



## Invited review article

# Current research trends and challenges in tissue engineering for mending broken hearts

Muhammad Qasim<sup>c,1</sup>, Pala Arunkumar<sup>a,1</sup>, Heather M. Powell<sup>d,e</sup>, Mahmood Khan<sup>a,b,\*</sup>

<sup>a</sup> Department of Emergency Medicine, College of Medicine, Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, United States

<sup>b</sup> Department of Physiology and Cell Biology, The Ohio State University Wexner Medical Center, Columbus, OH, United States

<sup>c</sup> Department of Stem Cell and Regenerative Biotechnology, Humanized Pig Research Center (SRC), Konkuk University, Seoul, Republic of Korea

<sup>d</sup> Department of Materials Science and Engineering, Department of Biomedical Engineering, The Ohio State University, Columbus, OH, United States

<sup>e</sup> Research Department, Shriners Hospitals for Children, Cincinnati, OH, United States

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## ABSTRACT

Cardiovascular disease (CVD) is among the leading causes of mortality worldwide. The shortage of donor hearts to treat end-stage heart failure patients is a critical problem. An average of 3500 heart transplant surgeries are performed globally, half of these transplants are performed in the US alone. Stem cell therapy is growing rapidly as an alternative strategy to repair or replace the damaged heart tissue after a myocardial infarction (MI). Nevertheless, the relatively poor survival of the stem cells in the ischemic heart is a major challenge to the therapeutic efficacy of stem-cell transplantation. Recent advancements in tissue engineering offer novel biomaterials and innovative technologies to improve upon the survival of stem cells as well as to repair the damaged heart tissue following a myocardial infarction (MI). However, there are several limitations in tissue engineering technologies to develop a fully functional, beating cardiac tissue. Therefore, the main goal of this review article is to address the current advancements and barriers in cardiac tissue engineering to augment the survival and retention of stem cells in the ischemic heart.

## 1. Introduction

Cardiovascular disease (CVD) is a major cause of mortality in the world. According to the Global Burden of Diseases, Injuries, and Risk Factors 2015 report, there were 17.92 million deaths due to CVD

worldwide [1]. The American Heart Association (AHA) estimated that 46.1% of US population will have some form of CVD by 2035 at which time, the total cost associated with CVD will be 1.1 trillion USD [2]. Among CVD in the US, coronary heart disease (CHD) accounts for 43.5% deaths followed by stroke (16.8%), high blood pressure (9.4%),

**Abbreviations:** CVDs, cardiovascular diseases; WHO, world health organization; CHD, coronary heart diseases; CDC, cardio sphere derived cells; 3D, three-dimensional; MI, Myocardial infarction; VF, Ventricular fibrillation; VT, Ventricular tachycardia; VA, Ventricular arrhythmias; ICD, implant of cardioverter defibrillator; SA, sinoatrial; AV, atrioventricular; ECM, extracellular matrix; PDMS, polydimethylsiloxane; PEG, poly(ethylene glycol); iPSCs, induced pluripotent stem cells; ESCs, embryonic stem cells; GSK3, glycogen synthase kinase 3; PGA, poly (glycolic acid); PCL, polycaprolactone; PLA, poly (lactic acid); PLGA, poly (D,L-lactico-glycolic acid); MeHA, methacrylated hyaluronic acid; GelMA, gelatin methacrylate; CMs, cardiomyocytes; PEG, polyethylene glycol; CPCL, carboxylated PCL; EBs, embryoid bodies; mESCs, murine ESCs; PPy, polypyrrole; hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; DCM, dilated cardiomyopathy; CTLs, cardiac tissue-like constructs; PMGI, polymethylglutarimide; AuNP, gold-nanoparticle; IDs, intercalated discs; TEM, transmission electron microscopy; CLSM, confocal laser scanning microscopy; SPNT, scanning probe nanotomography; PHEMA, poly(2-hydroxyethyl methacrylate); PVA, poly vinyl alcohol; pNIPAAm, poly (N-isopropylacrylamide); AAc, acrylic acid; pNIPAAm-AA, poly N-isopropylacrylamine-co- acrylic acid; SMT, standard medical therapy; hESCs, human Embryonic Stem Cells; hESC-CMs, human embryonic stem cells derived cardiomyocytes; cTnT, cardiac troponin T; ECs, endothelial cells; TEHV, tissue engineered heart valves; HAVIC, human aortic valve interstitial cells; hCMP, human cardiomyocytes patches; HMSCs, human mesenchymal stem cells; HEMSCs, human embryonic stem cells; HUVECs, human endothelial cells; PFC, perfluorocarbon; MTFs, muscular thin films; ECTCs, embryonic cardiac constructs; LCST, lower Critical Solution Temperature; Az-chitosan/Acr-PEG-RGD, azidobenzoic acid modified Chitosan with acryloyl-poly(ethylene glycol)-RGDS; OAC-PEG-OAC, oligo(acryloyl carbonate)-poly(ethylene glycol)-oligo(acryloyl carbonate) copolymer; PANi-Polyaniline, PPy-Polypyrrole; S/m, siemens per metre

\* Corresponding author at: Department of Emergency Medicine, Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical center, 473 W. 12th Avenue, Columbus, OH 43210, United States.

E-mail address: [mahmood.khan@osumc.edu](mailto:mahmood.khan@osumc.edu) (M. Khan).

<sup>1</sup> Equal contribution.

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heart failure (9.0%), diseases of the arteries (3.1%), and other CVDs (17.9%). CHD and stroke were found to be the first and fifth leading cause of deaths overall in the US [2].

CHD (aka ischemic heart disease) is caused by a buildup of plaque in the coronary artery (*i.e.* atherosclerosis), which gradually leads to partial or complete blockage of the artery with a concomitant reduction in blood flow to the heart muscle. The consequence can range from angina, shortness of breath, or myocardial infarction (MI) to heart failure in extreme cases [3]. As a result, medical and scientific communities worldwide are investigating strategies to reduce the burden of this disease. A major limitation with the conventional treatment strategies for CHD is that the heart muscle has little or no capability to recover after an episode of cardiac arrest, which ultimately leads to heart failure. Thus, heart transplantation remains the only viable option for treating end-stage heart disease. However, due to the low supply of donor hearts and high demand for transplantation [2,4], there is a pressing need to develop alternative strategies to treat CHD patients.

Cellular cardiomyoplasty in combination with tissue engineering offers a potential therapeutic option to repair the failing heart. In cell therapy, stem cells are injected into the infarcted heart to replace the damaged cells [5]. A recent progress report by Singh et al. summarized the importance of a variety of stem cells namely, mesenchymal stem cells (MSCs), cardiospheres derived cells (CDC) and cardiac stem cells for myocardial repair [6]. Additionally, these transplanted cardiac stem/progenitor cells can differentiate into a variety of cells like cardiomyocytes, endothelial and smooth muscle cells in the ischemic heart. Although stem cells have been successfully delivered into the infarcted region, the main mechanism of action is linked to the paracrine factors released by the transplanted cells, rather than cardiac regeneration [7]. To enhance the therapeutic effectiveness of stem cells, additional support in the form of scaffold/matrix is employed to improve the cell adhesion, viability and tissue regeneration [8]. Thus, nanofiber-based scaffolds and hydrogel-based extracellular matrices could play an important role in enhancing cell viability, retention and regenerative capability of transplanted stem cells in the damaged ischemic heart tissue.

In contrast to cell therapy alone, cardiovascular tissue engineering offers a promising integrative approach to build an artificial heart muscle using a combination of stem cells and biomimetic scaffolds [9]. Recent advances in nanotechnology and biomaterials provide a variety of different approaches to formulate scaffolds for tissue engineering, which include nanofibers [8], metallic nanoparticles [10], hydrogels [11], injectable gels [12], polymeric microparticles [13], nanogels [14], liposomes [15], and dendrimers [16], as shown in Fig. 1. Therefore, the main focus of this review article is to address the current limitations for treating CHD and novel tissue engineering approaches for cardiac regeneration as shown in Table 1. Furthermore, we will also discuss the current barriers in cell therapy and cardiac tissue engineering.

## 2. The conventional treatments for CHD and their limitations

Conventionally, there are three common ways to treat CHD: pharmacological treatment, surgical interventions with/without medical device implants and heart transplantation. Pharmacological treatments are commonly used to alleviate the early symptoms of angina, atrial fibrillation, peripheral artery diseases, swelling, dizziness and to prevent CHD. Some of the most widely prescribed medicines for the above-said complications fall under the category of anticoagulants, platelet inhibitors, cholesteryl ester transfer protein (CETP) inhibitors [17–19]. Additionally,  $\beta$ -blockers are administered to reduce the metabolic demands of the heart and to stabilize cell membrane [18], which reduces the risk of ventricular fibrillation (VF).

Medical devices and implants are primarily used to restore the heart functions. For instance, to restore cardiac rhythm, a pacemaker is used. Similarly, to restore blood flow and to reduce the shear stress in an

occluded artery, drug-eluting stents are used [17]. Surgical interventions like coronary artery bypass graft surgery (CABG) are performed to treat arteriosclerosis [20]. In the case of ventricular tachycardia (VT) and VF, an implantable cardioverter defibrillator (ICD) is used to stop the cardiac fibrillation, which prevents scar formation and restores the cardiac rhythm. However, ICDs cannot treat all the cases of VT and VF, which are associated with enhanced mortality and heart failure [24].

Overall, current therapies to treat CHD cannot sufficiently manage the progression and prevention of cardiovascular diseases; especially, pharmacological treatments and medical devices with interventional therapies are not a permanent solution and a number of follow-up surgeries are required. The only remaining option is heart transplantation, to replace the damaged heart with a healthy heart from a donor. However, several complications like immune reaction, organ rejection, immunosuppressive therapy and limited availability of donors hinder the success of heart transplantation. Due to these complications, heart transplantation remains an insufficient methodology and additional strategies are of utmost priority [21,22]. One such strategy is to engineer heart tissues and/or build a whole human heart using a combination of biomaterials and cells of the right type. Researchers across the globe are actively working in the area of bioengineering to develop functional human heart tissue. Before we discuss recent strategies, we will discuss in brief about the inherent challenges in tissue engineering to fabricate a functional cardiac tissue.

## 3. Challenges in cardiac tissue engineering

Current challenges in cardiac tissue engineering include the following: alignment and adhesion of cells on scaffolds, conduction of electrical impulse between the cells, optimization of scaffold mechanics to support cell mass and facilitate contraction, development of tissue junctions, the thickness of the tissue and the supply of oxygen [23–25]. It is extremely challenging to replicate the anisotropic structure of human heart tissue, as it requires an intricate, hierarchical control over cell alignment, cell-cell adhesion and, electrical conduction. Therefore, scaffolds should be designed to encourage cell adhesion and promote alignment of cardiomyocytes uniformly across the entire scaffold. Furthermore, acquiring a uniform contractility/cardiac rhythm from the seeded cardiomyocytes on a scaffold is very challenging as cardiomyocytes exist in an isolated form and exhibit mismatch with each other due to differences in their adhesion and cell-cell junctions [25]. Consequently, synchronized contractions of cardiomyocytes are critical for their response to electrical impulses and the ability to propagate an electric impulse [26,27]. Therefore, for a newly engineered cardiac tissue constructs to respond synchronously with the native tissue requires an impulse generator with the same frequency as that of the existing cardiac tissue [28]. The alignment of cardiomyocytes in the direction of the extracellular matrix (ECM) and scaffold's fibers is crucial as the scaffold fibers have to contract and relax along with the cells accompanied with no or minimal phase lag. The highly compact and aligned nature of cardiomyocytes in the myocardium results in cardiac tissue with anisotropic mechanical properties. Their alignment is not unilateral as in skeletal muscle, rather cardiac tissue shows complex patterns of cells that shifts to follow the principal direction of stress [29]. During cardiac contractility, cardiomyocytes contract in the direction of their alignment and expand radially outward, resulting in their host tissue following a similar pattern. Therefore, it is very challenging to design scaffolds, which could match the complexity, alignment, and functionalities of the cardiomyocytes in the native heart tissue [30].

In addition to the above-mentioned barriers, the thickness of the engineered cardiac patch is very critical [31]. It is easy to fabricate cardiac patches with the required surface area; however, fabricating a cardiac patch several millimeters thick is very challenging. It is reported that multilayered patches exhibit improved performance (in regards to uniform pulsations) when compared to single sheets of

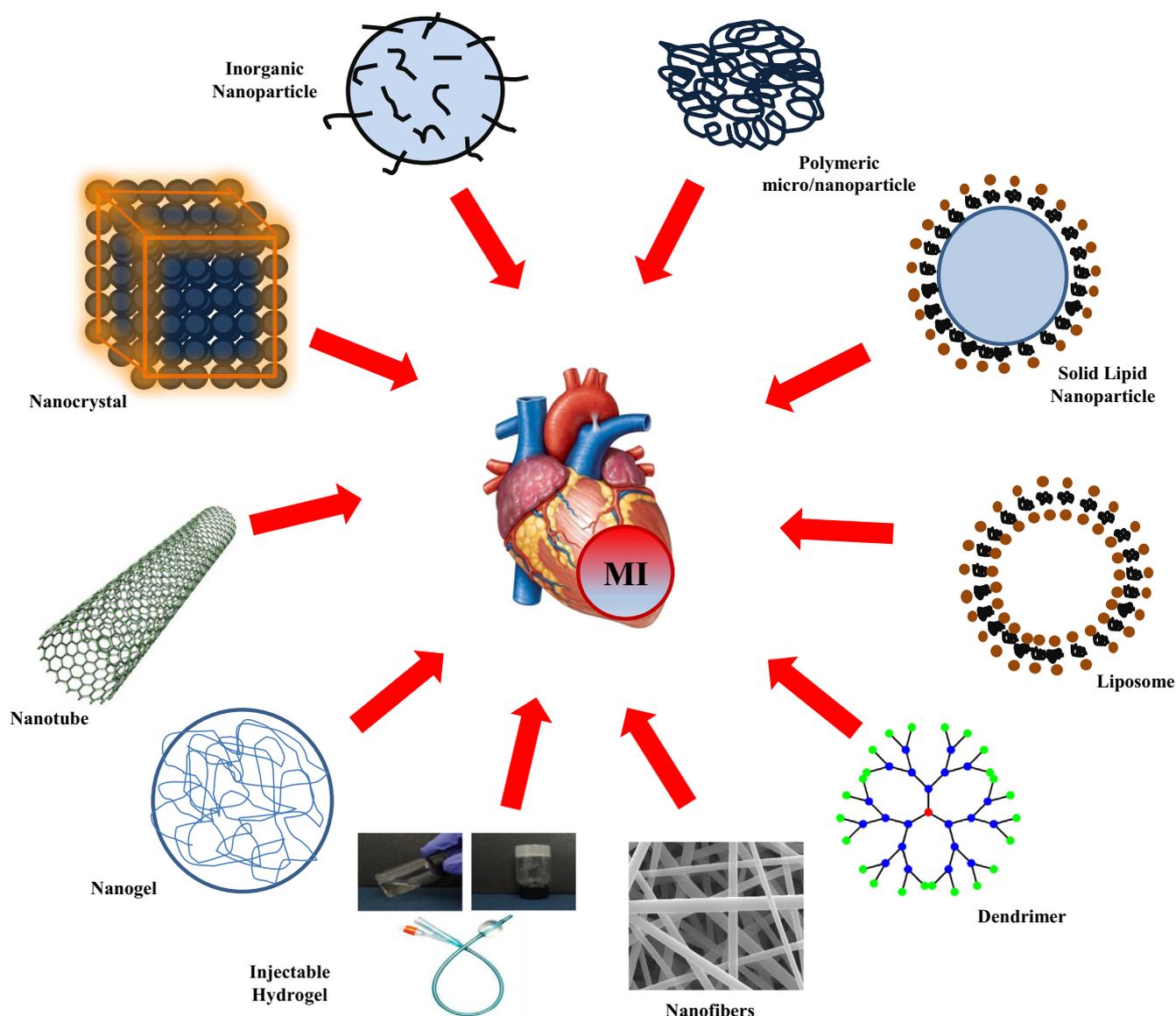


Fig. 1. Fabrication of different biomaterials for cell delivery, imaging and scaffold development for cardiac tissue engineering.

cardiomyocytes [32]. As the oxygen diffusion is limited to a thin sheet of the cardiac patch, vascularization may be required for implantation of thick sheets. For instance, a 100  $\mu\text{m}$  thick neonatal cardiomyocyte sheets (4–6 layers) was subcutaneously implanted into athymic nude rats showed survival for one year along with angiogenesis [33]. In another study, in order to have thickness closer to the normal human diastolic, left ventricular myocardium [34], four 100  $\mu\text{m}$  thick neonatal cardiomyocyte sheets were implanted. However, poor angiogenesis was shown and central necrosis was observed due to insufficient oxygen supply [35]. The supply of oxygen and nutrients to cardiomyocytes deep inside of the engineered patch is a major challenge [8,25] as most cardiac patches depend on diffusion to meet their oxygen demand [25]. Diffusion is not sufficient as there is a 200  $\mu\text{m}$  tissue depth limit for the diffusion of oxygen [34]. Thus, diffusion is only sufficient in a *in vitro* models where a thin layer of cardiomyocytes is in continuous contact with culture media [9,36]. It was reported that even for tissue constructs bathed in culture medium with harmonic or circulatory motion in case of vibratory or rotating bioreactors respectively, diffusion is the only mechanism to control oxygen exchange inside the construct [37–39]. The problem of oxygen supply can be resolved by vascularization of the synthetic cardiac patch *via* co-culture with endothelial

cells and/or release of angiogenesis growth factors [25,40]. While these neovascularization strategies have been widespread in tissue engineering, problems remain with inosculation of the *in vitro* formed vessels with the host vessels. Moreover, this requires establishing perfusion with multiple blood arteries and veins, and maintenance of co-culture environment, in addition to the coupling of electromechanical beating with host tissue [41]. To gain these functions, external electric stimulations and use of electro-conductive scaffold materials are required to improve the involuntary and synchronized beating of synthetic heart tissues [27,42,43]. Attaining precise control of all of these factors and optimizing them with native host tissue is very challenging and efforts continue in all these fronts to develop fully functional artificial heart constructs.

The other key challenges are the choice of right stem cell sources and their maturation aspect and incidence of cardiac arrhythmia after transplantation. We shall discuss briefly these issues in the following paragraphs.

**Table 1**  
Methods for cardiac tissue engineering.

Tissue engineering methods	Properties	Drawbacks	Challenges	References
Nanofibers	Provide natural ECM for cell migration and proliferation. Aligned structure, provide good mechanical support if they are fabricated with metallic particles.	Need special fabrication instrument. Difficult to get uniform nanofibers sheet. Need to be implanted-in Invasive procedure. Need of toxic solvents Need to be implanted Mostly invasive procedure. Very fast release kinetics	To obtain the desired porosity. Fabricating a thick nanofiber patch. Oxygen & nutrient diffusion in a thick patch.	[9,171–174]
Hydrogels	3D Natural ECM like structure. Can deliver growth factors. Easy surface modification. Versatile design.	Chance of blocking the vessels due to uncontrolled gelation Very fast release kinetics	Optimizing an optimal non-toxic cross-linkers for hydrogel gelation Electrical conductance	[142,144,146–149,152,154,156,158]
Injectable gels	Minimally invasive and Injected using a catheter. Thermo-sensitive and pH-sensitive properties. 3D Natural ECM like structure. Can deliver growth factors.	Availability of tissues, Washing step-very critical. Immune rejection. Specialized skills needed. Maintaining a sterile setup is not easy	Tuning an optimum gelation kinetics. Electrical conductance	[73,141,151,183]
Decellularization	Preserved architecture and mechanical properties. Electrical coupling with the host tissue.		Possibility of growth factor delivery Injectability	[148,162–166]
3D printing	Can lead to scaffolds with defined geometric dimensions Surface engineering possible Improved precision, can be coupled with microfluidics to develop heart-on-a-chip		Developing a fully function heart Alignment of cardiomyocytes in a 3D printing setup	[180,182]

### 3.1. Choice of stem cell sources and their maturation aspect for cardiac repair

The typical choice of donor cell sources for cardiac tissue engineering are: 1) Somatic muscle cells including fetal/neonatal cardiomyocytes and skeletal myoblasts. 2) Myocardium generating cells: Embryonic stem cells, pluripotent stem cells, adipose-derived stem cells, bone-marrow derived stem cells and cardiac progenitor cells, and 3) Angiogenesis-inducing cells such as fibroblasts and endothelial progenitor cells [44,45].

A growing body of evidence points towards the fact that the maturation stage of the stem cells play a vital role in their survival and host tissue integration [46–53]. An ideal maturation stage required for stem cell transplantation and cardiac tissue engineering studies is highly debatable. In the case of cardiomyocytes, early-stage cardiomyocytes (fetal/neonatal CM) were considered a better candidate than fully matured cardiomyocytes because of their better survival *in vivo* [54]. However, there are very few studies comparing the efficacy of these cell sources. One recent comparative study by Fernandes et al. [55] concluded that the human embryonic stem cells derived cardiomyocytes (hESC-CMs) and mesodermal cardiovascular progenitors (CPCs) performed better than the human bone marrow mononuclear cells (hBMMNCs), which is one of the most widely tested cells in the clinical trials. Interestingly, this study did not observe any difference in cardiac repair efficacy when hESC-CMs (more-matured) and CPC (less-matured) were compared [55]. However, this study had one limitation, it was not well established how matured hESC-CMs (with respect to their structure, sarcomere size, gene expression, and electrical conductance) when compared to the normal adult human cardiomyocytes. Similarly, another study demonstrated that induced pluripotent stem cells derived cardiomyocytes (iPSC-CMs) were typically immature in nature, when compared to adult cardiomyocytes [56]. Furthermore, these cells differ in terms of their structure/function, fetal gene expression, uneven morphology, dependence on glycolytic metabolism and poor contractile properties, which act as a critical barrier for *in vivo* survival of stem cells and host tissue integration [48,57–59]. Nonetheless, numerous studies have demonstrated various *in vitro* strategies to enhance maturation of cardiomyocytes by biochemical stimulations (using cytokines, hormones and other small molecules), biophysical stimulations (electric current and mechanical) and by providing necessary 3D micro-environments [48,51–53,56,60–62]. Overall, there is a paucity of information about desired maturation stage for successful engraftment of transplanted stem cells into the ischemic heart and further studies are warranted to compare transplantation of mature vs immature cells (human IPS-derived cardiomyocytes/CPCs) to establish the therapeutic efficacy of the stem cells for cardiac repair.

### 3.2. Transplantation of stem-cell patches and incidence of cardiac arrhythmias

The incidence of cardiac arrhythmias after transplantation of ESC/iPSC-derived cardiomyocytes cannot be ruled out [63–65]. Interestingly, arrhythmias were predominantly observed only in non-human primate models [64,65] and not in small animal models [66–68]. In the pigtail macaques MI model, Chong et al. demonstrated significant re-muscularization of the infarcted heart upon intramyocardial injection of one-billion hESC-CMs. However, they also observed non-fatal ventricular arrhythmias in hESC-CM engrafted primates [65]. In another study by Liu et al., similar graft-associated ventricular arrhythmias were observed when ~750 million hESC-CMs were intramyocardially injected in a non-human primate model [64]. In the contrary, it has also been reported that the cells grown on the patch showed a better host-tissue integration. For instance, a study by Ye et al. demonstrated that trilineage (2 million cells each; hiPSC-derived cardiomyocytes, endothelial cells and smooth muscle cells) 3D fibrin patch loaded with insulin growth factor (IGF)-encapsulated microspheres resulted in

significant improvement in the cardiac function. Furthermore, this study used a low dose of hiPSC-CMs (~100 fold lower than that used by Chong et al. [65]) and did not observe any cardiac arrhythmias [69]. Similarly, another study demonstrated that the cells grown on a patch caused a lower frequency of arrhythmias since the cells grown on a patch have structural and functional similarity to the host cardiac tissue [61]. A recent study by Shadrin et al. developed a large functional 3D patch called “Cardiopatch” cultured with hiPSC-CMs on a hydrogel and matured for up to 5 weeks. Using this approach, they fabricated highly functional clinically relevant cardiac patch of large dimension (36 mm × 36 mm). Additionally, these cardiac patches (0.5 mm, 7 × 7 mm) implanted onto rat heart following an MI showed improved cardiac function, with no incidence of cardiac arrhythmias [70]. Moreover, the cells seeded on the cardiac patches can be tuned to match the host-tissue structure and dynamics through designing a highly conductive non-isotropic cardiac patch. The conduction property of the patches can be enhanced by fabricating the patches with conducting materials like gold nanowires [71], carbon nanotubes [72], silica nanomaterials [73] or other conducting polymers [74,75]. These cardiac patches can also be functionalized to have growth factors or other chemotactic agents [69,76], which can attract host cells within the patch and assist in superior tissue integration and electrical conductance.

Menasche et al. in a recent clinical safety study demonstrated the successful transplantation of hESC derived cardiac progenitor cells in six patients having severe ischemic left ventricular dysfunction. The hESC derived cardiac progenitor cells co-expressing stage specific embryonic antigen-1 (SSEA-1) and cardiac transcription factor ISL-1 markers were specifically generated, encapsulated in fibrin patch and transplanted into patient's heart through epicardial delivery (median dose of 8.2 million cells) during a coronary artery bypass procedure. One patient died immediately after the surgery due to treatment-unrelated comorbidities. This study did not observe any tumors or arrhythmias in all of the remaining patient's follow-up for one year, which was the primary end point of their experiment. All treated patients showed improvement in their systolic motion, although one patient died due to heart failure after 22 months. This is the first study to demonstrate the safety and efficacy of hESC based cell therapy in an encapsulated fibrin patch [77].

In summary, we need to consider several factors to see beneficial effects of cardiac cell therapy and it is very crucial to examine the maturation stage of cardiomyocytes, and the total number of cells before implantation into the ischemic heart. Moreover, combination of a multiple lineage cells in a patch could have a significant clinical impact on the therapeutic outcomes for treating failing heart. Other considerations might be using a 3D conductive cardiac patch, which could improve the electrical conduction with the host-tissue and decrease the incidence of cardiac arrhythmias.

#### 4. Modern trends in cardiac tissue engineering

##### 4.1. Scaffold-free cardiac tissue engineering approaches

After an MI, there is a loss of up to one billion cardiomyocytes in the infarct zone. Thus the idea of myocardial regeneration by cell injection has emerged and was tested in several *in vivo* studies over the past two decades [54]. Scaffold-free approaches involve injection of cells either in 2D or 3D structures onto the infarct zone [78]. Scaffold-free approaches involve the fabrication of tissues by mimicking the tissue developmental processes, which follows a pattern of sequences like cell condensation, proliferation, differentiation, ECM production, and tissue maturation. Furthermore, self-organization and self-assembly are two distinct categories reported as scaffold-free tissue engineering. Self-organization is a process in which cellular organization is imparted via external energy or force is given to the system, which includes bioprinting and cell-sheet engineering. Cell aggregates are often formed in

culture by application of rotational force to suspended cells or by utilizing a non-adherent cell culture conditions, which can be categorized as self-organization. Due to this rotational motion, these culture experience improved diffusion and nutrient/gas exchange when compared to static cultures. In contrast, self-assembly does not require outside forces to form tissues. The tissue maturation follows a track which is identical to that of native tissue development, where cellular interactions and coming together (e.g. high-density cell culture) are directed by spontaneous minimization of free energy, followed by tissue-specific extracellular matrix (ECM) production *via* maturation process to form functional tissue [79].

These approaches of self-organization and self-assembly involve two methods which include engineering cell sheets and spheroids respectively [80,81]. Cell sheet engineering technique involves the generation of a sheet of confluent cells, which can be stacked on top of one another to develop a thick cardiac patch. Cell sheets are developed by seeding cells on a culture dish coated with temperature responsive polymeric layer [80]. Once a complete sheet is formed, the culture dish is placed in a chamber at reduced temperatures (below the polymer's LCST temperature) which alters the hydrophobicity of the polymer substrate and reduces the adhesion between cells sheet and the cell culture dish [82]. The sheet of cells is removed and stacked one another to assemble into a myocardium-like tissue. Vascularization in this tissue can be attained by sandwiching endothelial cell sheet between two cardiac cell sheets. Previously, an *in vitro* pre-vascularized 3D tissue construct was reported by using cell sheets developed with fibroblast and endothelial cells [83]. Similarly, cell sheet with aligned cells in a specific direction to develop anisotropic tissue construct was produced by using a patterned substrate [84]. These cell sheets can be configured into different shapes such as tubes or patches to attain the desired mechanical strength and functions *in vivo* without any synthetic polymer scaffold [85,86].

The second scaffold-free technique of tissue engineering works through the generation of multicellular masses of cells called spheroids [87,88]. Uniform embryoid bodies are obtained by seeding cells in microwells formed in containers comprised of polymers like PDMS and poly(ethylene glycol) (PEG) [89,90]. The physiology and morphology of these spheroids are based on the nature of seeding cell types, their density and the dimensions of the container (size, shape, volume). These resulted spheroids/embryoids have the ability to differentiate into a variety of cell types including cardiac cells, endothelial cells and nerve cells [91–93]. In a recent study, embryoids with a diameter of 450 μm demonstrated enhanced cardiogenesis, which was confirmed by increased expression of sarcomeric α-actinin and cardiomyogenic genes. Embryoids can be an important tool to study the initial phase of embryonic development and can mimic the cardiogenesis *in vitro* [94].

Multicellular spheroids are mostly used in scaffold-free tissue engineering due to their ease of handling and fast growth [95,96]. Various cell culture techniques utilized to prepare these multicellular spheroids include non-adherent cell culture plates, suspension cell culture, hanging drops and microfluidics devices [97,98]. These spheroids offer extra tissue regeneration capacity due to their intense cell-cell interactions and native ECM structure similar to the 3D scaffold [99]. Furthermore, they vascularize rapidly *in vivo* due to their high vasculogenic and angiogenic potential [100]. Recently, usage of spheroids for computer-aided tissue engineering technologies like 3D bioprinting have been reported. A complex computer-aided design (CAD) with full control on assembly of spheroids is now being used to develop fully functional 3D construct [101].

Although scaffold-free techniques offer advantages such as high levels of cell-cell interactions and production of native ECM, this strategy has limitations and further studies are warranted to optimize these techniques. Although, scaffold-free tissue engineering techniques showed success to some extent, they lack the native myocardium arrangement which is required to perform cardiac rhythmic contraction *in vivo* [102]. Other limitations include the weak mechanical strength of

its individual blocks and requirement of high initial cell density for spheroids [103]. In order to overcome limitations of mechanical strength, initial cell density, cells adhesions and ECM depositions in scaffold-free tissue engineering, scaffold-based tissue engineering approaches were developed as described below.

#### 4.2. Scaffold based cardiac tissue engineering approaches

Cell therapy studies for MI treatment resulted in poor survival of stem cells in the ischemic heart and majority of the transplanted cells were lost within hours to days after transplantation. Therefore, attempts have been made to overcome this challenge by using scaffolds to enhance the survival and retention and of transplanted stem cells in the infarcted tissue. These materials can be synthetic/natural in origin or a combination of both, they can further be modulated by surface modifications or by conjugating them with other required biological molecules for stimulating survival and proliferation of cells. These scaffolds can be seeded with autologous/allogeneic cells and transplanted onto the damaged site to promote tissue regeneration [104].

A myocardial tissue is dynamic in nature with continuous exposure to forces that alternately contract and relax the tissue. Thus, an ideal scaffold for cardiac tissue engineering should possess excellent mechanical properties and electrical conductivity to perform normal physiological functions of the heart [36,105]. Over a period of past two decades, a variety of scaffolds with high mechanical stability, electrical conductivity and biodegradability have been developed. Tables 2 & 3 lists the most widely used biomaterials (natural and synthetic) for cardiac tissue engineering with their properties. Materials utilized for cardiac tissue engineering include biocompatible natural polymers like gelatin, collagen, alginate, chitosan, fibrin, and hyaluronic acid [51,106–109]. Synthetic biodegradable polymers such as PGA, PCL, PLA, and PLGA have also been widely used for cardiac tissue engineering [8,93,106,110]. The major advantage of synthetic polymers

as compared to natural polymers is that they can be synthesized in a controlled manner and their properties can be fine-tuned with respect to the desired applications. For instance, PLGA degradation rate can be tuned by varying the ratio of glycolic acid to lactic acid [8]. This control of synthetic scaffold makes it possible to initially provide required cells support and then slowly degrade with the development of tissue and finally lead to the development of fully functional tissue. Furthermore, there are reports in which cell adhesion domains, biological cross-linking proteins/peptides and enzymatic cleavage sites are added to enhance cell attachment, migration, and to naturally dissolve the scaffolds [111].

To develop functional cardiac tissue, the scaffold must provide sufficient porosity for cell ingrowth, an architecture that aligns its cellular constituents, mechanical properties sufficient to facilitate surgical implantation, maintain/encourage the desired cell phenotype, and suitable surface characteristics to encourage firm cell adhesion. In order to fabricate such a scaffold, hybrid or composite scaffolds comprising of both natural and synthetic materials are currently developed. For instance, semi-interpenetrating networks of photo-crosslinkable methacrylated hyaluronic acid (MeHA) and collagen scaffold have been fabricated to attain better mechanical strength than collagen or MeHA alone, to maintain biocompatibility and cell adhesion [112]. Biomaterials have great potential to treat both myocardial conduction (AV block/VA) and contraction disorders. Polymers that exhibit high electrical conduction or composite materials with metallic nanoparticles can restore conduction pathways blocked either by ischemic heart diseases or by other physiological reasons. Extracellular matrix (ECM)-like structures made using conductive polymers can promote cell survival while establishing electrical bridges between the cells [71]. Researchers are trying to develop biological pacemakers, which can act as a permanent pacemaker. This proposed biological pacemaker will be able to respond to endogenous stimuli, which is triggered during increased/decreased cardiac activity [113].

**Table 2**  
Natural polymers for cardiac tissue engineering.

Natural polymers				
Polymer	Properties	Scaffold types	Functional outcomes	References
Alginate	Polysaccharide, Biocompatible, Biodegradable.	Nanofibers, Hydrogels, Injectable gels	Improved cardiac function, ventricular wall thickness, angiogenesis, cell survival, and decreased fibrosis and inflammation in the infarct zone. Sequential delivery of VEGF-A165 and PDGF-BB, increased vessels density and improved cardiac function.	[156]
Chitosan	Polysaccharide, Biocompatible, Biodegradable.	Nanofibers, Hydrogels, Injectable gels	Enhanced cardiac differentiation of adipose derived stem cells (BADSCs), increased angiogenesis.	[154,155]
Hyaluronic acid	Glycosaminoglycan, part of ECM, Support cell migration and proliferation	Nanofibers, Hydrogels, Injectable gels	Increased cell adhesion and proliferation	[112]
Collagen	Protein, ECM main component.	Nanofibers, Hydrogels, Injectable gels	Increased maturation of hESC-CMs. Increased cardiac re-modeling and angiogenesis. Cardiac differentiation of hESCs without the addition of soluble factors.	[51,107,112,123,148,151,152,175]
Gelatin	Peptide, Produced from collagen hydrolysis.	Nanofibers, Hydrogels	Increased cell attachment, proliferation, differentiation, alignment and contraction. Enhanced paracrine mechanisms such as tissue preservation and neovascularization.	[88,115,142,178]
Fibrin	Protein, Water insoluble.	Nanofibers, Hydrogels	Increased mechanical coupling of ECM. Improved cardiac function, ventricular wall thickness, angiogenesis, cell survival, decreased fibrosis and inflammation.	[116,144]
Cellulose	Polysaccharide, Strong mechanical properties.	Nanofibers, Hydrogels	Increased anisotropy.	[127]

**Table 3**  
Synthetic polymers for cardiac tissue engineering.

Synthetic polymers				
Polymer	Properties	Scaffold Types	Functional Outcomes	References
PLGA	FDA approved co-polymer, Biocompatible and biodegradable.	Nanofibers, Hydrogels, Microfluidics devices	Increased cardiomyocyte proliferation, decreased LV remodeling, and enhanced cardiac function.	[8,61]
PCL	Semi-crystalline and biodegradable polymer. Sustained drug delivery. High mechanical stability to support cardiac contractions.	Hydrogels, Nanofibers, Nanoparticles, Microfluidics devices	Increased alignment and maturation of cardiomyocytes.	[13,122]
PNIPAAm	Thermo-responsive, LCST can be tuned to 37°C. On-demand drug delivery.	Nanofibers, Nanogels, Hydrogels	Increased maturation and vascularization.	[14,153]
Gold Nanoparticles	Improved electrical conduction and enhanced coupling of tissue construct with host tissues.	Nanofibers, Hydrogels	Enhanced cardiomyogenic differentiation, enhanced MSC proliferation and differentiation into cardiomyocytes, increased contractile proteins synthesis and help to achieve typical cardiac phenotype.	[114,125,135]
Polypyrrole	Stimulating cardiomyocytes, regeneration of infarct myocardium and cardiac defects	Nanofibers	Promote cell attachment, proliferation, interaction, and expression of cardiac-specific proteins.	[132]
PVA	Water soluble, transparent, biodegradable and biocompatible. Co-culture membrane and excellent coating material.	Nanofibers Hydrogels	Stabilized cardiac function and attenuated dilatation.	[126,127]
CNT	Stimulating the proliferation of human cardiomyocyte, support improved alignment of cardiomyocyte, higher expression of cardiac-specific genes, <i>in vitro</i> cardio-myogenesis.	Nanofibers, Hydrogels	Enhanced cardiac differentiation and increased synthesis of contractile protein (cardiac myosin heavy chain).	[128,129]

#### 4.2.1. Synthetic scaffolds for tissue engineering

**4.2.1.1. Nanofibers.** Nanofibers can be defined as fibers that mimic the natural architecture of the extracellular matrix (ECM) and promotes a platform for cell adhesion in a 3-D microenvironment. They are fabricated using different methods, which include; electrospinning, self-assembly, drawing, template synthesis, and thermal-induced phase separation [114–116]. Nanofibers produced by these methods have a morphological resemblance to natural ECM [8] and provide special structural support for cell attachment, migration, and differentiation. The morphology, topology, and geometry of nanofibers are important for proper transport of biophysical factors and thus to maintain the functional cardiomyocyte physiology. Aligned nanofiber scaffolds made using Poly(L-lactic acid) (PLA) blended with polyaniline (PANI) have been reported to have enhanced conductivity with better beat frequency and good cell viability. The H9c2 cardiomyoblasts grown on these scaffolds were also found to have improved maturation and fusion index as well as better spontaneous beating due to the ECM mimicking properties of this scaffold [117]. To understand the mechanism by which cells interact with nanofibers, Balashov et al., performed a recent study to understand a single cell-single fiber interaction. They observed that cardiomyocyte with its cytoplasm, covers the entire nanofiber or nanofibers bundles in certain cases, forming a sheath-like structure, which enables the cell attachment over the entire fiber [118].

There are various electrospinning techniques to obtain nanofibers with different dimensions, morphologies, and porosity to most closely match natural ECM as shown in Fig. 2. Furthermore, a high level of porosity and improved oxygen supply to cardiomyocytes was observed in bioengineered scaffolds. PCL based nanofiber scaffolds are commonly used in tissue engineering applications, PCL nanofibers with its surface modified with gelatin coating increases its hydrophilicity, thereby enhancing cardiomyocyte attachment and proliferation [110]. In another study, polyethylene glycol (PEG), carboxylated PCL (CPCL), and PCL hybrid scaffold has been reported to develop embryoid bodies (EBs) from murine ESCs (mESCs). Additionally, this scaffold also facilitated the differentiation of mESCs towards functional cardiomyocytes. It was

also found that the enhanced CM differentiation was directly linked to the PCL-3D nanofiber scaffold and was triggered by the stimulation of canonical Wnt/ $\beta$ -catenin signaling during early differentiation [119]. A composite Polypyrrole (PPy), PCL and gelatin-based nanofibers were fabricated by mixing different concentrations of polypyrrole (PPy) to PCL/gelatin (PG) solution and tested for cardiac tissue engineering. This study showed a significant enhancement in attachment, proliferation, differentiation, and interactions of cardiomyocytes [120].

As discussed earlier, the alignment of nanofibers in the scaffold is also important for cardiac tissue engineering as shown in Fig. 3 [121]. Han et al. investigated the effect of aligned nanofibers on the maturation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The aligned nanofibers sheet showed highly synchronized sarcomeric organization when compared to controls. The morphology of cells on the aligned nanofibrous scaffold was also uniform and cell size was larger than controls ( $864 \pm 37 \mu\text{m}^2$  versus  $745 \pm 37 \mu\text{m}^2$ ). The hiPSC-CMs grown on aligned nanofibrous scaffold showed an increase in cardiomyocyte associated genes and protein expression as well as rapid response to treatment with cardiovascular drugs. However, modest changes were observed in regard to structural maturation as well as calcium transient were much slower than the control cells [122]. This study demonstrated that the aligned nanofibrous scaffolds despite its structural advantages, did not show any positive effects on the maturation of hiPSC-CMs. Similarly, Joanne et al. showed that collagen-based nanofibrous scaffold seeded with hiPSC-CMs attenuate dilated cardiomyopathy (DCM) mouse model. At two weeks of patch implantation, the cardiac function decreased in the control group (cell-free scaffold) while it was maintained in the experimental group. The main reason attributed to this difference was due to increased angiogenic activity of the cell-loaded scaffold when compared to the control. This study confirmed the potential use of hiPSC-CMs loaded nanofibrous scaffold to stabilize the DCM condition [123].

In another study, hiPSC-CMs were also used to develop cardiac tissue-like constructs (CTLs) by seeding them on a thin layer of poly (D, L-lactic-co-glycolic acid) (PLGA) nanofibers. These CTLs exhibited rapid host electrical integration with coupling times of about

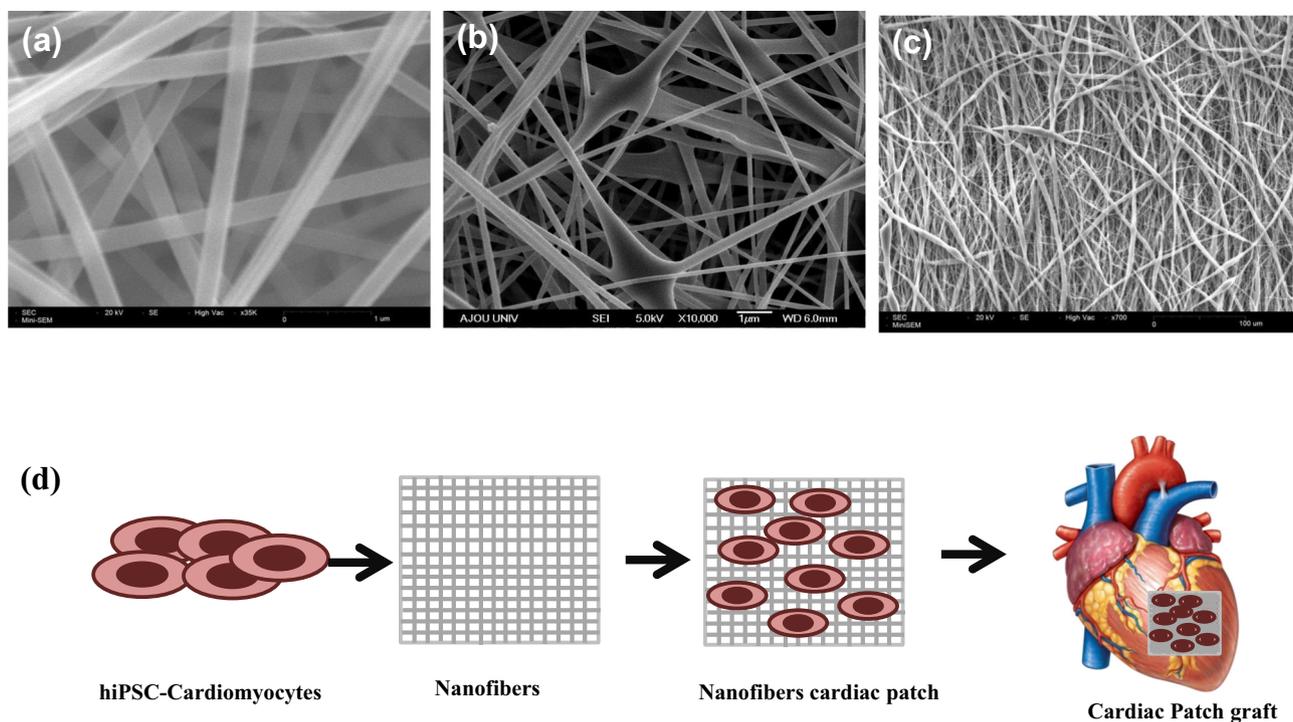


Fig. 2. Electrospinning techniques of nanofibers for cardiac applications. (a) Uniform nanofibers-having uniform size distribution (b) Hybrid-combination of nano and microfibers (c) Aligned nanofibers (d) Schematic of cells incorporated nanofiber based cardiac patch.

81 ± 49 min. The results from this study showed that the CTLC could integrate with the host CM sheets and help in establishing rapid electrical coupling between the interrupted areas in the heart tissue, suggesting a potential role of CTLCs in healing re-entrant cardiac arrhythmias [61]. They also tested and confirmed that CTLCs can be a better *in vitro* cardiac tissue model than the normal 2D culture [61]. Similarly, a recent study from our lab demonstrated the alignment of hiPSC-CM on a PLGA aligned nanofiber scaffold as shown in Fig. 3. Cardiomyocytes seeded on this scaffold showed well-organized sarcomeres and shorter calcium transients at 2 weeks of culture [121]. The successful integration of these scaffolds relies on their electrical coupling with the host myocardial tissues and they must facilitate the cell bundle extension to establish that connection [124]. Hybrid nanofibrous scaffolds with metallic particles and nanowires are reported to increase cell attachment, migration, proliferation, and electrical coupling with the host cells [72,125–129]. A coiled fiber scaffold with gold nanowire has been reported to enhance cardiac contraction, beating rate, and lower excitation thresholds. Gold nanowires help the cardiomyocytes to interact with each other and establish conduction bundles. Cardiac tissue grown on this hybrid scaffold showed anisotropic conduction of electrical signals with strong contraction force. These coiled nanofibrous scaffolds with nanowires was also reported to have better mechanical strength. These fibers stretch and recoil with the heart muscle and reinforce them with required mechanical strength for continuous rhythmic movements and conduction of impulses [130].

Fabrication of electrically conductive scaffolds having suitable mechanical strength, which can induce proper assembly of functioning cardiac tissue is a key challenge in tissue engineering of the heart [4]. Both natural and synthetic materials have their own limitations if they are used alone to fabricate scaffolds. The natural polymers suffer from low mechanical strength and immune reaction, while synthetic materials have issues of biocompatibility, biodegradability and low cell adhesions [5–8]. Therefore, a hybrid nanofibrous scaffold made of biocompatible, biodegradable polymers and metallic nanoparticles helps to overcome the shortcoming of individual material scaffold [72,131,132]. For instance, to develop a scaffold with robust

mechanical properties, carbon nanotubes are combined with a biocompatible polymer, chitosan, and this scaffold showed efficient electrical conduction with a conductivity of  $0.25 \pm 0.09$  S/m and superior mechanical strength to support cardiac tissue engineering. Furthermore, the carbon nanotubes impregnated chitosan scaffold was highly porous and had an elastic modulus of  $28.1 \pm 3.3$  kPa, which was similar to rat myocardium. This study confirmed that the introduction of conductive materials in nanofibrous scaffold increases their potential to mimic natural ECM especially in terms of their electrical conduction [42]. Further step ahead, small molecules like peptides [133] and functionalized gold nanoparticles were integrated with a nanofibrous scaffold of polymethylglutaramide (PMGI), these gold nanoparticles were functionalized with adhesive peptides to enhance the cell attachment. Additionally, these scaffolds were also found to promote the differentiation of hiPSCs towards cardiomyocytes [134]. Gold-nanoparticles (AuNP) have also been introduced in collagen nanofibrous scaffold to develop a hybrid scaffold (AuNP–Col). Interestingly, no differences were observed in the elastic modulus between the AuNP–Col and collagen only scaffold. However, they observed a significant difference in the local nanoscale stiffness between them, which might have contributed to the AuNP–Col's improved organization of cardiomyocyte and intercalated discs (ID) assembly [135]. In another study, the scaffold moduli were found to influence hiPSCs differentiation into cardiomyocytes. When maturation of hiPSC-CMs over two scaffolds: Matrigel-coated PDMS membranes and Matrigel-coated glass slide were compared, it was found that the action potentials was two-fold higher for cells grown on the low modulus PDMS membrane. However, the obtained action potential values were found to be just 57% of the standard *in vivo* value [136]. Robust iPSCs differentiation into cardiomyocytes was reported through the trigger of Wnt/Catenin signaling via inhibition of GSK3 in iPSCs grown on gelatin-coated PCL nanofiber scaffolds [110].

Although nanofibers-based scaffolds have tremendous potential, implantation of these scaffolds in the infarct heart requires invasive surgery. Therefore, hydrogels are currently being explored to mimic ECM for survival and retention of transplanted stem cells in the infarct

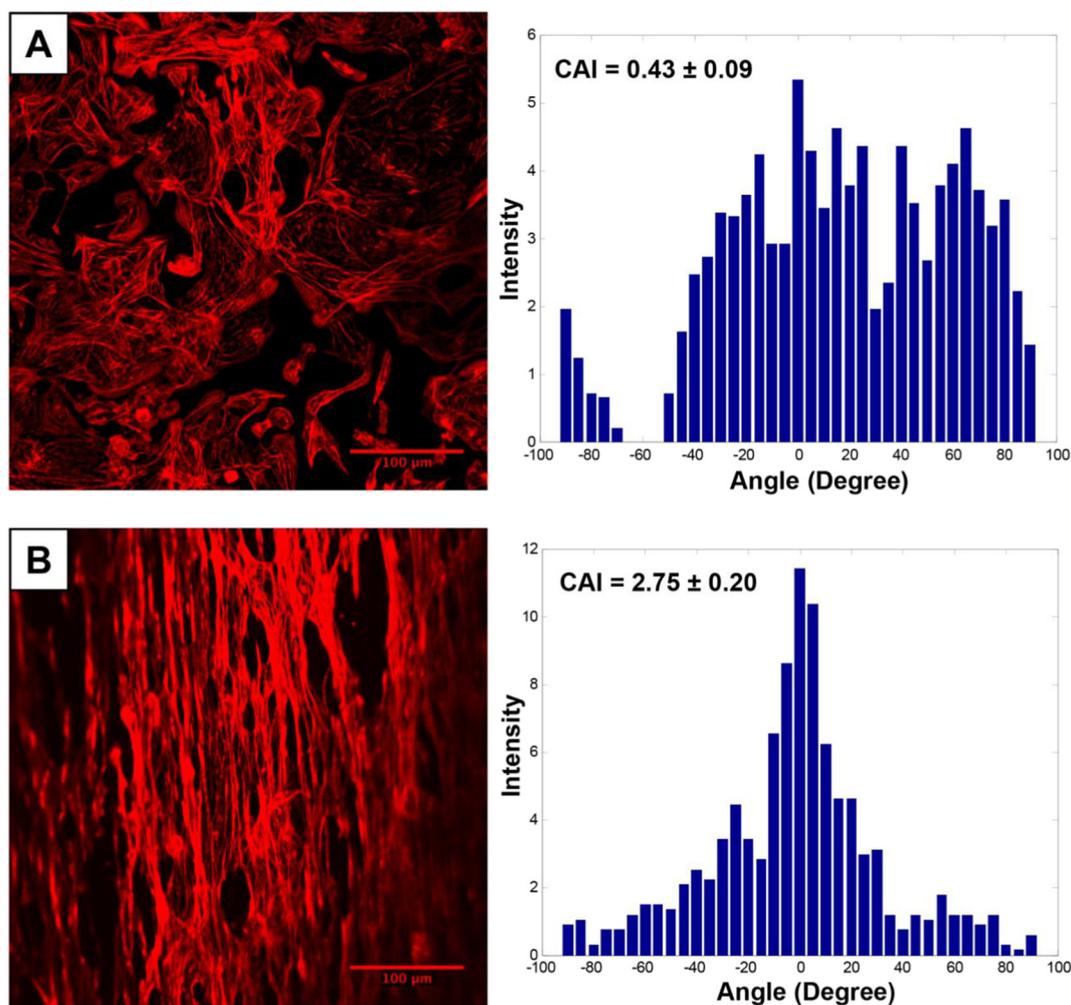


Fig. 3. Fluorescence imaging of hiPSC-CM labeled with phalloidin dye on a PLGA nanofiber scaffold. Cardiomyocytes seeded on the aligned nanofiber scaffold were aligned and elongated in the direction of the nanofiber, when compared to cells seeded on a flat cell culture plate. Alignment of cells on the scaffold was further confirmed by enhanced cell anisotropy index (CAI). Reprinted from Khan et al., PLOS One [121].

heart. In the below sections, we will discuss in detail various hydrogel-based strategies for cardiac tissue engineering.

**4.2.1.2. Hydrogels.** Hydrogels are three-dimensional hydrophilic polymeric networks, which are cross-linked by physical or/and chemical means and that can swell upon interaction with water [11]. They typically have a semi-solid/gel nature with very large content of water that mimics the natural ECM. Hydrogels can be fabricated using different polymers and their surface can be modified easily by the addition of co-monomers or crosslinkers [14]. There are a variety of approaches presented in the literature to use hydrogels in tissue engineering applications using natural and synthetic polymer-based hydrogels for cell delivery, synthetic peptide-based hydrogels to improve cell delivery [137,138], decellularized-heart derived hydrogels to enhance endogenous cardiac repair [139], ECM peptide-derived hydrogel for promoting endogenous cardiac repair and growth factor-releasing hydrogels for improved recovery from injury [140]. Hydrogels are typically used in two different forms: bulk/conventional hydrogels and injectable hydrogels/gels as shown in Fig. 4a [141]. Bulk hydrogels or interchangeably known as hydrogels, which are preformed *ex-situ* as a semi-solid/gel structure and are typically implanted at the site [142]. On the other hand, injectable hydrogels, are hydrophilic materials, which are in liquid form before injection and transform into a gel in the infarct region in response to change in physiological stimuli such as temperature/pH. The main advantage of

these injectable gels is that they can be delivered to the target site through a minimally invasive procedure *via* a catheter as shown in Fig. 5 [143].

In tissue engineering applications, hydrogels play a vital role in cell attachment, proliferation, and delivery of angiogenic growth factors [143,144]. In recent years, hydrogels have been used for a wide variety of cardiac applications. For instance, they are used to deliver vascular growth factors like VEGF, for enhanced angiogenesis inside the engineered cardiac patches, and to guide the alignment of cardiac cells to improve their functions [11,143,145]. In the case of drug delivery applications, these hydrogels are combined with micro/nanoparticles, to form a composite hydrogel system as shown in Fig. 4b [146–148]. These composite hydrogels can deliver multiple biomolecules for a longer duration in a controlled manner [149]. Some of the most commonly used hydrogels include alginate, matrigel, collagen, gelatin, chitosan, hyaluronic acid, fibrin, poly(2-hydroxyethyl methacrylate) (PHEMA), poly vinyl alcohol (PVA), PLGA, poly(N-isopropylacrylamide) (pNIPAAm), and poly(ethylene glycol) (PEG) [143,150,151]. These materials are used alone or in combination with other polymers and cells. Furthermore, hydrogel platforms can be further augmented by the incorporation of biological molecules, which act as a cross-linker for imparting biodegradable properties to the hydrogels [11]. Carbon nanotubes (CNT) can be combined with collagen hydrogels to enhance the electro-mechanical properties of the hydrogel and to improve cells viability of cardiac patch [152]. Additionally,

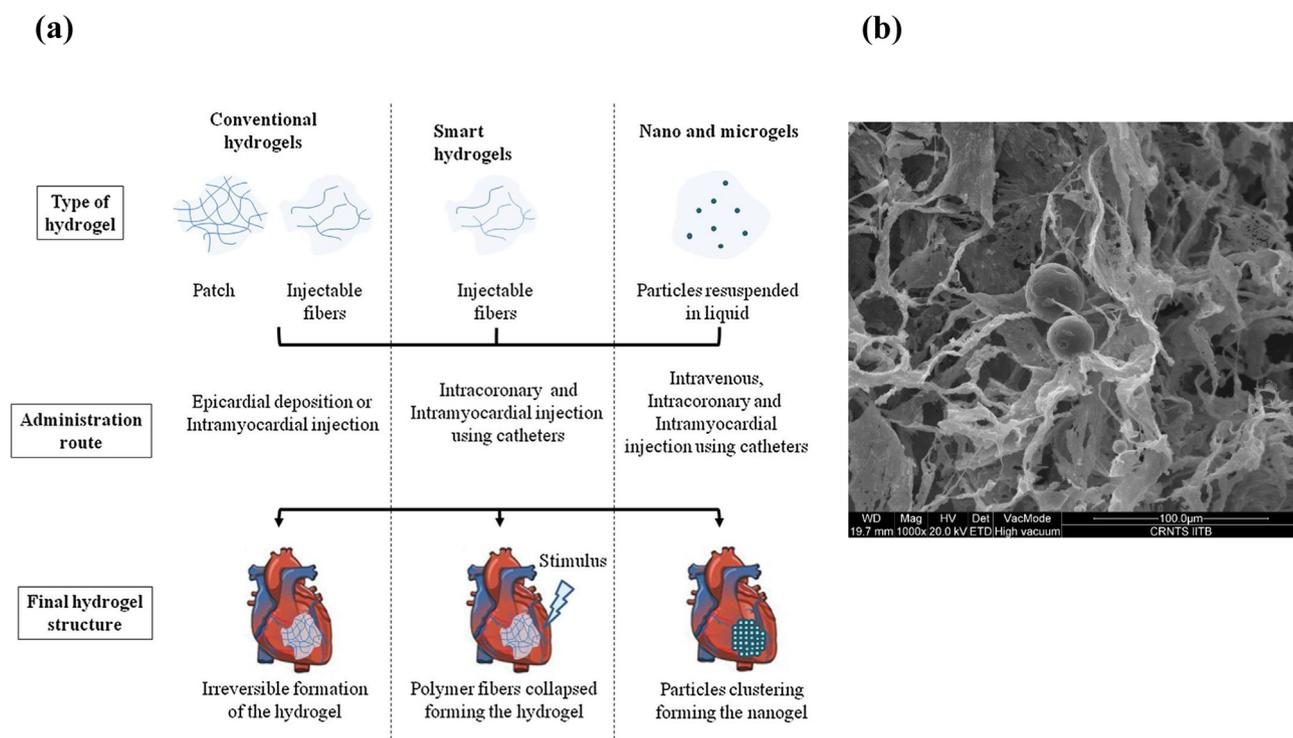


Fig. 4. (a) Hydrogel types and their delivery modes. Reprinted with permission from reference [143] (b) An example of composite injectable gels which has PCL-micro particles embedded in an injectable Chitosan hydrogel.

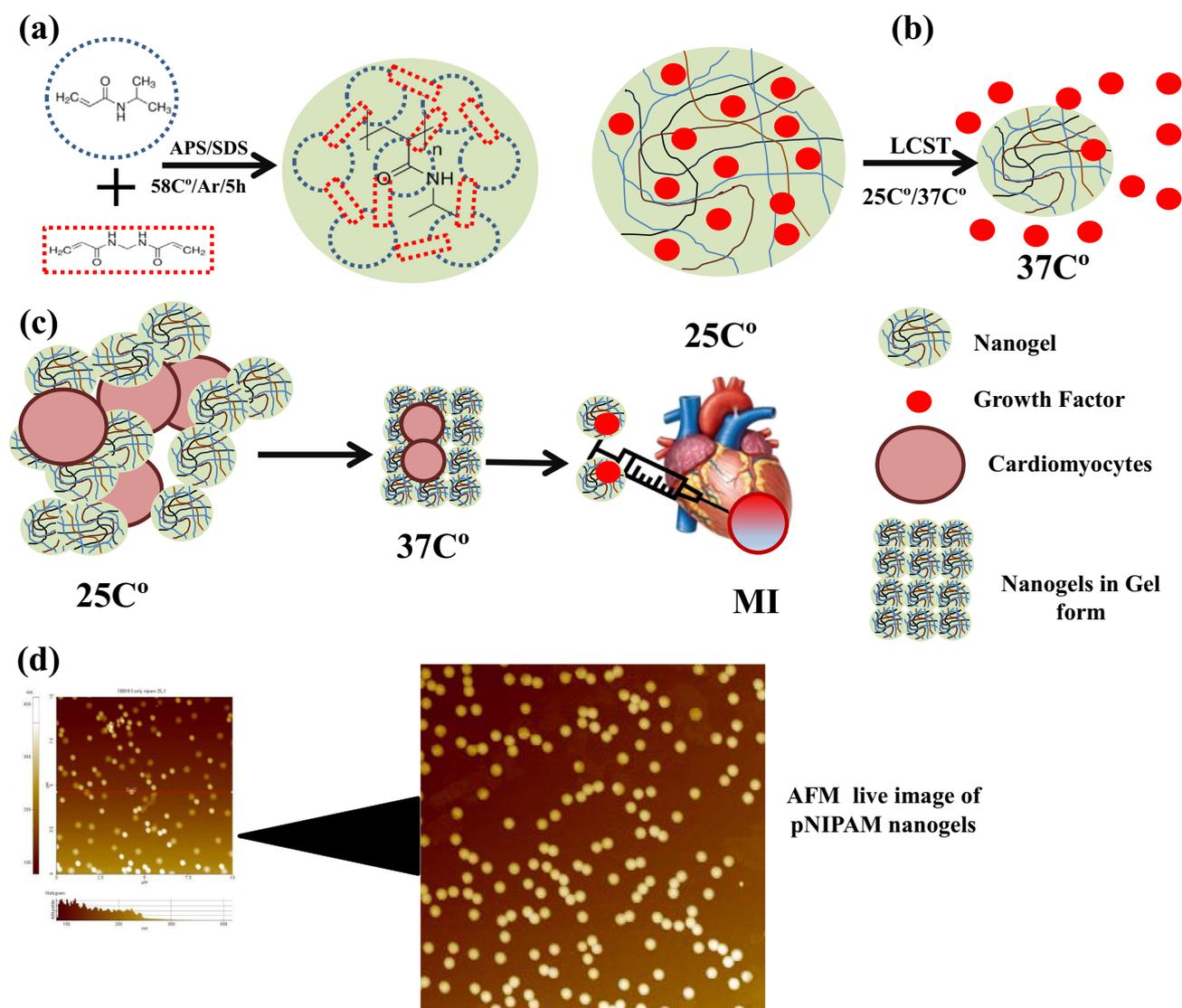
implantation of cell-free hydrogels has shown to improve cardiac function [11]. Hydrogels fabricated using pNIPAAm, a temperature-sensitive material, hydroxyethyl methacrylate-poly (trimethylene carbonate) and acrylic acid (AAc) was injected into infarct heart showed decreased left ventricular dilation and improved cardiac function. Similarly, pHEMA plastic-reinforced gels delivered in a canine model controlled the inflammation for one year and showed its potential as pericardial substitutes [11].

Hydrogels in nano-size are called nanogels, they can be used with nanofiber as hybrid materials for the development of artificial tissue engineered constructs [143]. A recent study demonstrated that cardiac stem cells encapsulated in the nanogels (pNIPAAm-AA) showed improved cell retention and decreased scar formation. Furthermore, the combination of cardiac stem cells and nanogels did not elicit an immune reaction in mouse and pig MI model [153]. Similarly, chitosan-based hydrogels also reportedly improved the healing after MI [154,155]. Though the clinical trials of biomaterials-based product for cardiac tissue engineering are rare, a recent clinical study on the alginate-based hydrogel along with standard medical therapy (SMT) to treat patients with advanced heart failure. Six months follow-up results showed that alginate-hydrogel is more effective in improving subjects exercising ability and as compared to standard medical treatment alone [156]. This clinical study suggests further potential clinical applications of hydrogels for cardiac repair. Decellularized heart matrix (decellularized ECM) and collagen type I hydrogels are reported to differentiate human embryonic stem cells (hESCs) to cardiomyocytes without the addition of growth factors. At two weeks after cell-seeding, improved contractile function and increased mRNA (cTnT) protein expression were observed in embryoid bodies. Therefore, the ability of native ECM-hydrogel for inducing hESCs cardiac differentiation in the absence of any soluble factors makes it an ideal candidate for cardiac repair applications [148].

On the other hand, injectable gels are much more attractive candidates than the hydrogels alone as they are easily translatable and can be delivered with minimally invasive delivery strategies. Smart polymers, which respond to either extrinsic or intrinsic stimuli as mentioned

before are explored as an injectable gelling system. The following are the list of various polymers (both natural as well as synthetic) which are used as injectable gels: alginate, chitosan, hyaluronic acid, matrigel, decellularized extracellular matrices, polyethylene glycol based copolymers, pNIPAAm based copolymers, Poly (2-hydroxyethyl methacrylate) (pHEMA), Poly(glycerol sebacate) (PGS), and self-assembling peptides. However, the main challenge in using these injectable hydrogels is the difficulty in tuning their viscosity, fluidity and gelation kinetics, which could lead blockade of blood vessels [143,157].

A recent study by Li et al. addressed the problem of the hydrogel in blocking vessels by controlling the gelation time of hydrogel. PAA, HEMA $\alpha$ TMC, MA-PEG, and NIPAAm were combined and polymerized to synthesize Poly (NIPAAm-co-PAA-co-HEMA- $\alpha$ TMC-co-MA-PEG) hydrogel with free radical polymerization method. This smart polymer behaves like a liquid at physiological pH of blood (pH 7.4) and behaves like a gel on pH of the infarcted heart (6–7). As this hydrogel behaves like a liquid at the physiological pH of blood and gels at the pH of an infarcted heart, it can be delivered safely to the infarct heart using a catheter. Furthermore, this study also demonstrated the successful loading, delivery, and differentiation of cardiosphere-derived cells (CDCs) in this hydrogel. CDCs were viable in this hydrogel for over 7 days period and increased maturation of these cells into cardiac cells was also observed [158]. In another study, a hydrogel-molding method was combined with dynamic culture to develop an *in vitro* human cardiac tissue maturation platform (cardiopatches) without exogenous stimulation. In this method, the human fibrinogen-based hydrogel-cells combination was added on to a PDMS mold (9x9mm) with a Nylon frame and it was crosslinked using thrombin. The cardiopatches thus obtained were removed from the mold and cultured on a 12-well plate with a rocking platform in the presence of cardiac medium containing aminocaproic acid to prevent fibrin degradation. Using multiple hiPSCs cell lines, it was shown that cardiopatches exhibit both electrical and mechanical properties similar to those of the adult human myocardium. Additionally, the scalability of the technique is shown by the developing a patch with a size of 4 × 4 cm that is clinically relevant, maintaining maturation and functional properties [70]. Recently, cylindrical



**Fig. 5.** (a) Nanogels fabrications, (b) biological molecules delivery from pNIPAM nanogels based on its LCST (c) Nanogels loaded with cardiac stem cells for MI treatment, (d) AFM images of pNIPAM nanogels.

heart tissue was developed by using free-floating dynamic cell culture. The resulted cardiac tissue mimicked the physiology and maturation as that of an adult cardiac tissue [159]. Although these laboratories-generated scaffolds have some success, they are not found to have natural architecture as well as complex signaling molecules as that of natural cardiac ECM. In order to emulate these properties in the scaffolds, decellularized scaffolds are developed, which we shall discuss in more detail in the following section.

#### 4.3. Decellularized scaffold

Decellularization is a process of isolating the ECM from the tissue after removing the majority of cellular debris. Unlike material based scaffolds, decellularization based scaffolds are made using natural decellularized matrix and repopulated with desired cell types [160]. Over the past decade, a variety of decellularization protocols have been successfully developed [161]. In decellularization process, the xenogenic are washed out from sacrificial tissue/organ using surfactants like sodium dodecyl sulfate (SDS), Triton X-100, or enzymes like trypsin and collagenase type I and II, as shown in Fig. 6 to get the cell-free scaffold [162]. It is a prerequisite that the washing process for decellularization has to be repeated until complete removal of the xenogenic materials,

without damaging the ECM composition and integrity. The key advantage of the decellularized scaffolds over their synthetic counterparts is that they maintain the native ECM with site-specific proteins for cellular adhesion. The decellularized xenogenic or allogenic ECM-based scaffolds have been proved to be less immunogenic and were found to display precise biophysical stimuli and signaling molecules that could stimulate normal physiology of tissues. Moreover, the decellularized scaffolds also mimic the natural ECM and thus have better biocompatibility [148]. Therefore, the manipulation of the decellularized heart as a scaffold for heart tissue engineering is an ideal case considering the complexity to regenerate heart tissues. For instance, improvement in cardiac function and its pumping capacity was observed when a decellularized heart ECM was used as a scaffold for seeding of rat CMs and ECs [163]. In another study, decellularized mouse hearts have been used as a scaffold to culture human iPSCs-derived multipotential cardiovascular progenitor cells, which showed uniform migration, proliferation, and differentiation into CMs, ECs and SMCs [164]. Similarly, porcine heart slices (150  $\mu\text{m}$  thick) were decellularized and seeded with either rat or human cardiac cells. This study demonstrated that engineered heart tissues (EHTs) beat synchronously at 5 days after seeding with cardiac cells and displayed a robust length-dependent activation at 21 days. The cells were aligned in the direction

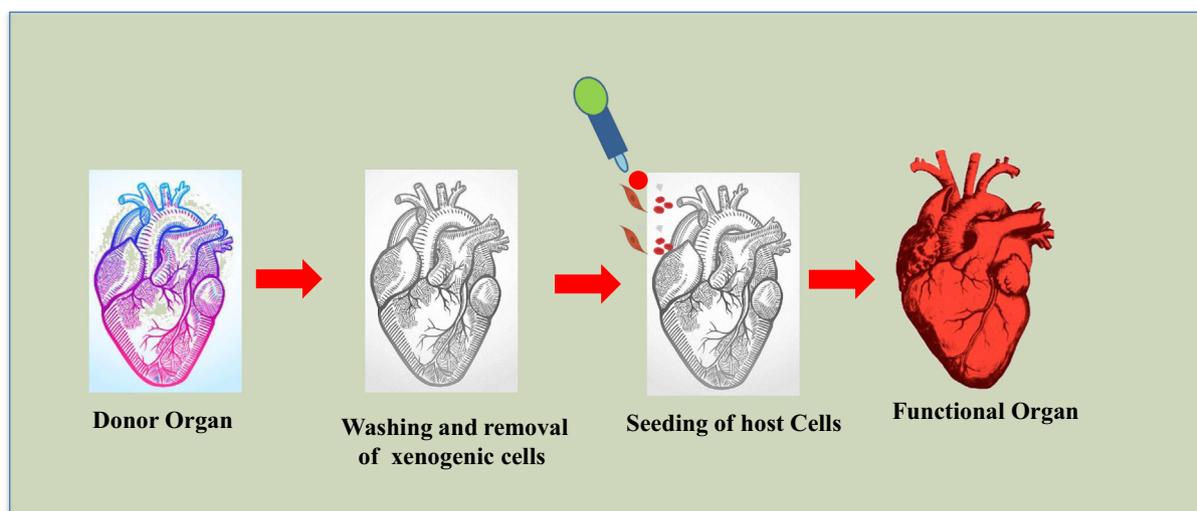


Fig. 6. Schematic representation of decellularization process for a human heart.

of the fiber exhibiting physiological contractile anisotropy [165].

To evaluate the potential of decellularized plant tissues to regenerate cardiac tissues, researchers have used decellularized plant leaves as scaffolds. Gershlak et al. used vascularized leaves surface as a scaffold with fluid perfusion ability. They used various types of leaves with different vascular patterns and found that these leaves remained intact as a decellularized scaffold and their perfusion channels were able to transport fluids. Furthermore, hiPSC-CMs, human endothelial cells (HUVECs) and HMSCs were seeded on the leaf scaffold. After a few days of culture, HUVEC occupied the inner surface of leaf vasculature and the other two types of cells migrated to the outer surface of the leaf. After three weeks, hiPSC-CMs showed spontaneous contraction and expressed calcium handling properties. In addition, the hiPSC-CMs adhere to surface of the leaf and formed clusters after three days and showed contraction one day after the culture [166].

Despite the successful improvement in the functions of the heart using decellularized heart-based scaffolds, there are several drawbacks associated with it as follows: 1) the site-specific repopulation of cells and obtaining the appropriate mechanical strength for pumping the blood [139,160,167]; 2) limited organ donors to get decellularized scaffolds and cross-species source may face potential immune rejection. Therefore, researchers around the world are investigating newer synthetic biodegradable scaffolds and technologies, which could mimic natural ECM. One such approach is the 3D bioprinting of cardiac tissue, which will be discussed in greater detail in the following section below.

#### 4.4. 3D bio-printing based cardiac tissue engineering approaches

3D bio-printing is a computer-controlled process in which materials are combined in a 3D pattern to form a 3D object [168,169]. A recent study demonstrated a 3D printing technique to improve tissue vascularization and rapid vascular integration *in vivo* [170]. The scaffolds with defined geometric dimensions, which mimic the natural ECM morphology are efficiently developed using 3D printing technologies [171]. Surface engineering, also known as topological or surface guidance in 3D printing is used to print grooves and channels in scaffolds, which are found to influence cell migration, differentiation, morphology, phenotype, and physiology [169,172,173]. In regards to this, there are reports in the literature that showed a resolution of  $\leq 1 \mu\text{m}$  and thus are able to maintain intact and distinct channels [174,175]. Similarly, CMs seeded on a 3D printed scaffold showed synchronized contractions with improved cell adhesion within the scaffold [176]. In another technique called 3D sacrificial molding, a rigid 3D lattice of sugar filaments are printed and embedded within an ECM-like matrix

with cells and subsequently dissolved to leave behind a micro-channel network for endothelialization [177]. Similarly, 3D printed gelatin hydrogel cross-linked with an enzyme mTgase with microchannels was developed. These microchannels in the hydrogel were found to orient hMSCs, promote myocardial lineage commitment and improve the cardiomyocyte functionality and orientation [178]. One-step-ahead to develop a fully functional heart, researchers have utilized 3D bioprinting to develop a microfluidic chip-based *in vitro* model of heart as shown in the schematic in Fig. 7 [174,179]. Another study by Lind et al. demonstrated a 3D printed microfluidics device, which could continuously monitor electronic readouts of the contractile stress in 3D printed microfluidics channels. The 3D printed microfluidics device imparts a successful platform to record the contractile function of cardiac tissues and drug screening platform in a controlled micro-environment [180].

In another strategy, bioink which is a mixture of cells and desired hydrogel polymers, are directly printed as scaffolds to develop partial or full organ [168]. A recent study by Gao et al. showed that a novel way of 3D printing technique using multiphoton-excitation to print ECM based scaffold with unique high resolution. The 3D printed scaffold was used to develop a human cardiomyocytes patch, and implantation of this patch onto the mouse MI heart showed increased engraftment of transplanted cells in the patch [181]. Zhang et al. used the composite bioink composed of endothelial cells and microfibrillar hydrogel in 3D printing to fabricate a myocardium. Due to the gravity effect and difference in density, endothelial cells aligned with the periphery of microfibrils and form a symmetrical endothelium layer. Above this layer, a layer consisting of cardiomyocytes was fabricated with controlled anisotropy which gave rise to an aligned functional myocardium with synchronized rhythmic beating [174]. To develop a functional cardiac tissue, a hybrid hydrogel system based on three components *i.e.* PCL, sacrificial hydrogel and cell-laden hydrogel was developed. The sacrificial and cell-laden hydrogel was made using fibrin and cross-linked using thrombin. This hydrogel along with  $10 \times 10^6$  cells/ml acted as a bioink to print a 3D cardiac construct at  $18^\circ\text{C}$ . During the 3D printing process, three different dispensing modules were used for each type of hydrogel and PCL. Overall, the bioengineered cardiac tissue patches showed a synchronized beatings in culture, indicating the potential for cardiac tissue maturation *in vitro* [169].

Use of biomaterials or polymers as constituents of bioink have issues associated with their biodegradability, immune reactions, and toxicities. In order to address these drawbacks, Ong et al. demonstrated a unique strategy to directly deliver stem cells using 3D bio-printing

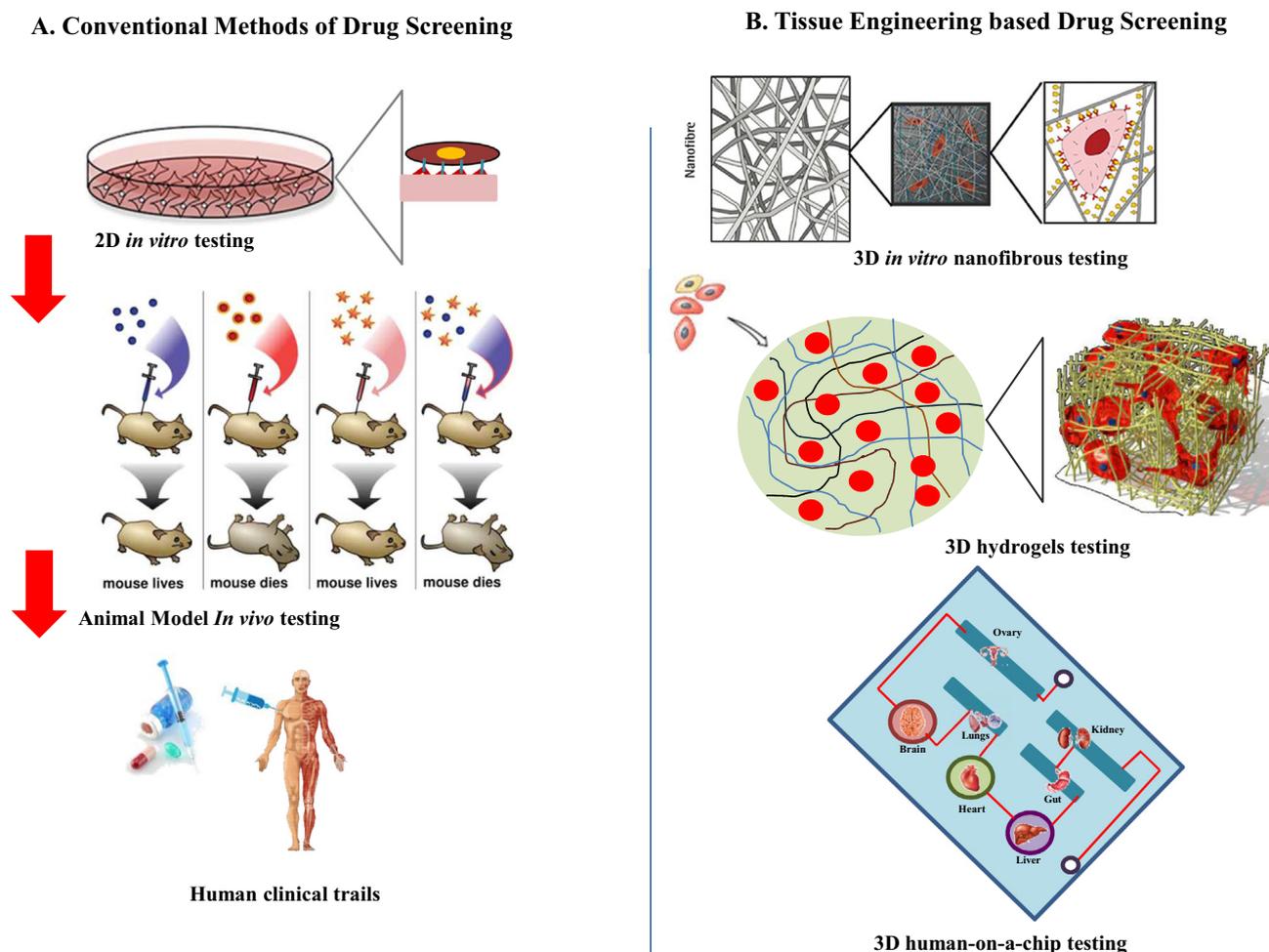


Fig. 7. A) Conventional and B) 3D-tissue engineering based approaches for drug screening applications.

technique without the addition of biomaterials. To develop this cardiac patch, hiPSC-CMs, fibroblast and endothelial cells (ECs) were combined in ratios (CM: FB: EC = 70:15:15, 70:0:30, 45:40:15) to create spheroids and these spheroids were used for 3D printing of cardiac patches. At day 3, these patches initiated its beating and fusion of voids between these patches was also observed resulting in electrical integration of the patch. Additionally, these cardiac patches demonstrated action potential waveforms similar to ventricular cardiomyocytes, along with constant cell alignment and electrical conduction in the patch [182].

## 5. Conclusions

Several potential tissue-engineering approaches have been employed to treat the damaged heart *via* a combination of stem cells and biomaterials-based 3D scaffolds. However, replicating the anisotropy of a fully functional cardiac tissue is a major challenge in the field of cardiac tissue engineering. Further studies are needed to optimize the scaffold and the scaffold-free approaches of tissue engineering. There is an unmet need to develop biomaterials, which could rapidly integrate with the body's host immune system and synchronize with native cardiac tissue for electro-mechanical coupling. Furthermore, developing scaffolds with angiogenic properties will promote vascularization to supply oxygen and nutrients to the ischemic cardiac tissue. Scaffolds also need to have properties to support native ECM development and recruitment of an appropriate number of cells to promote regeneration. The 3D printing of scaffolds offers a promising approach to develop functional cardiac tissue. Overall, the current research trends in cardiac tissue engineering are pointing towards a new hope for developing

novel biomimetic cardiac scaffolds in the near future for treating damaged heart tissue.

## Declaration of Competing Interest

All the authors of this manuscript have declared no conflict of interest.

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## Author contributions

MQ, PA, MK wrote the manuscript draft, designed the outline of the paper, figures and tables. MK and HP revised the manuscript draft. All authors have read and approved the final version of the manuscript.

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