 were used to reprogram mouse fibroblasts into neurons without using exogenous cell-fate-specific factors (microRNAs) [83]. Fu et al. [84] reported the direct conversion of mouse fibroblasts into cardiomyocytes-like cells using a combination of CHIR99021 (C), RepSox (R), Forskolin (F), VPA (V), Parnate (P) and TTNPB (T) (abbreviated as CRFVPT). Other studies also showed that synthetic agents such as CHIR99021 and IWR-1endo that modulate Wnt signaling can support cardiac differentiation of stem cells [85]. A dianthins G analog, [Glu2]-dianthins G (1), was also used successfully for *in vitro* cardiac differentiation of rat bone marrow MSCs into cardiomyocytes [86]. These cells presented with high expression levels of cardiac-specific genes including cardiac troponin T (cTnT), GATA4, cardiac myosin heavy chain (MHC), cardiac troponin I (cTnI), and connexin 43.

Despite the plethora of approaches used, the use of chemical cocktails to promote cardiac differentiation of stem cells/trans-differentiation of other cells is highly promising for the regeneration of damaged cardiac tissue due to the cost effectiveness as well as the long term stability.

2.6. The influence of electrical microenvironment on stem cell differentiation

The development and maintenance of an appropriate electrical microenvironment that can mimic the physicochemical and functional properties of a specific tissue/organ is a major challenge in cardiac tissue engineering. The primary goal for the use of electroactive biomaterials in tissue engineering is to recapitulate the electrical properties and functional characteristics of the natural ECM and native tissue [87]. In order to generate an optimal electric microenvironment, conducting scaffolds can be utilized to provide an external electrical stimulation capable of activating growing cells. For instance, when stem cell-derived cardiomyocytes were cultured on graphene-loaded polycaprolactone scaffolds, cells exhibited cardiomyocyte phenotype and contracted spontaneously [88]. Another recent approach tested was based on piezoelectric scaffolds that can generate electrical charges upon mechanical deformations or vibrations [89,90]. Piezoelectric tissue engineering scaffolds with intrinsic capacity of electric signal generation could promote directed stem cell differentiation without external electrical power supply [91]. Considering that the myocardium is an electroactive tissue, this unique feature of a piezoelectric scaffold is highly relevant in cardiovascular tissue engineering [92]. Both stem cell-derived cardiomyocytes and endothelial cells cultured on piezoelectric polyvinylidene fluoride-trifluoroethylene [P(VDF-TrFE)] scaffolds appear to display the structural and functional properties of physiological cardiovascular cells [93]. Cardiomyocytes on P (VDF-TrFE) scaffolds expressed classic cardiac specific markers, formed well-organized sarcomeres and displayed rapid contractility.

Moreover, cardiomyocytes cultured on poly(caprolactone) nanofilm coated with a thin layer of P(VDF-TrFE) microfibrils could preserve contractility for more than a week [94]. In addition to these effects, the contractile function of cells can be monitored by the integrated piezoelectric sensors in the scaffold itself [95].

Due to the ability of these platforms to support stem cell proliferation, differentiation, cardiomyocyte adherence and provide a synchronized contractile function, use of stem cells and piezoelectric scaffolds in conjunction are hypothesized to be highly promising for repairing damaged heart [96].

3. Cardiac tissue engineering and clinical potential

Tissue engineered heart valves have been tested in patients since the beginning of the 21st century [97]. While decellularized tissues provide attenuated host immunological reaction, the first porcine decellularized valve (SynerGraft) failed early in the clinical trials with fatal consequences, due to strong inflammatory response [98]. Significant improvements in decellularization approaches have been achieved thereafter, which in turn improved their performance [99–101]. A recent meta-analysis revealed lower mortality and hospitalization rates related to the use of decellularized heart valves compared to commercially-available bio-prosthetic valves [102]. Decellularized valves are often applied to the right ventricular outflow tract position in clinical trials, whereas valve replacement in clinical practice takes place at the aortic or mitral position, where valves degenerate more rapidly. Several clinical studies employing decellularized valves for aortic valve replacement reported promising outcomes [103–105], however further clinical studies are needed to test tissue engineered valves for aortic or mitral valve replacement, particularly for the evaluation of their long term durability.

Another strategy for valve replacement focused on seeding cells in scaffolds. The first study was reported by Serghei et al., [106] using decellularized human pulmonary valve allografts and autologous endothelial progenitor cells. These valves demonstrated good hemodynamic performance and the capacity to grow aligned with the growth of young patients [107]. Similar strategies using pre-seeded endothelial cells or incorporating factors that can recruit circulating endothelial progenitor cells have also been reported [108]. Furthermore, when bioresorbable valve was fabricated by combining bone marrow-derived stem cells and biodegradable polymers, it presented with several limitations including valve calcification and leaflet remodeling [109].

Generation of viable tissues for heart failure patients after MI is another major focus in the field of cardiac tissue engineering. This approach aims to repair myocardial lesion by introducing cells, biomaterials or a combination of the two within a cardiac patch or injection. In 2008, Chachques et al. [64] first utilized collagen matrix seeded with autologous bone marrow stem cells (BMSCs) applied to left ventricular post-ischemic myocardial scars. In this study, the cell seeded matrix increased muscle wall thickness and improved diastolic function. Other stem cells and biomaterials have been tested in clinical trials for the treatment of MI and showed moderate improvement of myocardial function [110–113]. Although these beneficial effects remained moderate, it is believed that stem cells may play a major role after MI. One of the reasons for the limited success of this approach is the rapid clearance of stem cells from the site of application, together with limited cell survival and vascularization within the infarct zone [111]. To address this concern, cardiac patch using bioabsorbable polymeric scaffolds or decellularized scaffolds seeded with stem cells have been tested. However, the only commercially available cardiac patch (CorTMpatch) for MI is not pre-seeded with cells and thus seeding with stem cells can improve the biological activity [114].

These studies, as well as those focused on using combination of stem cells and biomaterials, demonstrated limited improvement of cardiac function after MI. However, in a study by Molkenkin et al. [115], cardiac function was improved by intra-cardiac injection of adult stem cells,

possibly due to the acute immune response being beneficial to the muscle wall in this context. Nevertheless, better understanding of the role and mechanism of stem cells and biomaterials in myocardial repair, could provide new insights in determining the clinical potential of tissue engineered cardiac products and their future application in this field.

4. Utilizing stem cells in cardiac tissue engineering

Several cardiac tissue engineering approaches using stem cells have been explored to meet the increasing demand for a novel therapeutic option for cardiac regeneration [116,117]. These bioengineered tissues could have a potential application for repairing the infarcted myocardium and damaged heart valves. The appropriateness of the cell type and other components within the tissue platform are dependent on the heart condition being treated. End-stage heart failure following MI is characterized by a dramatic loss of viable cardiomyocytes and in order to overcome the shortage of available donor hearts for transplantation, the administration of functional cardiomyocytes or cardiac progenitor cells has been explored as a potential approach to replace dead cells [118]. In particular, the generation of a tissue engineered patch containing healthy cardiomyocytes has been evaluated [119]. However, for heart valve replacement, an engineered heart valve with a more complex organization including multi-layered cellular organization, specific leaflet shape (tri or bicuspid) and multiple cell types (e.g. valvular endothelial cells and interstitial cells), is required [120]. An overview of the methodology incorporating stem cells within cardiac tissue engineering platforms is presented in Fig. 2. In autologous stem cell-based cardiac tissue engineering models, stem cells can be isolated from bone marrow, adipose tissue, or blood. Various adult stem cells,

including BMSCs or adipose tissue-derived MSCs, have been utilized in cardiac tissue engineering [121,122]. Table 1 summarizes findings from various studies that investigated different sources of stem cells in cardiac tissue engineering.

Another promising approach is the use of iPSCs, which are generated through reprogramming of adult mature cells using a set of transcriptional factors, Oct4, Sox2, Klf4, and c-Myc [123,124]. A number of growth factors, including TGF- β and cytokines (e.g. soluble intercellular adhesion molecule-1, sICAM-1; platelet-derived chemokine, CXCL7) are considered important for the differentiation of stem cells into specific cardiac lineages, including cardiomyocytes, cardiac smooth muscle cells and cardiac fibroblasts [125]. Approaches to activate maturation related pathways such as TLR3-NF κ B pathway is of prime importance to achieve proper maturation of stem cells or precursors into functional cardiomyocytes [126]. Studies also indicated that together with mechanical cues (e.g. nanofibrous architecture, stiffness), biophysical factors (e.g. electric stimulations), substrate properties (eg, stiffness), biochemical parameters (eg, use of fatty acids/hormones), genetic manipulation (eg, miRNA overexpression), and coculture with other cell types (eg, endothelial cells/fibroblasts) are important in achieving cardiomyocyte maturation [127]. With prolonged culture, hiPSC-derived cardiomyocytes (hiPSC-CMs) displayed more mature phenotypes (larger cell size), structure (myofibril density, alignment, microscopically visible sarcomere), and physiology (calcium handling and β -adrenergic response). Triiodothyronine (T3) treatment makes hiPSC-CMs bigger, with elongated morphology and longer sarcomeres, higher mitochondrial activity and improved calcium handling along with higher contractile function [128]. Passive stretching of engineered heart muscle composed of hiPSCs improves its maturation and functional

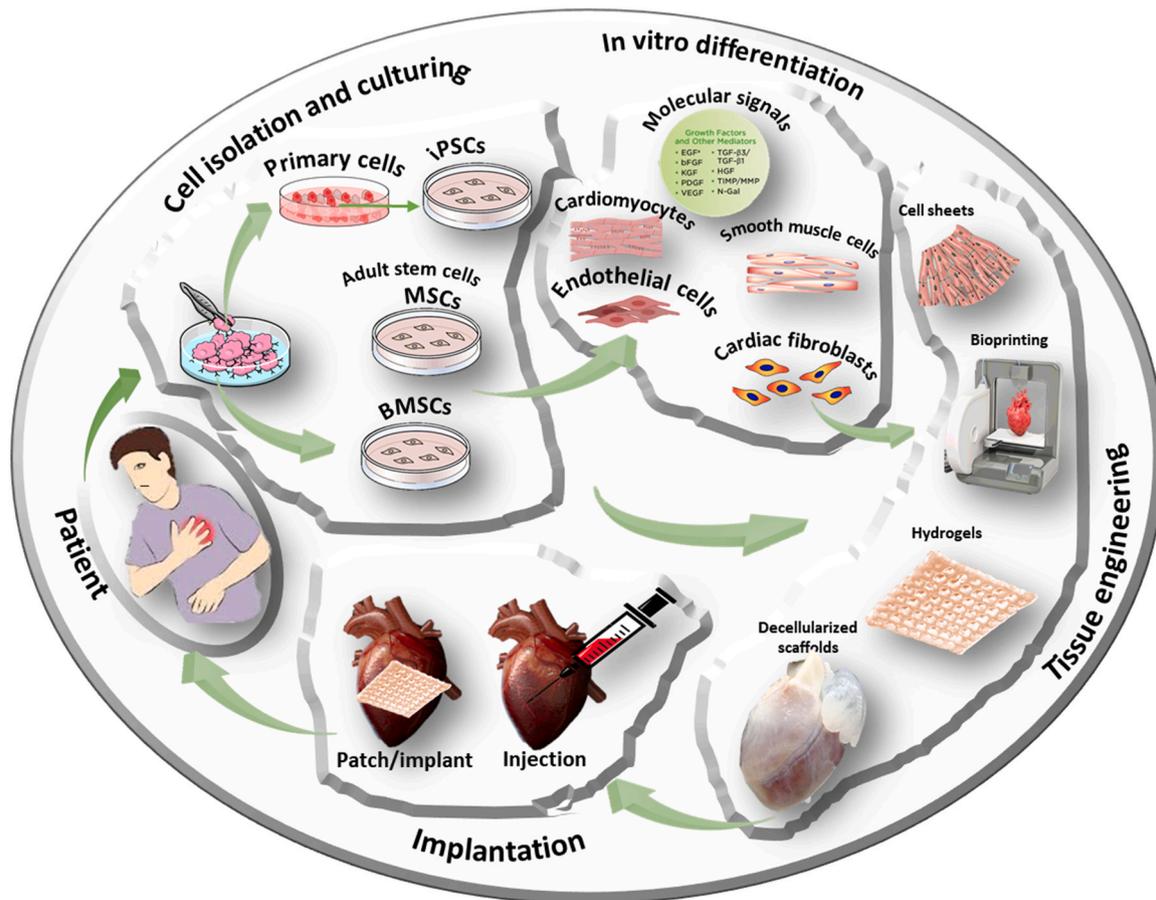


Fig. 2. Stem cell-based approaches in cardiac regeneration and tissue engineering. MSCs: mesenchymal stem cells, BMSCs: bone marrow stem cells, iPSCs: induced pluripotent stem cells.

Table 1
Summary of the studies using different origins of stem cells in cardiac tissue engineering.

Stem cell type	Method/biomaterial (s) used	Testing model	Application	Outcome of the experiment			Ref.
				Stem cell engraftment	Vascularization	Improvement in cardiac function	
Bone marrow mesenchymal stem cells	Type 1 collagen	Acute rat MI	MI repair	-	+	+	[143]
	Type I collagen-(GAG) patch	Acute rat MI	MI repair	+	+	NE	[144]
	Acellular pericardia patch and cell sheet	Chronic rat MI	MI repair	+	+	NE	[145]
	Acellular pericardia patch and folded cell sheet	Chronic rat MI	MI repair	+	+	-	[146]
	Collagen injection	Acute rat MI	MI repair	+	NE	-	[147]
	Poly (lactide-co-ε-caprolactone) patch	Acute rat MI	MI repair	+	NE	+	[148]
	Acellular porcine pericardium	Rat subcutaneous model	Aortic valves	+	+	NE	[149]
Adipose tissue mesenchymal stem cells	Cell sheet fragments	Lewis rat acute MI	MI repair	+	+	+	[150]
	Cell sheet fragments	Lanyu mini-pig MI	MI repair	+	+	+	[151]
	Cell sheet monolayer	Chronic rat MI	MI repair	+	+	+	[152]
	Type I collagen patch	Rat/pig MI	MI repair	+	+	+	[153]
	Type I collagen injection	Rat MI	MI repair	+	+	+	[154]
Human induced pluripotent stem cell	Cell sheet	Rat sub-acute MI	MI repair	+	+	+	[155]
	Cell sheet	Mini pig MI	MI repair	+	+	+	[156]
	Cell sheet	Mouse MI	MI repair	+	+	+	[157]
	Cell sheet	Mouse MI	MI repair	+	+	NA	[158]

MI: Myocardial infarction, NE: Not evaluated, NA: Not applicable, '+': Positive result obtained, '-': Negative result obtained.

performance [129].

As discussed above, electrical stimulation can also be used to direct stem cell differentiation into cardiomyocytes in vitro [92,130]. Cardiac cells can then be directly injected intramyocardially into damaged cardiac tissue along with a suitable hydrogel or seeded onto an engineered porous scaffold, before implantation [131,132]. Undifferentiated stem cells can also be applied onto the damaged heart to achieve local tissue repair mainly by paracrine action [3,133,134]. Various approaches including cell sheet technology [135], bioprinting [136], hydrogel patches [137], and decellularized scaffolds [138] have been used to develop three dimensional cardiac tissue constructs. Implantation of engineered tissue can be performed by either direct injection of cell/hydrogel mixture into the injured area [139] or via a transcatheter implantation of the patch or bioprinted scaffolds [140].

Despite the fact that the mammalian heart is a highly complex organ, a number of viable strategies have been employed to better recapitulate both cellular complexity and tissue architecture of the heart. These also include co-culturing cardiomyocytes with other important cardiac cells including fibroblasts, endothelial and/or immune cells as 3D vascularized cardiac spheroids that could be bioprinted using patient-derived stem cells [141,142]. Nevertheless, there are a number of challenges with cardiac bioengineering including appropriate design of the constructs, viability of the implanted cells, limited regenerative capacity, safety concerns with undifferentiated cells and potential immunogenic response [142].

4.1. Injecting stem cells directly into damaged myocardium

A number of studies have investigated the regenerative potential of stem cell therapy in CVD including in patients with an acute MI or chronic ischemic heart failure either by intravenous or intramyocardial injection/infusion [3]. As discussed above, myocardial ischemia results in myocardial tissue and cell death, particularly affecting cardiomyocytes viability. Cardiac tissue appears to have a poor capacity to self-regenerate with only 1–2% of new cardiomyocyte being produced each year. This is not achieved through regeneration but rather proliferation of existing cardiomyocytes [159], suggesting the lack of endogenous stem cell pool within this tissue [160]. Thus, utilizing cardiac stem cells for myocardial regeneration is not an effective approach compared to using stem cells from other sources in conjunction with suitable biomaterials and growth factors targeted at repairing injured

myocardium. Fig. 3 outlines the methodology and mechanism of action of injection-based stem cell therapy for MI. Use of a suitable hydrogel with required porosity, flexibility, biocompatibility, in situ gelation capacity and controlled degradation rate, can act as a medium for cell injection, protecting the cells during injection and facilitating subsequent proliferation in vivo [161]. Tsou et al. extensively reviewed and discussed how hydrogels as bioactive materials support stem cells and determine stem cell fate [162]. Hydrogels support stem cell proliferation and differentiation by providing an ECM three-dimensional microenvironment. During injection, hydrogels act as a soft cushion and protect embedded cells from shear-stress induced damage. In vivo they provide a scaffold where stem cell can proliferate, migrate and differentiate into cardiac lineages [163]. Different types of polymers such as alginate, gelatin methacryloyl (GelMA), hyaluronic acid and chitosan can also be utilized as injectable hydrogels in stem cell cardiac tissue repair [164].

BMSCs are the most frequently investigated both in pre-clinical and clinical studies. However, mechanistic pre-clinical studies suggest that BMSCs are capable of both differentiating into cardiomyocytes [165], as well as secreting molecular cargo within their extracellular vesicles that can repair the tissue by immunomodulation, stimulating angiogenesis, inhibiting inflammation and oxidative stress; all of these mechanisms are important for regenerating myocardial tissue and improving cardiac function [166,167]. On the other hand, several clinical trials showed promising therapeutic potential of BMSCs in acute MI. As early as 2006, a clinical trial using intramyocardial administration of BMSCs showed improvement in left ventricular function in patients following acute MI [168]. Administrations of BMSCs was also demonstrated to have good safety and efficacy profile following both intravenous infusion [169], and trans-endocardial injection [170]. These results together demonstrated that BMSCs could improve patients' cardiac function and ventricular remodeling by repairing or reducing myocardial infarct size.

On the other hand, pluripotent stem cells (both ESCs and iPSCs) can differentiate into cardiomyocyte with efficiency exceeding 80%, the characteristic that deems them promising candidates in cardiac tissue engineering. In pre-clinical studies of ischemia/reperfusion models in non-human primates, intramyocardial administration of clinical scale human ESCs-derived cardiomyocytes was capable of remuscularization of the infarcted heart tissue. Moreover, transplanted area was perfused by the host vasculature, and the left ventricular ejection fraction was improved, however non-fatal ventricular arrhythmias were observed in this study [171,172]. Furthermore, the beneficial effects of iPSCs in MI

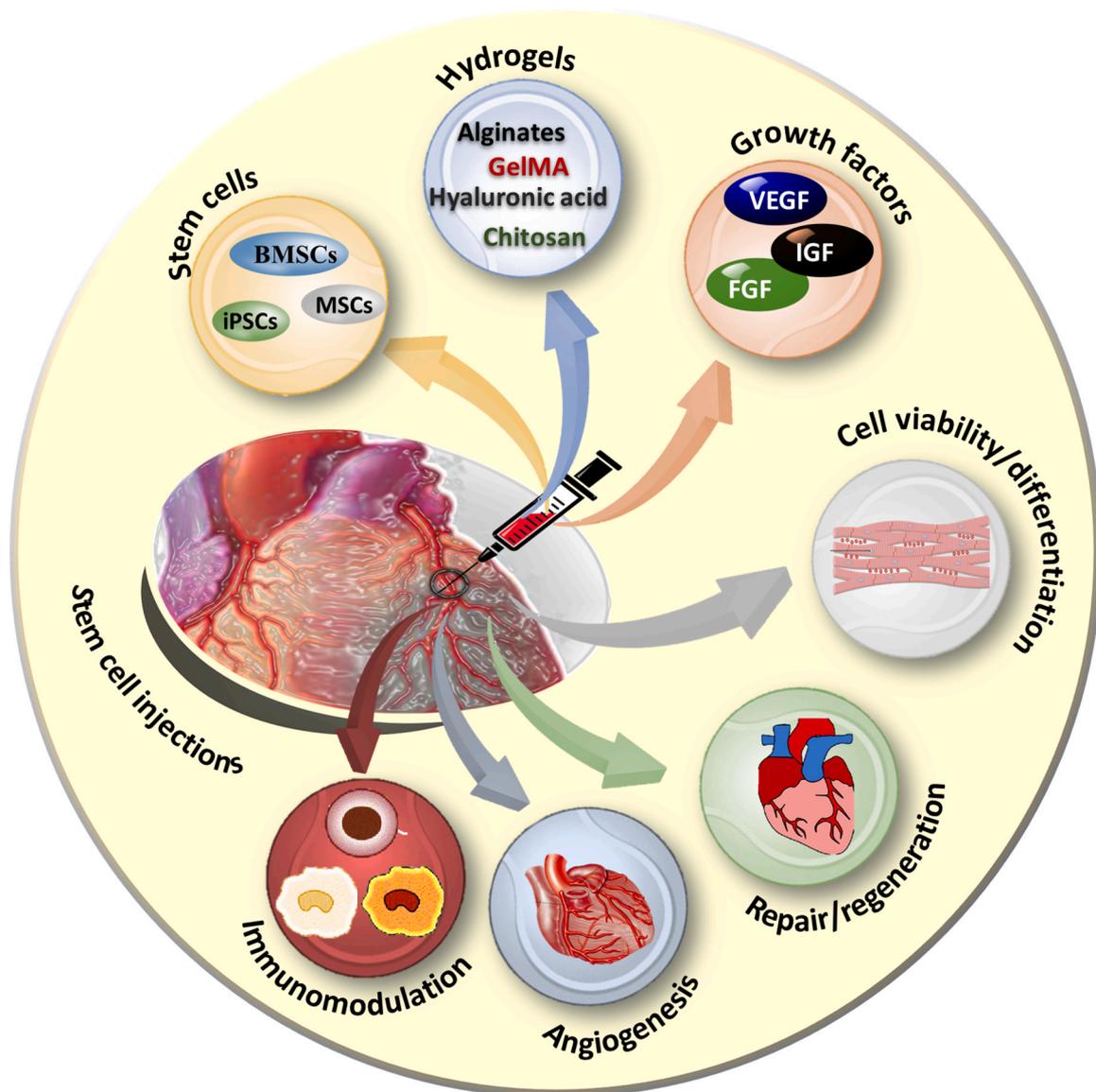


Fig. 3. A summary of various methodological approaches used for administering injectable stem-cell therapeutics for cardiac repair. Stem cells including bone marrow stem cells (BMSCs), mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs) are currently being developed and used for cardiac repair. Adequate numbers of cells are mixed with a suitable hydrogel (e.g. alginates, gelatin methacryloyl (GelMA), hyaluronic acid, chitosan) often containing biological signaling molecules including growth factors [vascular endothelial growth factor (VEGF), insulin growth factor (IGF) or fibroblast growth factor (FGF)] before being injected into the region of the ischemic heart tissue [173]. Injected stem cells, hydrogels and biological factors together provide an effective microenvironment for differentiation, repair/regeneration, immunomodulation, and angiogenesis [174].

were also attributed to their paracrine action, through the release of reparative cargo within extracellular vesicles [133].

Although direct intravenous administration of stem cells has been investigated in a number of pre-clinical and clinical models of CVD, there are several limitations associated with this mode of delivery including poor survival rate of the cells following injection and at the site of repair.

4.2. Spheroids in cardiac repair

Scaffold-free cardiac tissue engineering using multicellular spheroids has been investigated and developed utilizing the technology based on the fundamental characteristics of the self-assembling properties of cells [175–177]. These can be generated by using either: (i) low attachment plates; (ii) hanging drop cultures; (iii) rotating bioreactors; (iv) microfluidics devices; (v) magnetic-based cell coating [141]. Overall, hanging drop cultures enable the optimal control of the cell number, spheroid

shape, and live imaging [141]. Initial experiments generated contractile cardiac spheroids by plating a mixture of rat neonatal ventricular cardiomyocytes, human dermal fibroblasts, and human coronary artery endothelial cells on ultralow attachment plates. More recently, full vascularization of cardiac spheroids has been achieved by co-culturing iPSC-derived cardiomyocytes (iPSC-CMs), human cardiac fibroblasts and coronary artery endothelial cells at a ratio approximately the same as found in vivo [176]. These vascularized cardiac spheroids (or VCSs) recapitulate biochemical, morphological, physiological features of typical human heart in vivo. Human VCSs have been utilized to identify the molecular and cellular targets of doxorubicin, a well-known cardiotoxic drug used to treat leukemia, lymphoma and breast cancer [176]. Given their unique properties to mimic the human heart microenvironment, VCSs have also been used to model cardiac fibrosis, characterized by an excessive ECM deposition as a consequence of MI or in end-stage heart failure [142]. More recently, the use of differentiated autologous stem cells within cardiac spheroids appears to improve

cardiac cell viability and function when tested for cardiac repair application [142]. Recent studies indicated that the incorporation of electrically conductive silicon nanowires to hiPSC cardiac spheroids led to advanced structural and functional development of hiPSC-CMs by improving the endogenous electrical microenvironment [178]. A combination of nanowires and electrical stimulation enhanced cell-cell junction formation, improved development of contractile machinery, and led to a significant decrease in the spontaneous beat rate of hiPSC cardiac spheroids [178]. Vascularized spheroids possess liquid-like properties that deem them suitable for use as “building blocks” for the engineering of thick viable and functional vascularized tissues [142,175,179–181]. For this reason, scaffold-free spheroids have been employed as “bioinks” in 3D bioprinting technology [182]. This concept can select individual spheroids using vacuum suction before positioning them on a needle-based array, which is inspired by the needle arrays known as “kenzan” from the ancient Japanese art of flower arrangement, ikebana. This technology allows spheroids to be precisely positioned in any configuration and results in individual spheroids fusing together over a short period of time to create a 3D bioprinted cardiac tissue. This method thus allows spheroids to be manipulated with ease that has potential implications for the future of scaffold-free organ biofabrication.

4.3. Cell sheets in cardiac repair

Similar to cardiac spheroids, cell sheet engineering has emerged as a scaffold-free approach for the engineering of heart tissues [183,184]. It utilizes a temperature-responsive cell culture surface (e.g. methylcellulose hydrogel), where the cells can be cultured and separated out as cell sheets by reducing the temperature [185]. The advantage of this approach is that multiple thin cell sheets can be stacked together to generate cell-dense thick tissues. Several reports investigated the development of myocardial cell sheets based on various types of autologous stem cells that generated electrically active cardiac constructs [152,183,186]. More recently, the same group transferred the cell sheet onto a stretchable silicon base that was then replaced by a cell shifter before it was transplanted in a rat model [187]. This approach allows optimal delivery of cells with minimal disruption of the tissue and orientation of cells suggesting cell maturation *in vivo*. ‘HeartSheet’ (Terumo, Tokyo, Japan) is a human skeletal muscle derived cell sheet product, which is already approved in Japan for the treatment of severe heart failure [188].

Transplantation of cell sheets onto damaged cardiac tissue improved heart function in various animal models. An interesting study showed that adipose-derived mesenchymal stem cells (ADMSCs)-based cell sheet could induce angiogenesis, reverse wall thinning in the scar area and improve cardiac function in rat MI models [152]. In another study, Wang et al. generated MSC cell sheet fragments of submillimeter size and injected these into the peri-infarct area of injured rat hearts [150]. Interestingly, MSCs as cell sheets differentiated into Nkx-2.5 and α -sarcomeric actinin positive cardiomyocyte-like cells, myofibroblasts and vascular cells. Compared to MSC cell suspension injections, MSC-based cell sheet fragments demonstrated higher angiogenesis potential, an improvement in heart function, and decreased dilation. Under the influence of connexin 43, the cells in multiple sheets can produce engineered constructs with spontaneous pulsative properties, visible even with the naked eye [186,189]. In another study, BMSCs-based cell sheets were capable of differentiating into smooth muscle cells and endothelial cells, forming vasculature and restoring subsequently regional blood perfusion hence ameliorating post-infarcted cardiac function [151]. Using mouse muscle-derived single-layer stem cell sheet in a chronic MI mouse model led to considerable improvements in ejection fraction (without the presence of cardiac arrhythmias) [190]. iPSC-derived cell sheets are also showing promising outcomes for the treatment of MI [156,191–195]. In an MI porcine model, it was reported that providing an omental flap could significantly increase cell survival of transplanted

human iPSC-CM sheets [196]. It is likely that secreted factors from omental cells could provide a suitable microenvironment for cell survival and differentiation. Another study with human iPSC-CMs sheets in MI models, reported the restoration of cardiac function and electrical rhythm for as long as 2 months after transplantation [155]. Controlling the microenvironment by using fibrin-based hydrogels also showed improved survival of human iPSC-CM sheets leading to increased angiogenesis and cell engraftment, and decreased cardiac infarction in MI mouse models [157]. These results demonstrate that electrically conductive, pulsatile three-dimensional cardiac constructs can be achieved, both *in vitro* and *in vivo*, by stacking monolayers of stem cell sheets that can be implanted in damaged areas of cardiac tissue leading to myocardial regeneration. Recent clinical trials that evaluated cell sheets for treating cardiomyopathy also shed light onto the possibility of bench to bed side translation of this approach [191,197]. Overall, using stem cell-based cell sheets could be a robust approach for repairing damaged hearts, improving cardiac function and hence the quality of the life and survival of patients. However, concerns regarding the safety and effectiveness of these approaches still remains [198]. A thorough evaluation including end-points such as cardiac and all-cause mortality, together with a placebo-controlled double-blind group would be ideal to critically assess these results.

4.4. Cardiac repair patches

Other approaches used to overcome the challenges with stem cell survival following injection/infusion include the use of a combination of biomaterials and growth factors within a patch. This enables protection of stem cells during *in vivo* administration to the damaged cardiac tissue [142,199]. Patches have higher surface area to volume ratio and can cover a large portion of myocardial area, hence retaining more progenitor/stem cells, delivering cells in a controlled manner, and providing suitable architecture and topography for the differentiation and proliferation of stem cell within the implanted area [200]. Due to the preassembled nature of patches, as biomimetic scaffolds for stem cell-based cardiac tissue engineering, these provide a relatively pre-defined microenvironment for stem cell proliferation and differentiation resulting in the regeneration of intended functional cardiac tissue [200, 201].

Various hydrogel-based scaffolds loaded with stem cells were developed and tested in various MI models. For example, hydrogel-containing alginates are capable of ameliorating oxidative stress, thus improving cell viability and survival, and facilitating cardiac repair [202]. Hydrogels can also be used as a delivery system for biological molecules or stem cells, or delivered alone to the injured myocardium [203]. Nevertheless, successful propagation of stem cells, effective implantation, and maintenance of cell viability in a large tissue engineered cardiac patch is still challenging. Entrapment of stem cells within the pores of self-assembling peptide gels coupled with large sized poly(ethyl acrylate) (PEA) scaffolds (5 × 5 cm) effectively supports tissue regeneration, capable of covering a large area of MI [204]. One of the key factors for successful implantation of stem-cell loaded cardiac patches is prompt integration into host tissue facilitated by vascularization, which is critical for maintaining the viability and survival of cells within the cardiac patches [44]. *In vivo* studies using bioabsorbable polycaprolactone elastomeric scaffolds containing PuraMatrix peptide hydrogel seeded with adipose tissue-derived progenitor cells were effective at decreasing fibrosis, reducing infarct scar expansion and lowering post-ischemic adverse left ventricular remodeling [205]. Results of this study clearly indicated the successful development of a vascular network between the patches and native cardiac tissue. Similarly, when iPSCs were used in an animal model of MI, an improvement in left ventricular function, decrease infarct size and reduce apoptosis within the periscar border zone of myocardium was observed [206]. Achieving synchronized contractility of cardiomyocytes inside engineered constructs along with physiological cardiac tissue is also vital.

Establishing a confluent growth of differentiated cardiomyocytes capable of electrical signal conduction can provide simultaneous contraction of engineered myocardial tissues [186]. Following successful in vitro and in vivo studies in terms of efficacy and safety, this concept was investigated in clinical trials. One of the first clinical trials that started in 2008 investigating the regenerative effect of collagen matrix loaded with bone marrow cells in patients with post ischemic myocardial scars showed an increase in scar thickness and led to improved diastolic function with limited ventricular remodeling [64]. Another clinical trial was performed in France in 2015 with cardiac progenitor cells produced from ESCs; these cells were delivered in a fibrin-based hydrogel patch to patients who demonstrated improvement in symptoms and contractile function in severe heart failure patients [110]. However, a clinical trial using bioabsorbable cardiac matrix without stem cells failed to demonstrate any beneficial effects in patients following MI [207].

4.5. Tissue engineered heart valves

It is estimated that over 182,000 heart valve replacements per year are performed in the US [208]; current biological heart valves are made from porcine or bovine pericardium. The main concern with existing biological heart valves is their rapid degeneration due to the absence of self-renewing cells. Tissue engineering could address this problem by utilizing a combinations of stem cells and biomaterials to construct heart valves [209]. The general concept of valvar tissue engineering is to construct a 3D extracellular scaffold, which is responsible for the mechanic support and leaflet motion, in combination with two type of cells found in physiological heart valve: the valvular interstitial and endothelial cells, these cells are responsible for the structural integrity and vascular homeostasis, respectively. Similar to other tissue engineering fields, valvular cell differentiation from a number of different types of stem cells, has been widely studied [210,211]; suitable biomaterials important for valvular hemodynamics have also been widely investigated. The current challenge in valvular tissue engineering is finding the

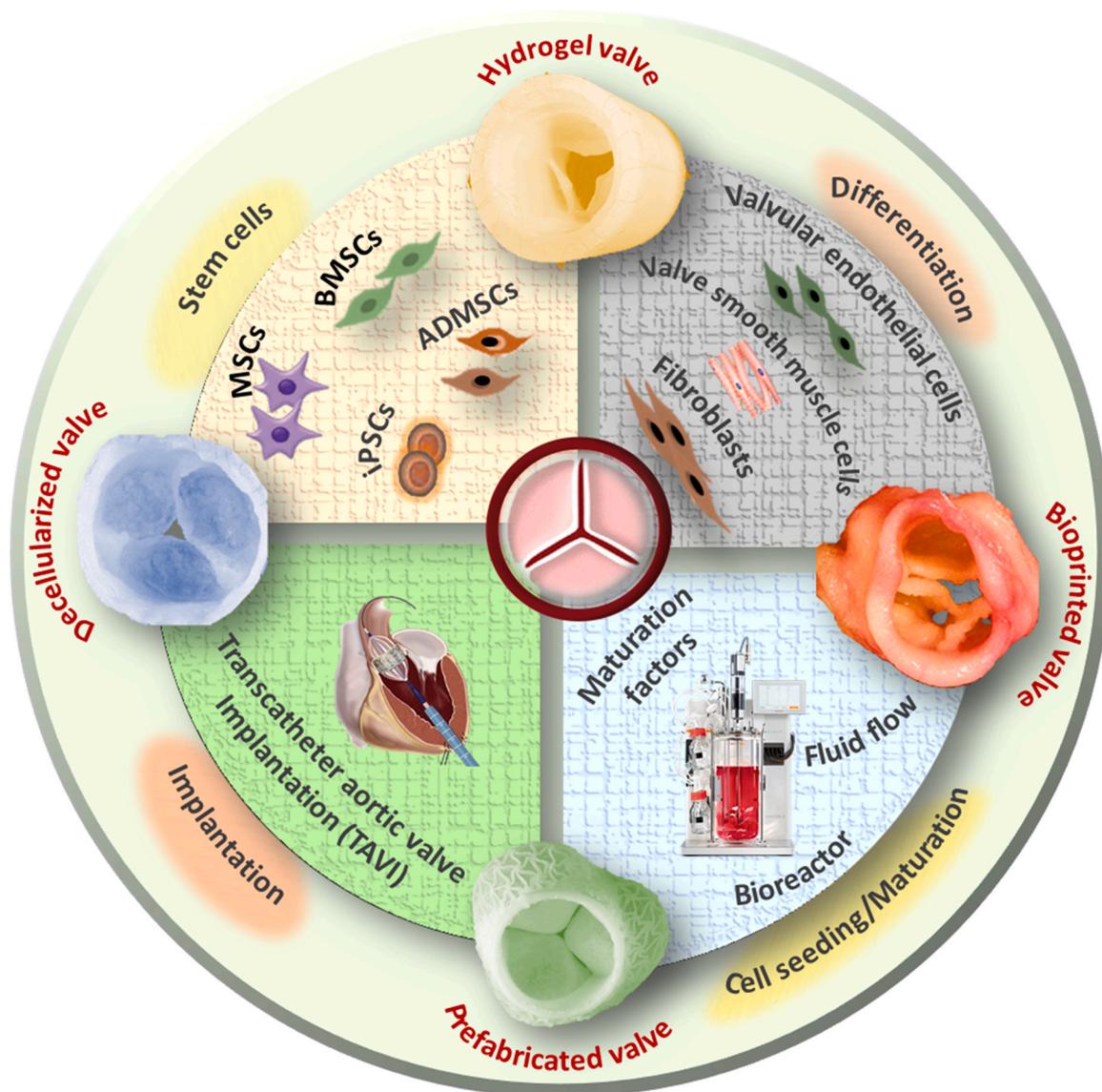


Fig. 4. Heart valve tissue engineering uses various scaffolding approaches including molded hydrogels, decellularized scaffolds, and prefabricated porous valve scaffolds. Recently 3D bioprinted heart valves were also investigated. Stem cells (MSCs, BMSCs, iPSCs, ADMSCs) are supported with biomolecular signals to induce differentiation into valvular endothelial cells, valve smooth muscle cells and fibroblasts that are incorporated into the scaffold to generate bioengineered tissue. Maturation of tissue engineered valves can be achieved by maintaining these in a bioreactor, which can provide optimum microenvironment for appropriate proliferation and maturation of cells before implantation.

most optimal combination of cells and scaffolds that can offer long-term sustainability, especially in relation to vascular homeostasis and resistance to degeneration. Both natural and synthetic materials that can provide a suitable microenvironment for proliferation and differentiation of stem cells are largely studied [212]. Several approaches including hydrogel-based scaffolds, 3D bioprinting and decellularization platforms are used to develop bioengineered heart valves (Fig. 4). Driessen-Mol et al. used a homologous decellularized valve in sheep for a long-term evaluation, observing good functionality of the valve with matrix remodeling and host cell repopulation at the end of 24 weeks follow-up period [213]. Another tissue engineered valve was implanted in a sheep model for 6 months demonstrating the presence of interstitial-like cells and vascularization of the bioengineered matrix [214]. Jolanda et al. demonstrated an outstanding heart valve design using synthetic materials; this tissue engineered valve showed sustained functionality up to 12 month, with gradual ECM deposition [215]. Duan et al. detailed the challenging task of developing stem cell based functional living tissue-engineered heart valves and their integration with the native cardiac tissue to demonstrate the long term functioning [216]. They used MSCs isolated from both bone marrows (BMMSC) and adipose tissue (ADMSC) to construct heart valves. When they compared the functional capacity of pediatric human aortic valve interstitial cells (pHAVIC) in 3D environment, it was found that BMMSC and ADMSC showed more differentiation capacity into valvular cells than pHAVIC. They also found that FGF can drive the differentiation of ADMSC, BMMSC and pHAVIC into fibroblast cells. Such multi-lineage differentiation potential of stem cells in the presence of specific signaling molecules indicate the possibility to generate multiple heart valve associated cells by tuning the microenvironment. With ongoing advances in material science and stem cell research, it is likely that the current biological porcine or bovine heart valve will be soon replaced with patient specific tissue engineered heart valve.

4.6. Bioprinting of myocardial tissues for transplantation

The mortality associated with heart failure is astounding 30–40% within 1 year of diagnosis [217]. For end-stage heart failure patients the lasting option is heart transplant. However, long waiting lists, limited donors and associated morbidity and mortality with heart transplant, make this option very challenging [218]. Therefore, there is an urgent need for other more suitable strategies such as bioengineered hearts. As described previously, by providing necessary microenvironmental cues, stem cells from various sources can be differentiated to generate functional cardiomyocytes. For instance, Witty et al. established a method to differentiate iPSCs into epicardial-like cells, which contribute to the formation of mesenchymal vascular structures and other non-myocyte cells [219]. This is considered an important step towards the development of a fully functional “artificial” heart [220]. 3D bioprinting is an emerging area that has been substantially progressed and could lead to generation of complex organs including the heart [221,222]. 3D bioprinting can be used to produce a defined shape of a tissue construct matching the original target organ/tissue. This process integrates stem cells into appropriate microenvironment, hence generating morphologically, anatomically and functionally matching tissue engineered construct [223]. 3D bioprinting-based approaches involve the generation of heterogeneous hydrogel matrix containing multiple factors including ECM proteins, growth factors, chemokines, nutrients, oxygen releasing agents at pre-defined ratios to regulate stem cells migration, proliferation and differentiation into defined phenotypes generating multicellular heart [224,225]. However, developing functional vasculature within large bioengineered tissues such as heart is a challenging task [226]. Maiullari et al. generated vascularized cardiac tissues with the help of multicellular bioprinted constructs including human umbilical vein endothelial cells (HUVECs) and iPSC-CMs [227]. HUVECs and iPSC-CMs were encapsulated inside hydrogels composed of alginate and PEG-fibrinogen (PF), which were then extruded through the printing

head producing vascularized cardiac tissue constructs with predefined shapes. Attempts are also being made to overcome these challenges with omental tissue derived iPSCs-CMs and endothelial cells, by providing a suitable microenvironment using either personalized ECM hydrogels or an in vivo bioreactor, i.e. the omentum [228]. Using two separate bioinks for the parenchymal cardiac tissue (containing redifferentiated cardiomyocytes) and blood vessels (containing redifferentiated endothelial cells), functional and personalized vascularized bioprinted cardiac tissue could be successfully generated, anatomical recapitulating the human heart (Fig. 5).

In addition to the maintenance of cell viability and appropriate differentiation of stem cells upon implantation, it is equally important to achieve a synchronized interaction between multiple cells within bioengineered cardiac construct and the cells of the host tissue, as discussed above. Thus, it is vital to investigate the functional maturation of cells in bioengineered cardiac tissues, their communication with the host cells, and understand the cardiac electromechanical function at multiple spatial levels [229]. In order to achieve adequate cardiac function, a bioengineered cardiac construct should: (a) be composed of closely packed, aligned, highly differentiated and electromechanically connected cardiomyocytes, (b) have rapid action potential conduction with high velocities, and (c) possess comparable contractile forces with that of the physiological cardiac tissue [230].

5. Challenges and prospects in cardiac tissue engineering

Despite substantial progress in cardiac bioengineering, several challenges remain including the difficulties with isolating stem cells, maintaining their stemness and differentiation potential, controlling differentiation towards specific lineages, and scaling up the production of large number of cells that are adequate for the construction of a tissue or organ [77]. The utilization of adult stem cells over ESCs circumvents ethical concerns despite the multiple challenges with their replication and differentiation. Recent advances in the development of iPSCs have addressed some of these challenges including cumbersome isolation process, reduced availability of autologous stem cells with differentiation potential in older patients, and limited differentiation potential [142]. For instance, iPSCs can be created in large amounts in the laboratory from omentum tissue, maintained in undifferentiated state for many generations and differentiated into cardiomyocytes or endothelial cells for the development of engineered cardiac tissue [228].

BMSCs have shown promising potential for cardiac tissue engineering [33]. However, these cells are capable of differentiating into chondrocytes [231]. Unintended cell differentiation could also happen with MSCs [232,233] and iPSCs [234]. Therefore, controlling cell microenvironment can potentially prevent undesirable cell differentiation. However, it is often difficult to determine the optimal number of supporting biomolecules in a spatiotemporal manner in order to achieve desired lineage of cell differentiation.

Although injectable hydrogel-stem cell constructs are highly promising in cardiac tissue regeneration due to the ease of application to the damaged area, achieving specific differentiation in vivo is challenging. In this context, considering the role of regulatory genes in surrounding tissues and native cells at the implantation site is important to facilitate appropriate cell differentiation. Moreover, some studies have investigated the effectiveness of locally delivered bioactive agents that may be capable of facilitating the recruitment of endogenous stem cells to the areas in the need of tissue regeneration. These approaches could aid clinical translation of stem cell-based tissue engineering approaches.

Despite some advancements, creating a complex tissue architecture of the heart continues to be a challenge. Bioprinting technologies have demonstrated progress in generating required tissue architecture for this purpose. However, designing the internal structure of the heart including valves, aorta, septum using computer-aided design with patient specific dimensions as well as maintaining the morphology of bioprinted internal structures without collapsing, has not been managed

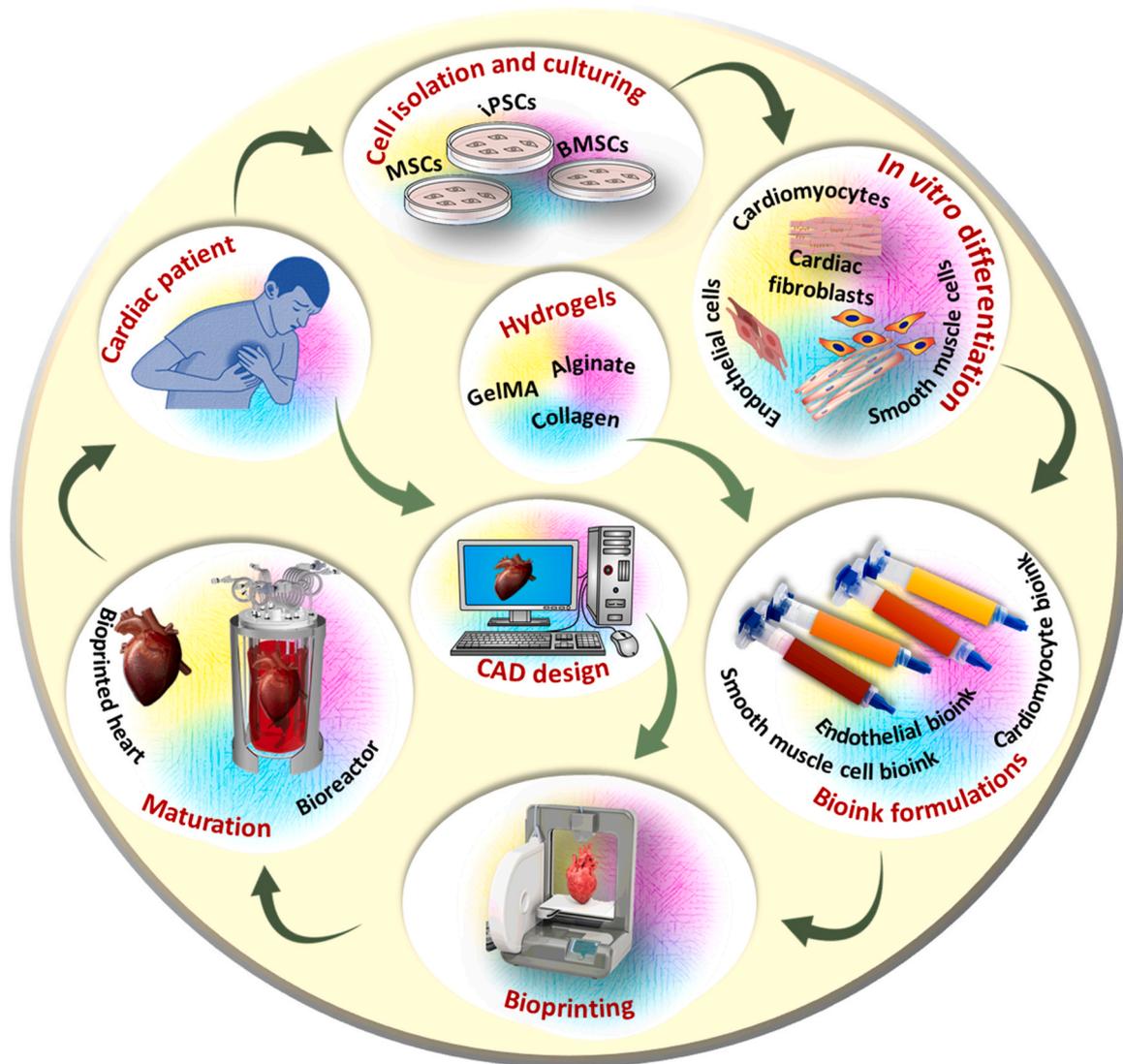


Fig. 5. Scheme showing the overall concept of the development of a bioprinted heart transplant (A). Cells can be isolated from the patient while the matrix can be developed from natural or synthetic hydrogels providing a suitable microenvironment for cell proliferation in the bioengineered heart. Either mature cells can be reprogrammed to iPSCs and then differentiated to cardiomyocytes (CM), cardiac fibroblasts and endothelial cells (EC) or stem cells such as MSCs and BMSCs can be used. The bioinks are developed using differentiated cells and hydrogel, then printed to obtain vascularized heart based on a CAD model. In order to repair or replace the damaged heart, this bioprinted heart can be transplanted back into the patient.

yet.

In addition to the scientific and technical challenges, there are several regulatory barriers hindering the translation of stem cell-based therapies and tissue engineered constructs to the clinic. There is an urgent need for the development of clear guidelines by the regulatory bodies with adequate and feasible recommendations for these emerging therapeutic approaches.

6. Conclusions

Effective therapeutic strategies capable of repairing cardiac tissues impacted by infarction and heart failure or death are still lacking, hence impacting patients' quality of life and prognosis. Recent developments in stem cell-based tissue bioengineering offer promising options for restoring the functions of damaged heart. New knowledge and improved understanding of the interactions and signaling mechanisms between stem cells and their niche or microenvironment within the *in vivo* setting, have improved the bioengineering capabilities for optimal tuning of the stem cell microenvironment *in vitro*. Regardless of the type of bioengineered tissue, the success of stem cell-based tissue engineering

relies on the ability to fine tune the spatiotemporal cell differentiation and phenotype based on the tissue type by providing the most suitable microenvironment *in vitro*. Research shows that stem cell differentiation is highly dependent upon the biological, chemical, mechanical, and electrical cues from the microenvironment. To explore these stem cell-microenvironment interactions and tailor the development of bioengineered tissue for a specific purpose, it is critical to establish appropriate scaffold architecture with optimal physicochemical properties and supporting biomolecules. The vast majority of current tissue engineering approaches involves *in vitro* differentiation of patient-derived stem cells, combining these with a suitable biomaterial scaffold or hydrogel, and *in vitro* maturation before implantation into the patient (Fig. 4). Recent reports provide solid rationales for the use of various types of stem cells including BMSc, iPSCs and MSCs in cardiac tissue repair, heart valve tissue engineering and heart reconstruction. Although clinical application of these bioengineered cardiac platforms is still in its infancy, substantial progress has been made in optimizing the use of stem cells in conjunction with suitable biomaterial-based scaffolds that have adequate physicochemical properties recapitulating closely human physiological cardiac architecture.

Author Contributions

Robin Augustine: Conceptualization. **Robin Augustine, Pan Dan:** Investigation. **Robin Augustine, Pan Dan:** Resources. **Robin Augustine, Pan Dan, Lana McClements, Carmine Gentile:** Writing - original draft. **Robin Augustine, Lana McClements, Pan Dan, Israa Magdi Khalaf, Parvathy Prasad, Kajal Ghosal, Carmine Gentile, Pablo Maureira:** Writing - review & editing. **Robin Augustine:** Visualization. **Robin Augustine, Pablo Maureira, Anwarul Hasan:** Supervision. **Robin Augustine:** Project coordination. **Anwarul Hasan:** Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Declaration of conflicting interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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