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REVIEW

Application of Human Induced Pluripotent Stem Cells for Tissue Engineered Cardiomyocyte Modelling

Puspita A. Katili[1,2] · Amira P. Karima[3] · Winda Azwani[3] · Radiana D. Antarianto[4,5] · Mulyadi M. Djer[6]

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Abstract

Purpose Cardiactissue engineering opens up opportunities for regenerative therapy in heart diseases. Current technologies improve engineered cardiac tissue characteristics by combining human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with non-cardiomyocytes, selective biomaterials, and additional growth factors. Animal models are still required to determine cardiac patches' overall in vivo effect before initiating human trials. Here, we review the current in vivo studies of cardiac patches using hiPSC-CMs.

Methods We performed a literature search for studies on cardiac patch in vivo application and compared outcomes based on cell engraftment, functional changes, and safety profiles.

Results Present studies confirm the beneficial results of combining hiPSC-CMs with other cardiac cell lineages and biomaterials. They improved the functional capacity of the heart, showed a reduction in infarct size, and initiated an adaptive inflammatory process through neovascularisation.

Conclusion The cardiac patch is currently the most effective delivery system, proving safety and improvements in animal models, which are suggested to be the role of the paracrine mechanism. Further studies should focus on honing in vitro patch characteristics to achieve ideal results.

Lay Summary Cardiac tissue engineering answers the demand for regenerative therapy in heart diseases. Combining human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with biomaterials and growth factors in cardiac patches improves the heart's structural and functional characteristics. This delivery system is safe and efficient for delivering many cells and minimising cellular loss in vivo. Rat and porcine models of ischemic and non-ischemic heart diseases demonstrated the benefits of this therapy, which include cell engraftment, reduced infarct size, and increased left ventricular (LV) systolic function, with no reported critical adverse events. These reports sufficiently provide evidence of feasible improvements to proceed towards further trials.

Keywords Human induced pluripotent stem cells · Cardiovascular disease · Cell therapy · Cardiac patch · Animal models

Puspita A. Katili and Amira P. Karima contributed equally to this work.

✉ Mulyadi M. Djer
muldjer@yahoo.com

[1] Division of Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia

[2] Research Center for Biomedical Engineering (RCBE), Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia

[3] Harapan Kita Women and Children's Hospital, Jakarta 11420, Indonesia

[4] Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia

[5] Stem Cells and Tissue Engineering Cluster, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia

[6] Division of Pediatric Cardiology, Department of Pediatric, Faculty of Medicine, Universitas Indonesia-Dr, Cipto Mangunkusumo Hospital, Jakarta 10430, Indonesia

Introduction

Heart failure remains a massive burden in cardiovascular diseases, contributing 6.5 million cases in the USA in 2015–2018 [1]. Despite significant development in pharmacological therapy and assist devices, mortality remains high. A heart transplant provides the only way to eliminate pathological changes in the diseased heart; however, donor availability is unpredictable [2]. Novel advances are still needed to improve mortality and reduce morbidity, improve patients' quality of life, and find a treatment that is cost-effective and accessible to all layers of society. This problem emphasises the need for a multidisciplinary, regenerative therapy that incorporates cell-based methods and tissue engineering.

The heart suffers permanent damage following myocardial injury due to its inability to repair and replace damaged cells and tissues. This pathological process involves cell death, fibrosis, calcium regulation, metabolic changes, and neurohormonal activity in an inflammatory cascade [3]. While it initially aims to preserve the ventricles' structural integrity and pumping capacity, the resulting fibrosis creates scar tissue that distorts cardiac architecture [4]. Additionally, remote fibroblast activation and constant inflammatory cytokines produce a continuous pathological stimulus which leads to left ventricle remodelling. The self-renewal capability of cardiomyocytes is reported to be only < 1% in adulthood to only 0.45% at 75 years of age, which is highly inadequate to repair cardiac anatomy to its original state [5, 6]. The lack of cardiomyocyte regeneration and permanent fibrosis follows declining heart function, which eventually manifests as heart failure.

Tissue engineering emerges as the solution to this problem, in which its three main components (cells, scaffold, and growth factors) simultaneously act as a functioning tissue to replace damaged counterparts in vivo [7]. Multiple stem cell lines have progressed to clinical trials using mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), umbilical cord stem cells (UCSCs), bone marrow-derived stem cells (BMSCs), and cardiosphere-derived cells in the paediatric and adult population [7, 8]. Theories emerged that these cells initiate a paracrine effect that inhibits apoptosis and fibrosis, enhances contractility, and activates tissue regeneration. Numerous efforts have been made to maximise these effects through cardiac cell maturation, selecting suitable scaffolds, and identifying biochemical cues that most mimic adult cardiac tissue. This technology is far from advanced, and improvements are required to recreate identical heart tissue in vitro before starting human trials. Currently, animal trials have been conducted using engineered cardiac tissue that may eventually be implanted in a human heart. This review touches upon the application of cardiac tissue engineering and available 3D heart models, outlining findings in current small or large animal models of heart diseases.

The Triad of Tissue Engineering

Tissue engineering is an interdisciplinary approach to replicating native tissue to replace damaged tissues or organs. Tissue engineering combines the three main components in a tissue: cells, scaffold, and biochemical signals (Fig. 1). Each element may be designed individually or in combination, owing to the supportive relationship of either two parts. Numerous advances have been made to overcome challenges and limitations, including cell sources, biomaterial compatibility, and the biomechanical properties of each component. To qualify as a treatment in human subjects, engineered tissue should be capable of large-scale production, widely available, and cost-effective so that it is reachable to all layers of society. Pluripotent or multipotent cells offer innumerable options for in vitro applications due to their expandable source, differentiation into multiple types of cell lines, and similarity to native human cells as opposed to animal sources. Stem cells derived from a patient's tissue also carry epigenetic factors, which are valuable for in vitro disease modelling in genetic diseases. The industrialised use of engineered cardiac tissue is also underway, presenting this technology for drug screening and family-specific disease modelling purposes.

Cellular Sources

Multiple cell sources are available for cellular therapy, but the major challenge resides in finding an inexhaustible cell source with high pluripotency capacity while maintaining minimal immune response. The successful isolation of embryonic stem cells (ESCs), developed by Thomson in 1998, is significant progress as pluripotent embryonic stem cells can generate into three germinal layers [9]. ESCs are non-differentiated, posing a high potential to differentiate into various cell types, even with the risk of developing teratomas. However, ESC extraction from the inner cell mass of human embryos carries an ethical dilemma. Mesenchymal stem cells (MSCs), derived from bone marrow, umbilical cord, or adipose tissue, are a widely available, plastic-adherent cell population with the ability to differentiate into multiple cell lineages of mesodermal origin with a lower risk for teratoma. MSC extraction can be done in adult tissues, promoting an excellent alternative for extraction. MSCs also exert a strong potential for immunomodulation by inhibiting lymphocyte proliferation, mainly major histocompatibility complex (MHC) class II, leading to better integration in

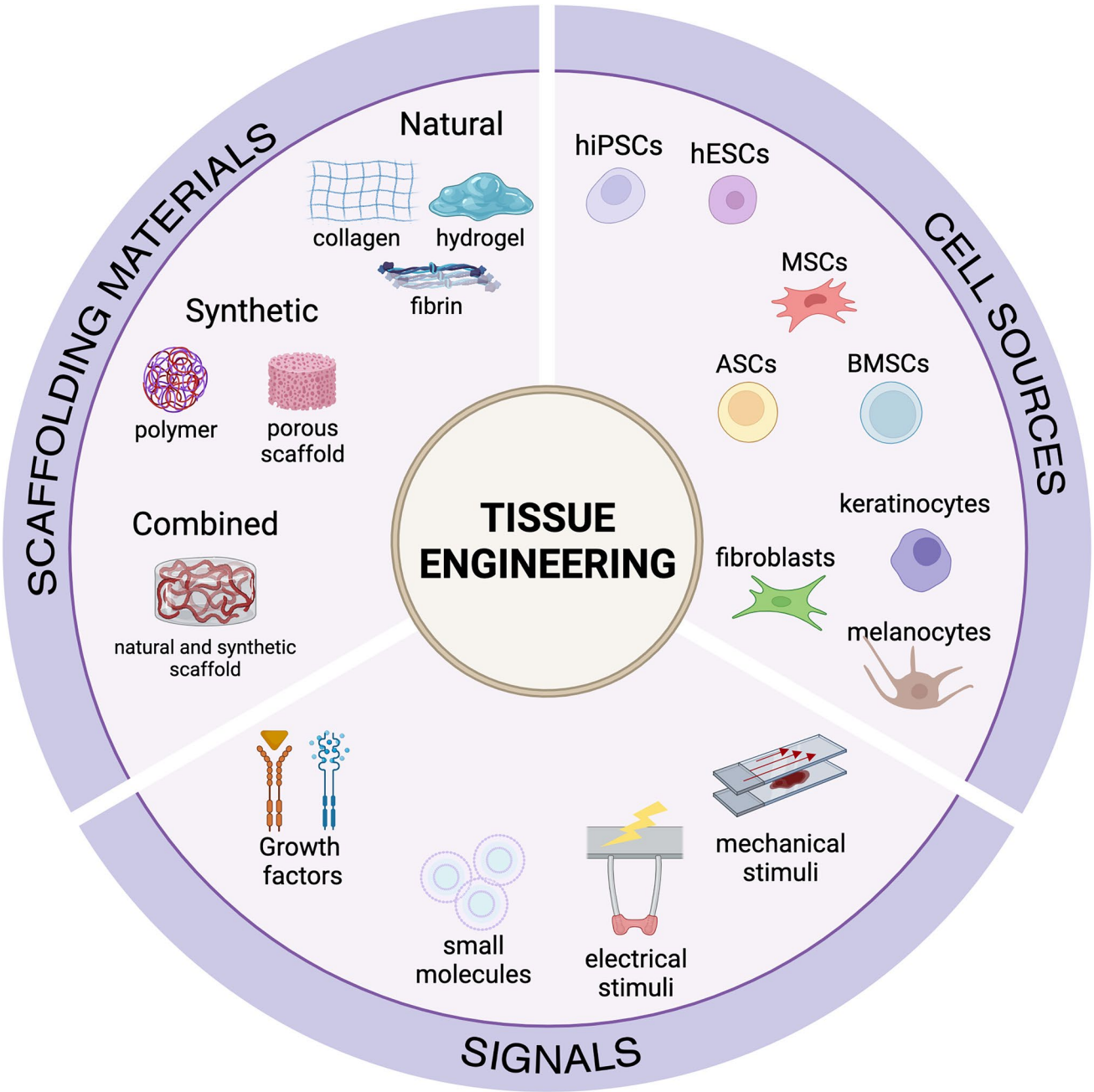

Fig. 1 Schematic representation of the components in tissue engineering. Cells on a biomaterial scaffold are supplied with biochemical stimuli to promote interaction between tissue components and mimic the physiology of the intended tissue. Alteration of each element (cells, materials, and signals) can be carried out independently or in combination to accomplish therapeutic goals. Abbreviations: hiPSCs, human induced pluripotent stem cells; hESCs, human embryonic stem cells; MSCs, mesenchymal stem cells; BMSCs, bone marrow stem cells; ASCs, adipose-derived stem cells. Created with BioRender.com

native tissues [10]. These characteristics are attractive as these cells offer the option of stem cell banking for tissue engineering purposes.

A promising advance to improve these drawbacks has been made with the discovery of human induced pluripotent stem cells (hiPSCs), in which adult somatic stem cells are reprogrammed to their pluripotent state using four reprogramming factors (Oct3/4, Sox2, c-Myc, Klf4) [11]. This paved a new road to developing a patient-specific therapy sourced from the patients' cells (autologous cells) to get rid of immunologic rejection. Alternatively, allogeneic cells from other humans have similar potential but are also immunogenic and still require immunosuppressive therapy. To this day, hiPSCs offer the safest advantage over other

cell sources for patient-specific treatment. hiPSCs also offer limitless cell sources for drug screening and disease modelling applications. The use of hiPSCs in heart diseases was led by the successful differentiation into hiPSC-derived cardiomyocytes (hiPSC-CMs) from various cell lines [12]. Differentiation protocols have successfully achieved 90% cardiomyocyte purity in a previous publication [13]. Successful differentiation to specific cardiac subtypes was reported using retinoic acid for atrial CMs and IWR-1 for ventricular CMs [14, 15]. Cardiomyocyte differentiation was also explored using bone marrow mesenchymal stem cells from pigtail macaque (*Macaca nemestrina*) that exhibited connexin 43 [16]. Despite that, hiPSC-CMs pose some significant challenges: immature characteristics compared to adult human cardiomyocytes and, for 2D cultures, the inability to reenact interactions in the three-dimensional heart. Immature cardiomyocytes hinder drug screening and disease modelling usage. Various attempts have been made towards ameliorating hiPSC-CMs maturation in vitro to better reenact the cardiac anatomy and physiology of the native heart.

hiPSC-CMs Maturation Affect Cardiac Physiology in Engineered Tissue

Distinct morphological features of the adult cardiomyocyte (rod-shaped, multinucleated, with organised sarcomere and well-developed sarcoplasmic reticulum and T-tubules system) are undetected in 2D cultures of hiPSC-CMs. 2D-cultured hiPSC-CMs showed typical foetal-like phenotypes such as reduced cell size, immature myofibrillar alignment, underdeveloped sarcoplasmic reticulum (SR), and absence of T-tubules [17]. This morphological immaturity also showed impaired calcium handling, further supported by differing numbers of SERCA2, RyR1, L-type channels, and other vital proteins. Comparing gene expressions of mature cardiac proteins such as ssTnI (foetal isoform) vs cTnI (adult isoform), adult cardiac myosin heavy chain genes (MYH6) vs foetal (MYH7), and titin isoforms (N2B, N2BA) in hiPSC-CMs can be used to assess cardiomyocyte maturity [18]. Comparison of gene expression in a time-based manner showed troponin isoform ssTnI (TNNI1, foetal isoform) is higher in hiPSC-CMs than that of cTnI (TNNI3, adult isoform) [19]. The incomplete development of these proteins hinders a fully functioning calcium pump and structures essential for cardiomyocyte contraction. Immaturity impairs the usage of hiPSC-CMs in drug screening, disease modelling, and tissue engineering purposes. Further assessment using human foetal and neonatal cardiomyocytes is needed to create a standardised maturation parameter.

Mechanisms to promote maturation include prolonging the culture period and altering the biophysical environment. Kamakura et al. have demonstrated that increasing maturation is possible by extending culture time; however, this method is labour-extensive and financially unfit for industrial uses [20]. Other ways of optimising maturation include altering biochemical signalling, enhancing cell-to-cell interaction, and stimulating electrical and mechanical systems. Giacomelli et al. reported improved maturation in hiPSC-CMs co-cultured with cardiac fibroblasts, cardiac endothelial cells, or both in a scaffold-free microtissue. These improvements were accompanied by electrical and mechanical maturation [21]. Mills et al. tried to induce cell cycle exit by inducing a metabolism switch from glycolysis to fatty acid. As it turns out, the resulting hiPSC-CMs showed expression of metabolism maturation and increased expression of titin, troponin I, and myosin heavy chain to the respective adult isoform [22]. Mirroring electrical field stimulation during in vivo hiPSC-CMs differentiation is brought to light using Biowire by seeding hiPSCs along an electrified, sterile surgical suture coated by type I collagen gels [23]. External electrical stimulation had successfully induced maturation towards a conduction system phenotype until recently. These cells expressed more of the gap junction protein connexin 40 and exhibited faster action potential (AP) depolarisation alongside other conduction-like properties [24]. Branco et al. reported that the formation of 3D aggregates before cardiac differentiation had reported better results when compared to a 2D culture system due to priming of the hiPSCs into a mesendodermal lineage differentiation. This method generated over 90% $cTnT^{+}$ cells and showed higher structural and functional maturation levels than a parallel 2D culture [25]. This result indicated that cellular interactions are better recapitulated using in vitro 3D models as their spatial configuration and biophysical stimuli are more similar to the native heart.

Mimicking Extracellular Matrix Characteristics In Vitro

Extracellular matrix (ECM) is widely known for its function in cell-to-cell communication, aiding proliferation, differentiation, and cell regeneration and supporting the mechanical functionality of a tissue. Cellular alignment that determines the mechanical and physiological properties of the heart is dictated by the ECM, which researchers are trying to remake in a cardiac in vitro model. Scaffold is an essential component to redefine these characteristics in vitro. Scaffold may help cell growth and retention, where dynamic interactions with adjacent tissues might speed up cell wash-out. Scaffolds can be made with natural or synthetic biomaterials, even both, to maximise advantages. Natural scaffolds such as fibrin, collagen, hydrogel, or Matrigel are biodegradable but with low structural integrity. Meanwhile, synthetic fibres such as polylactide (PLA), polyglycolic acid

(PGA), poly-lactic-co-glycolic acid (PLGA), polyethylene glycol (PEG), polyurethane, and polycaprolactone (PCL) have a more robust tensile capacity with less immunologic responses but take longer to degrade [26]. Mechanical strength and a better conduction capacity supported by the biodegradability and low toxicity of a natural scaffold make an ideal hybrid polymer. Crucial to cell survival, a vascular network needs to be created to provide cells with nutrients and eliminate waste, and this network requires space to fill in.

The production of a porous scaffold was made by a hybrid of gas foaming and freeze-drying using polyvinyl alcohol (PVA) [27]. The scaffold presented an irregular porous structure ranging from 10 to 370 nm in size with similar morphology in each sample, showing the potential of a high reproducibility scaffold. These samples integrate well with iPSCs, showing successful differentiation expressing cardiac-specific markers and observed cell beating. PVA shows excellent potential for use in cardiovascular medicine due to its biodegradability and flexibility to incorporate with other biomaterials. In hybrid form, the combination of PVA and decellularised human fibroblast has been previously reported [28]. PLGA also significantly affected maturation on an aligned nanofiber cardiac patch. The structural analysis reported longer sarcomere length and organised myofibril with clear Z-discs than a standard flat culture dish, with a subsequent faster calcium cycling rate that allows cells to contract at a higher frequency. Coordinated and synchronised beating on the entire hiPSC-CMs aligned nanofiber patch was reported at 2 weeks, while hiPSC-CMs on a flat culture only showed nonsynchronous beating. This study showed the possible effects of anisotropic mechanical properties of the cardiomyocytes, in which a more aligned structure improves mechanical function [29]. Combining scaffold with another bioengineering method is achieved by Noor et al. by creating 3D thick cardiac patches using a bioprinter. This method developed fully personalised hydrogel from the patient's fatty tissue that is later combined with autologous hiPSC-CMs to provide bioink for cardiac 3D bioprinting, in this case, parenchymal cardiac tissue and blood vessels [30]. They reported thick vascularised cardiac tissue that matched the original cells' anatomical, immunological, cellular, and biomechanical properties.

Growth Factor Usage in Engineered Tissue

Cellular interaction is mediated by signalling molecules in a paracrine manner. Growth factors (GF) are essential in paracrine signalling that it regulates various cellular processes, such as proliferation, regeneration, migration, or differentiation. As growth factors are released by cells, researchers have tried co-culturing hiPSC-CMs with endothelial cells, fibroblasts, or the growth factors themselves, such as IGF-1 and thyroid hormone, to assess the gravity of these interactions [31, 32, 33]. MSCs have been reported to secrete exosomes and extracellular vesicles that produce proangiogenic markers, such as vascular endothelial growth factor (VEGF) and placental growth factor (PGF). Nanotechnology has been developed to deliver growth factors in ECM, simultaneously providing cells with signalling molecules and a 3D network. The potential for growth factors is further supported by using an acellular matrix of porcine small intestinal submucosal ECM (SIS-ECM) implanted in the infarcted heart. The use of the acellular matrix directs a more adaptive and more functional response without using stem cells yet. They reported that FGF2 and TLR2 potentiate VEGF production from fibroblast, therefore inducing angiogenesis and reducing fibrosis [34].

The therapeutic potency of growth factors has been hindered due to its relatively unstable conditions: short half-life, rapid enzymatic degradation, and low protein stability. The challenge remains in developing efficient administration methods to locally achieve optimal concentrations, sustain its therapeutic effects, and control its release without repeated administration. Multiple efforts have been underway to overcome these limitations, physical encapsulation, covalent immobilisation, and ECM-like adhesive proteins, all of which control the binding, delivery, and gradual release of multiple growth factors. The drug delivery system using nanoparticles was recently adapted to control GF release from scaffolds using natural and synthetic materials, including protein-, polysaccharides-, and lipid-based NPs, and polymer nanocapsules such as PLGA [35]. These micro-sized vehicles allow higher dose-loading, responsive envelope surface characteristics, with precise control of local GF release. The use of insulin growth factor-1 (IGF-1) using encapsulated microspheres has improved cardiomyocyte differentiation and maturation in a functioning tissue [33]. Current in vivo applications of growth factor-loaded biomaterials with stem cells have also shown cardioprotective effects in multiple MI models [36]. NPs provide numerous advantages that help enhance GF delivery and achieve targeted effects, in combination with advanced tissue engineering techniques to enhance tissue repair.

Methods of Cardiac Tissue Engineering

The ideal platform should include multiple cardiac cell lineage, including atrial and ventricular cardiomyocytes, to articulate myocardial structure and functionality. Incorporating atrial and ventricular cardiomyocytes is critical due to the difference in electrophysiology and ion current profiles, which translate to a different drug toxicity response. Cell maturation is also a factor to be determined; the foetal

phenotype of hiPSC-CMs may not best characterise the response of a working heart. Prolonged cell culture with additional external electrical stimulation is proven to employ the phenotype of an adult human myocardium, making a better model for cardiac toxicity study or drug screening and disease modelling [20]. This emphasises the need for an in vitro cardiac model with high precision to native heart tissue, combined with multiple tissue engineering technology. Engineered tissue methods and uses are summarised in Fig. 2.

The most straightforward combination of cells and scaffold, first developed by Zimmerman et al. using neonatal rat cardiomyocytes on a synthetic extracellular matrix (Matrigel®), resulted in synchronously beating engineered heart tissues (EHTs) [37]. Protocols for force-generating EHTs using hiPSC-CMs have been previously published, including cardiomyocytes in casting moulds with a defined preload [38]. Previous EHT models showed well-developed cardiomyocyte organisation that recapitulated the appropriate inotropic responses similar to native cardiac tissue [39]. Additional to force, calcium transient analysis reported a positive force-frequency relationship within the physiological human beat rate in hiPSC-CMs EHTs [40]. This evidence acknowledges its use for drug screening and disease modelling purposes. In another study, the EHT platform for positive inotropes was reported to have low accuracy but reducing spontaneous beat rate increases sensitivity [41]. In addition, EHTs are not suitable for analysing action potential, making them only suitable for steady-state conditions. Despite that, EHTs provide the chance to minimise

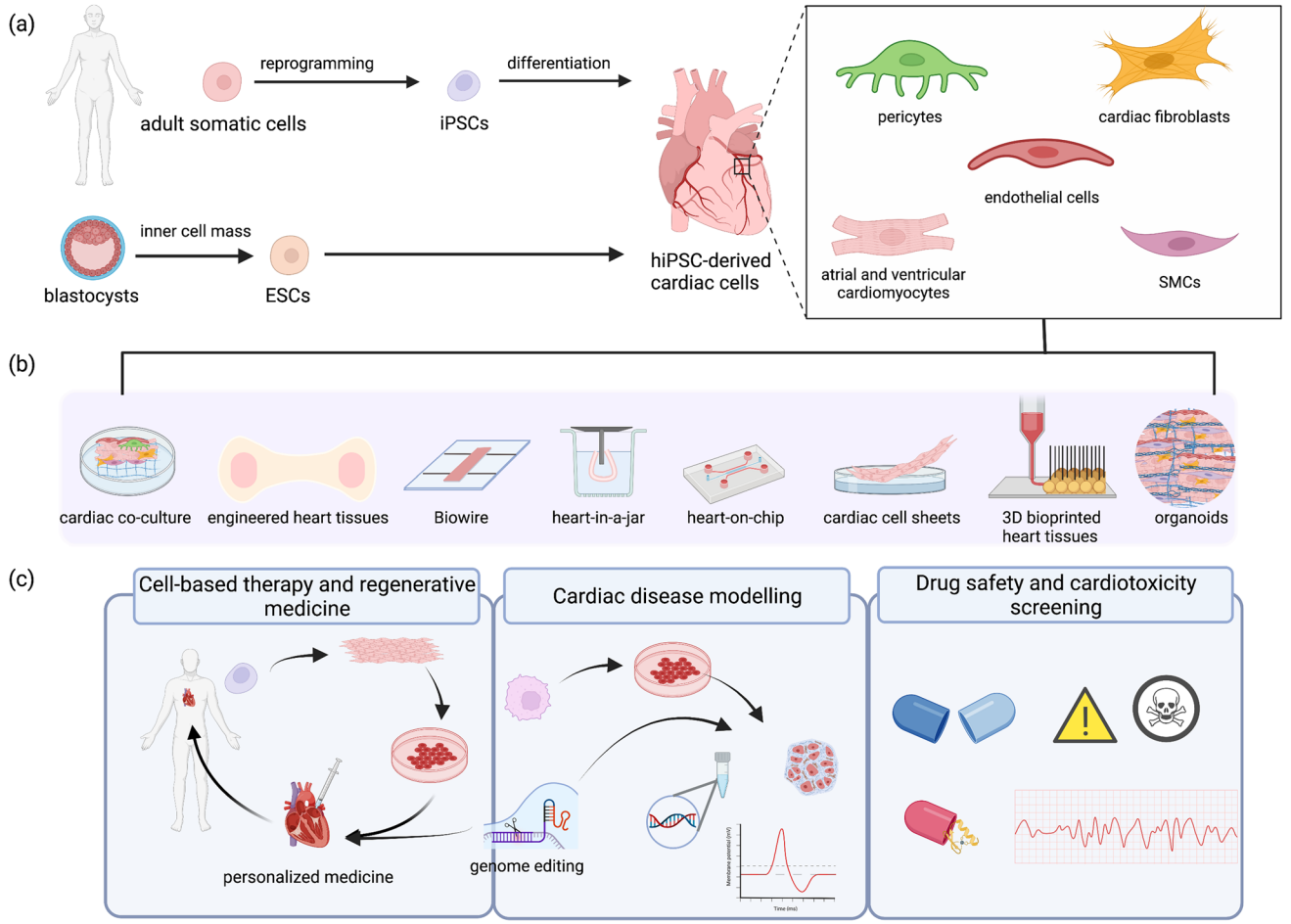

Fig. 2 Current application and future prospects of hiPSC-derived cardiac cells. **a** hiPSCs and hESCs can generate multiple cardiac lineage cells, namely cardiomyocytes, endothelial cells, fibroblast, smooth muscle cells, and pericytes. **b** Tissue engineering strategies facilitate the expanded use of these cells from conventional 2D cultures to a more complex 3D platform, incorporating biochemical and mechanical cues to generate physiological 3D platforms. These models include cardiac co-culture systems, engineered heart tissues (EHTs), Biowire, heart-in-a-jar, heart-on-chip, cardiac cell sheets, 3D bioprinted heart tissues, and organoids. **c** These technologies uncover the potential of developing personalised biomedicine, discovering complex disease processes, and defining the safety and cardiotoxicity profiles of novel pharmacological agents. Abbreviations: iPSCs, induced pluripotent stem cells; ESCs, embryonic stem cells; SMCs, smooth muscle cells. Created with BioRender.com

the economic loss in drug development which makes this platform famous for industrial uses.

Simultaneous contractility analysis can be performed using the heart-on-chip technology, in which multiple muscular thin films (MTFs) layered with polydimethylsiloxane (PDMS) are constructed using fibronectin layers. This construct allows 3D deformation of the two-dimensional tissue, in which MTFs curl up in systole and flatten during diastole, subsequently allowing measurement of contractile strength and AP morphology [42]. This technology was fabricated using microfluidic chips, cells/microtissues, microactuators, and microsensors. Microfluidic channels resemble the in vivo interaction of the organ to blood vessels and modulate cellular interaction, microactuators expose the cells to electrical and mechanical stimulation to resemble the heart's natural physiology, and microsensors detect cell status that aids in the functional analysis [43]. Heart-on-chip technology has been used for drug screening platforms for isoproterenol and in a higher-throughput model of 4 MTFs of 12 assessed drugs [41, 42]. Heart-on-chip has also been used to assess molecular interaction in an ischemic-reperfusion injury model and analyse pathophysiology in Barth syndrome [43, 44].

As 3D cultures prove their superiority over 2D cultures in recapitulating complex cardiac environments, a scaffold-free culture (termed organoid) has also been considered for an in vitro cardiac model. This method comprises pre-differentiated cardiomyocytes in a 3D aggregate instead of embryoid bodies, which start as non-differentiated hiPSC-CMs. Currently published models have recently reported the optimal cell ratio of using four predominant cardiac lineages (cardiomyocytes, epicardial cells, cardiac fibroblasts, and endothelial cells) and method of suspension gravity, which results in an organoid expressing cardiac markers cTnT, CD31, WWT1, Cx-43, and α-SMA [45]. Further efforts to manipulate engineered tissue architecture made tremendous progress with the introduction of 3D bioprinting and bioreactor. Bioreactors are utilised to replicate tissue conditions in vivo by mechanical stimulation, which has been reported to improve cell proliferation, growth, and functionality. The culture process in a bioreactor was carried out using hiPSCs which were then layered to create a cardiac cell sheet, combining the two components of tissue engineering. Almost 80% of hiPSCs were successfully differentiated into cardiomyocytes, expressing cTnT with a foetal phenotype marked by the expression of Nkx2.5 and SM22. Cell proliferation was sufficient, from 2×10^7 cells to 4×10^7 cells on day 3, increased to 8×10^7 cells on day 14, and the EB showed spontaneous beating. In a monolayer-based method, the flow cytometric analysis also showed cTnT expression in 80% hiPSCs, and these cells express sarcomeric alpha-actinin with a fine-striated pattern [46]. When the cell sheets were layered on top of each other, the confocal analysis showed 80–90% cTnT-positive cells and sarcomeric α-actinin. Findings using both methods proved that either approach is suitable for differentiating hiPSCs.

Bioprinting 3D cardiac models allow the generation of complex microtissue architecture using the desired biomaterial, in which cell alignment is required for a uniform contraction. Cardiac tissue constructs using this method have been reported for various types of bioink, such as fatty cell-derived ECM, which produces thick, vascularised cardiac tissue [30]. The fabrication of scaffold-free bioprinted cardiac sheets is also possible using the co-culture of hiPSC-CMs, cardiac fibroblast, and endothelial cells. Cardiospheres were first cultured in U-wells, which served as the blueprint for cardiac sheet creation using a needle array method. After 1 week of printing, these cells showed 93.3% viability and were found positive for troponin T, vimentin, CD31, and Cx43. A scaffold-free method minimises biomaterial-related issues such as biodegradability, immunogenicity, fibrous tissue formation, and toxicity of byproducts [47].

Another issue arises as economic limitations require high-throughput, high-quality data that simple 2D models cannot produce. With this in mind, novel technology is developed to simultaneously assess tissue functionality in human-like in vitro heart tissue. Biowire II is introduced by Zhao et al. as a non-invasive, multi-parametric readout of physiological responses [48]. This platform enables the growth of cylindrical tissues suspended between two wires, allowing simultaneous quantification of force and Ca^{2+} transients. Characterisation of these cells confirms that atrial cells beat at a different rate than ventricular cardiomyocytes, similar to human myocardium. Ca^{2+} transients also rose more quickly with a shorter AP on the atrial end. Adding inotropic drugs to the cells produces the same result as the original drug effects in vivo [23]. Biowire II acts as a liable method for generating atrial and ventricular tissue and characterising chamber-specific drug responses simultaneously in a chronically stimulated tissue. The increasing need to characterise the heart's physiology as a pump further pushes innovation to allow better characterisation of in vivo cardiac models. The fundamental analysis of Ca^{2+} transients and expression of Ca^{2+} regulating genes cannot fully represent the tissue's ability to contract and eject fluid, and cell beating only analyses twitch force. This problem precipitates the heart-in-a-jar technology, or human ventricle-like cardiac organoid chamber (hvCOC), a miniature heart chamber that allows volumetric-based assessment to determine the heart's pumping ability such as ejection fraction, stroke force, and pressure–volume assessment and eventually to assess drug responses. Li et al. developed this method using human pluripotent stem cell cardiomyocytes embedded in a hydrogel matrix constructed in a custom bioreactor to create a tissue mould with subsequent mechanical and electrical stimulation [49]. The resulting model comprises a 3D

cardiac tissue with a hollow chamber geometry that can generate pressure during each beat. This model exhibited a key feature of the heart, the Frank-Starling mechanism, showing a linear relationship between developed pressure and the previous pressure load. Clinical cardiac function parameters such as ejection fraction, cardiac output, stroke volume, end-diastolic and end-systolic pressure, and pressure–volume loops can now be measured using a single in vitro human heart model. In combination with structural and electrophysiological markers, this model offers a comprehensive assessment of the 3D cardiac in vitro model as an organ.

Drug Screening and Disease Modelling Uses

The possibility of an in vitro drug screening model was assessed soon after the successful induction of hiPSC-CMs to prevent economic loss due to pharmacological discoveries. The commercial drug industry is expensive to research. About 18% of marketed drugs had to be withdrawn due to safety and toxicology concerns, and about 1 in 7 publicly marketed drugs was withdrawn from 1980 to 2009 despite having passed phase III clinical trials [50]. The use of animal trials had been a routine in the past, but this method is just as costly, requiring 11.3 million USD in 2015 alone [51]. Safety and toxicology results cannot be entirely accurate and applicable in humans regarding major molecular and physiological differences, which deems animal trials unnecessary. Drug toxicity is especially sensitive for cardiac ion channel modifiers, as an alteration in electrophysiology cannot be concluded using an in vitro single ion assay. This problem concerns mainly drugs that delay the myocyte repolarisation process that, if left undetected, may lead to Torsades de Pointes (TdP). This concern prompted the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, which attempted to establish a standardised and reproducible approach to assess the impact of cardiac drugs on the electrophysiology of hiPSC-CMs and to detect key cardiac channels that may have an impact on the heart by candidate drugs [52]. Twenty-eight drugs screened using hiPSC-CMs across diverse laboratories have been reported to have widely consistent results with 87% accuracy in predicting a low risk, intermediate, and high risk of arrhythmia [53]. Efforts to unify operating procedures for drug screening were also conducted by Saleem et al., which focused on using various 2D and 3D platforms to quantify contractility and other electrophysiological measurements. Combining different cells and platforms reported an initial 44–85% accuracy in predicting the correct inotropy [41]. For the 36 drugs that were screened, initial results showed a low predictivity score for positive inotropes, which was then altered by reducing baseline spontaneous beat rate. Accuracy was increased up to 85–93%. This analysis enabled the use of hiPSC-CMs for a drug safety evaluation in the future and convinced that simple platform refinements can successfully increase sensitivity in a drug screening model [41]. Mannhardt et al. provided a systematic comparison of using different cell lines from 10 commercial and academic hiPSC-CMs for drug screening. hiPSC-CM was studied in EHT format given BayK-8644, nifedipine, EMD-57033, isoprenaline, digoxin, thapsigargin, and ryanodine as indicators of inotropic response. There were differences in baseline contractility and duration of AP between 10 different cell lines. The variation in the kinetic response of these drugs ranges from 80 to 93% [54]. Analysing confounders such as cardiomyocyte purity, differentiation protocol, and prolonged culture time did not explain the large variability. Nonetheless, the canonical drug response was seen in most of the EHT cells used. Evidence supports the importance of using different cell lines and determining the difference relative to the baseline hiPSC-CMs in each drug screening procedure, while some studies have even suggested determining control rates. Isogenic control should also be obtained to detect the phenotypic differences that will emerge. Although the developed model needs further improvement, the use of hiPSC-CMs in drug screening has paved the way for detecting the overall effect of drugs before starting human clinical trials.

hiPSC-CMs have been used to model several inherited cardiac diseases under the classification of channelopathies and cardiomyopathy. Disease models of cardiac diseases had been reported for long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT), Friedreich's ataxia and Barth syndrome, and syndromic diagnosis associated with cardiomyopathy (LEOPARD syndrome, Pompe disease, laminopathies) [52–55]. These diagnoses comprise largely of genetic mutations; however, direct causality between genetic and environmental factors that affect disease phenotypes is still largely unknown, and until recently, there has been no reliable, human-sourced model to reenact disease progression outside the human body. hiPSCs allow the collection of diseased cell types to be investigated as cardiomyocytes develop in vitro, enabling the investigation of molecular and cellular mechanisms that contribute to pathological changes in an individual context.

Current studies focus on identifying new genetic mutations and reproducing their phenotypes in vitro using cell lines from diagnosed patients and their families. Disease models of hypertrophic cardiomyopathy (HCM) showed hypertrophy of cardiomyocytes, irregular sarcomere, and interstitial fibrosis in a hiPSC-CMs model. In the study by Lan et al., genetic analysis was carried out on ten patients in 2nd- and 3rd-generation families where one family member had been diagnosed with HCM. A missense mutation confirmed genetic aetiology in the myosin heavy chain (MYH7 gene) in 5 family members, but only one family member

showed clinical manifestation [56]. Arrhythmias and irregular calcium handling were also found in the cellular level analysis. Genetic mutations without clinical phenotypes are an exciting area of research, and mechanisms regarding environmental influence on the genetic background are yet to be discovered. To confirm this hypothesis, Tanaka et al. analysed the influence of multiple hypertrophy-promoting factors in the hiPSC-CMs disease model from 3 known HCM patients, in which two of them were negative for known sarcomeric mutations. hiPSC-CMs HCM model treated with endothelin-1 (ET-1) showed disorganised cell hypertrophy and myofibrils compared to negative controls. They concluded that ET-1 triggered the phenotype of HCM-related gene mutations, supported by findings of increased cell surface area, poor sarcomere arrangement, and impaired action potentials [57]. hiPSC-CMs further expand their role to trace the cause of impaired contractility through the detection of novel sarcomeric mutation in cases of adult-onset cardiomyopathy. Two siblings were diagnosed with overt heart failure with reduced ejection fraction but showed no evidence of left ventricular hypertrophy (LVH) or chamber dilation; therefore, cardiomyopathy was never detected. A heterozygous missense mutation at E848G on MYH7 was found in both patients. hiPSC-CMs derived from these patients showed impaired fractional shortening on a cellular level, and on EHT, contractility analysis reported four times lower capacity than controls [58]. Impaired contractility was not present in early cultures of hiPSC-CMs, but after further maturation efforts (prolonged culture and tissue engineering), a contractility deficit was evident. Sun et al. have demonstrated the critical role of calcium regulation in dilated cardiomyopathy. In this study, cardiomyocytes from iPSCs of patients with R173W mutation in the troponin T showed impaired calcium, decreased contractility, and abnormal distribution of alpha-actinin sarcomeres compared to controls from other family members. When the hiPSC-DCM model was given the beta-blocker metoprolol for 1 week, the number of hiPSC-CMs single cells with irregular sarcomere was reduced. Although not yet statistically significant, this model shows a reduced chronotropic effect and a transient increase in calcium in hiPSC-CMs DCM. When SERCA was delivered with an adenoviral vector, the gene expression in the hiPSC-CMs DCM model changed to resemble that of the control hiPSC-CMs. Overexpression of SERCA is thought to improve calcium regulation, thereby improving functional capacity in cardiomyopathic patients [59].

A systematic review by Eschenhagen et al. reported consistent abnormalities in both HCM and DCM, changes in sarcomere arrangement, increased expression of NPPA or NPPB genes, and proarrhythmic activity. HCM showed increased cell size, MYH7 gene expression, and accumulation of the transcription factor NFAT. Mutations in the MYH7 and MYPBC3 genes were the most common mutations, followed by the troponin T (TNNT2) and troponin I (TNNI3) genes, and mutations in the actin and myosin light chain genes [60]. A consistent finding in DCM is a decrease in the force-generating capacity of contraction. These findings are premature but still potentially meaningful for analysing the deleterious disease process and finding new therapeutic targets.

Cardiac Patch Applications in Animal Models

Stem cell applications in regenerative medicine have begun applications in human trials using stem cells delivered through intracoronary infusion or intracoronary injection. MSC injection in ischemic and non-ischemic heart diseases showed safety and successfully improved outcomes [61]. Another ongoing phase II clinical trial also offers a combination of MSC + CPC (CONCERT-HF) [62]. In paediatric heart failure, the use of MSCs, bone marrow mononuclear cells (BM-MNCs), and umbilical cord blood stem cells (UCBs) has been reported with inconclusive results due to the small sample size in each study [63]. The problem encountered in both methods is rapid cell wash-out due to a lack of mechanical support, which may lead to an unsustainable therapeutic effect. Cardiac patch technology is expected to counter this problem as it suspends the cells while also meeting cell metabolic demands, much like the ECM. Cardiac patch implantations in vivo have started a few animal trials using many methods, as it is only a matter of time to discover what method suits best for clinical use [63]. Figure 3 illustrates the use of the cardiac patch in present studies of animal models.

Cardiac patch uses a combination of scaffold and cells as one of the efforts to achieve better cell retention in vivo by mimicking the native ECM. The method of removing live cells from a human-derived tissue, called decellularisation, is on the rise as it carries the exact ECM component, mechanical characteristics, and biomolecular signals as their in vivo counterparts. This method gives the best possible ECM substitute to compensate for the decreased functionality in a diseased heart. The integrated native biochemical signals also enhance cell proliferation and infiltration to the targeted tissue. This led to the use of decellularised rat placenta as a scaffold for a cardiac patch with hiPSC-CMs in a rat infarct model [64]. After 7 days of hiPSC-CMs seeding, the cells express cTnT, thus successfully creating a bioengineered cardiac patch. The patch spontaneously contracted at 40–100 bpm, with an active response to external stimulation. Several growth factors (VEGF, angiogenin, angiopoietin-2, and hepatocyte growth factor) significantly increased after hiPSC-CMs seeding onto the placenta; it was concluded that hiPSC-CMs seeding could enhance the paracrine effect through several angiogenic growth factors. Cardiomyocyte

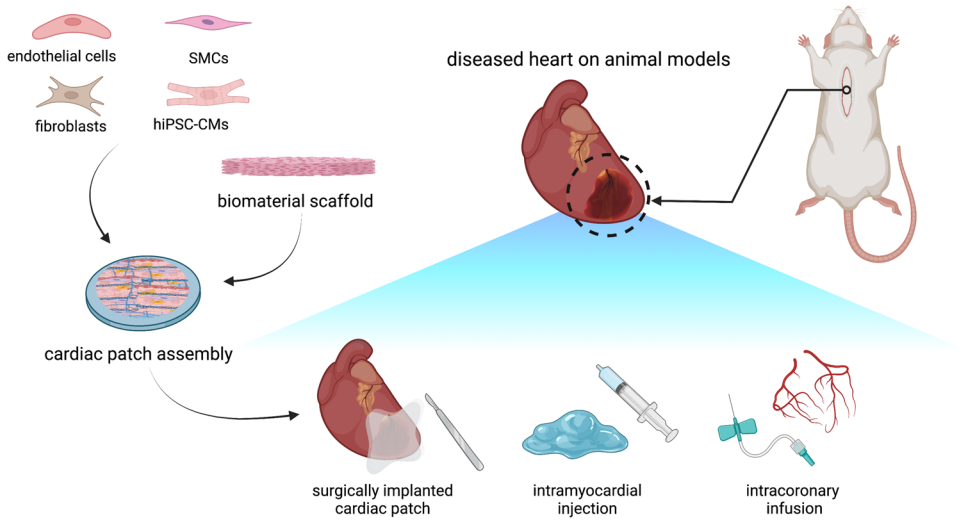

Fig. 3 Cardiac patch implantation in animal models. Diseased animal heart models undergo stem cell therapy using intramyocardial injection, intracoronary infusion, or surgical implantation of a cardiac patch [63]. The cardiac patch is assembled using cells and biomaterial scaffold to provide mechanical support and improve cell retention in vivo. Abbreviations: hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; SMCs, smooth muscle cells. Created with BioRender.com

structural, conduction-related, and cellular metabolism-related genes are upregulated in the bioengineered patch compared to the hiPSC-CMs monolayer. In the functional analysis, patch implantation improved left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) while also reducing left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD). The use of a cardiac patch also improved engraftment at the peri-infarct area, alongside increased vessel density compared to placenta or hiPSC-CMs alone. This method was also performed by Wang et al., who combined hiPSC-CMs and hiPSC-derived $CD90^+$ cells onto decellularised rat placenta [65]. The use of $CD90^+$ fibroblast from the same individual is expected to facilitate tissue engraftment and minimise immunogenicity. This cardiac patch enhanced in vitro maturation, as confirmed by expressions of cardiac and contractile genes, with increased ratio of MYH7/MYH6 and MYL2/MYL7. Implantation in male Sprague–Dawley rats with a myocardial infarction showed functional improvement (LVEF and LVFS) after 4 weeks, with neovascularisation in the peri-infarct area. Moreover, staining with human-specific nuclear antigen at 4 weeks showed that implanted cardiac cells were still viable at the transplant site. Other reported studies of cardiac patch in vivo analysis are concluded in Table 1.

The flexibility of decellularisation allows multiple ECM sources to be created into scaffolds. The spleen houses haematopoietic stem cells, which serve as the basis for developing spleen ECM-derived thermoresponsive hydrogel (SpGel) from the porcine spleen [66]. Thermoresponsive hydrogel remains liquid at room temperatures; therefore, it can be injected in a liquid form and solidify at 37 °C to form a patch. SpGel was combined with hiPSC-CMs and hiPSC-ECs (endothelial cells) and then injected intramyocardially in male mice models of MI. In line with other publications, the SpGel + CMs + ECs group had the greatest increase of LVEF ($54.53 \pm 2.09\%$) at 4 weeks compared to the other groups, with decreased LV systolic and diastolic diameter. Cardiac fibrosis was also significantly reduced compared to other treatment groups. Pre-implantation in vitro analysis proved the cytoprotective mechanism of cells encapsulated in SpGel, which translated to improved cell survival and integration in vivo.

Fan et al. provided data on hiPSC-CMs fibrin-based patch with added nanoparticles CHIR99021 and FGF1 to maximise their regenerative potential in a rat MI model. Aside from improved echocardiography parameters (LVEF, LVFS, LVEDD, LVESD), the number of engrafted hiPSC-CMs was higher, and graft size was 3–4 times greater in the patch group than in controls. The increase of engrafted hiPSC-CMs from day 3 to day 28 in the patch group suggests that cell proliferation might have contributed to this finding, even though structural analysis exhibited immature hiPSC-CMs on the graft [67]. Lancaster et al. have developed an absorbable material (polyglactin 910) co-cultured with human neonatal fibroblast and hiPSC-CMs, which is later used to treat Sprague–Dawley rats with induced congestive heart failure (CHF) [68]. Fibroblast was used mainly due to its proangiogenic effect that increased blood flow to help with patch survival and signal transport. The contraction was observed on the in vitro patch, and in vivo electrophysiological mapping showed synchronous contraction between the patch and the underlying myocardium in sinus rhythm. Patch-treated rats reported decreased left ventricular end-diastolic pressure (LVEDP) and left ventricular diastolic time constant (Tau), with improved diastolic function. hiPSC-CMs were not detected 21 days after implantation, but positive functional changes remained. This points to the possibility that the functional improvement seen in animal models using hiPSCs might be derived from the paracrine and angiogenic effect of the newly introduced cells, not by the integration of the cell itself.

Table 1 Published reports of small and large animal models using a hiPSC-CMs-based cardiac patch. [a]Human induced pluripotent stem cell-derived cardiomyocytes, [b]myocardial infarction, [c]left ventricular ejection fraction, [d]fractional shortening, [e]human induced pluripotent stem cell-derived endothelial cells, [f]spleen ECM-derived thermoresponsive hydrogel, [g]congestive heart failure, [h]left ventricular end-diastolic pressure, [i]pericytes, [j]human engineered heart tissues, [k]fractional area change, [l]human induced pluripotent stem cell-derived smooth muscle cells, [m]mesenchymal stem cells, [n]gelatin methacrylate, [o]polyethylene glycol diacrylate

Animal model	Cell types	Cell volume	Biomaterial	Diagnosis	Follow-up period	Outcomes	Reference
Rats	hiPSC-CMs[a]	1×10^6 cells/cm^2	Decellularised rat placenta	MI[b]	4 weeks	↑ LVEF[c], FS[d], decreased infract size	[64]
SD rats	hiPSC-CMs, hiPSC-CD90$^+$ cells	3:1 ratio, 10^4 cells/mm^2	Decellularised heart rat matrix	MI	4 weeks	↑ LVEF, FS	[65]
Mice	hiPSC-CMs, hiPSC-ECs[e]	2×10^5 CM, 1×10^5 ECs	SpGel[f]	MI	4 weeks	↑ LVEF, reduced fibrosis	[66]
Mouse	hiPSC-CMs	1×10^6 cells per patch	Fibrin	MI	4 weeks	↑ LVEF, FS, higher engraftment of hiPSC-CMs than control	[67]
SD rats	hiPSC-CMs, human neonatal fibroblast	Not disclosed	Polyglactin 910	CHF[g]	3 weeks	No significant change of LVEF, decreased LVEDP[h], improved diastolic function	[68]
SD rats	hiPSC-CMs, human pericytes	2.2×10^6, hiPSC-CMs, 3.4×10^6 PCs[i]	Fibrin gel	MI	4 weeks	↑ LVEF, FS lower than baseline, reduced infarct size	[69]
Guinea pigs	hiPSC-CMs, hiPSC-ECs	5×10^6 CMs, 2×10^6 ECs	EHTs[j]	Cryoinjury	4 weeks	Decreased FAC[k]	[70]
Female swine	hiPSC-CMs, hiPSC-ECs, hiPSC-SMCs[l]	1:1:1, 6×10^6 cells total	Fibrin patch	MI	4 weeks	↑ LVEF, FS, reduced infarct size	[33]
Swine	hiPSC-CMs, hiPSC-ECs, hiPSC-SMCs	4×10^6 CMs, 2×10^6 ECs, 2×10^6 SMCs	Fibrin matrix, thrombin	MI	4 weeks	↑ LVEF, reduced infarct size	[71]
Murine	hiPSC-CMs, hiPSC-ECs, h-MSCs[m]	1×10^6 per ml	GelMA[n] and PEGDA[o] bioprinted in 4D	MI	4 months	No significant change in LVEF, reduction of infarct size, cell viability	[72]

Another application of cardiac patch using hiPSC-CMs and fibrin gel was analysed in vivo using a rat infarct model. Lab-grown hiPSC-CMs were co-cultured with pericytes as a vascular support cell on a fibrin gel [69]. The structural analysis showed organised sarcomeres in the CM + PC patch, generated sufficient forces to contract, and deposited ECM, mostly fibrin. The functional analysis showed LVEF and LVFS reduction in all groups at 1-week postoperative period. At 4 weeks, CM + patch group displayed higher LVEF and LVFS than the other groups, even though they remained lower than baseline values. The area of the engrafted patch had a pale, thin tissue layer covering the infarct area, and consisted of scar tissue and CM. Trichrome staining found the patch to be quite collagenous after implantation, even though the cTnT nuclei in the patch increased twofold (102.9%) after 4 weeks in vivo. They found active vascularisation originating from the host, especially the border zone in the myocardium, suggesting a paracrine-like mechanism in cell therapy. Pericytes of the PC-only patch did not survive at 4 weeks in vivo but were present in the CM + PC patch, strengthening its function in compaction and alignment of fibrin gel in the patch.

Although rat models showed a positive result, larger animal models are required to detect side effects, such as arrhythmia, that would otherwise be masked in a small animal model. A guinea pig model was chosen in a protocol by Weinberger et al. to implant hiPSC-CMs and hiPSC-ECs in an engineered heart tissue (EHT) after induced myocardial injury [70]. Compared to rats or mice, guinea pigs resemble much more of the electrophysiologic characteristics of the heart. Their heart rate ranges about 250 beats/min, with a ventricular plateau phase and distinct potassium current that are important to detect repolarisation. The implantation occurred 1 week after infarct induction, in which there were already large transmural scars on the heart. Echocardiography showed an improved fractional area change (FAC) by 31%, vascularisation of host origin, and synchronised electrical coupling in the guinea pigs' hearts 4 weeks after implant.

Ye et al. reported an in vivo study of hiPSC-CMs, hiPSC-ECs, and hiPSC-SMCs (smooth muscle cells) on a fibrin patch with IGF-1 microspheres in porcine models of MI. A total of 6 million cells were transplanted into the heart, and after 4 weeks of implantation, cell survival reached 8.97% ± 1.8% [33]. Vascular networks of host origin were detected in the border zone of the infarcted area, which may have contributed to improving cardiac function. Arrhythmias and ST-segment elevation were present during the occlusion and reperfusion, but no animals developed further arrhythmias in the 4-week follow-up period. Gao et al. reported similar results in porcine models of MI using a large patch (4 cm × 2 cm × 1.25 mm) made from hiPSC-CMs, hiPSC-ECs, and hiPSC-SMCs on a fibrin matrix [71]. Engraftment rate reached 11%, along with improved echocardiography parameters, reduced infarct size, and reduced apoptosis. In both studies, the reported engraftment rate was too low to have impacted functional assessment, and it was thought that the paracrine effect might pose a role in this finding. Arrhythmia and ST-segment elevation only occurred during left anterior descending (LAD) artery ligation and within 14 days after MI, and similarly, no animal in the following 14-day follow-up period developed ventricular arrhythmia. These reported studies emphasised the safety and feasibility of using larger animal models that resemble the human heart to reproduce physiological conditions in vivo and eventually replace damaged cardiac tissue.

Reported in vivo animal trials only had results on a short-term follow-up period; therefore, the long-term functional improvement or side effects have not yet been elucidated. A study using murine models of myocardial infarct attempted to implant a 4D hydrogel-based cardiac patch in a 4-month follow-up period using a combination of hiPSC-CMs, endothelial cells, and mesenchymal stem cells. Cardiac patch was designed in a computer-aided design (CAD) beam-scanning stereolithography to create bioengineered tissue that is adaptable to the physiology of the heart. Cardiac patch architecture was designed to correspond to the spiral left-to-right fibre alignment with varied fibre orientations ranging from + 60 to − 60°, and a mesh pattern in the native myocardium, which allows maximum force generation in systole and diastole according to the native heart. This produces a highly adaptable structure that eventually improves cardiac functionality in the diseased heart. Epicardial engraftment on top of the infarcted region showed GFP^+ hiPSC-CMs retention after 3 weeks, with regions of neovascularisation. Infarct sizes of the implant group showed a reduction after 10 weeks compared to controls. After a 4-month follow-up period, the engrafted patch consistently showed a smaller infarcted area, and hiPSC-CMs retained their viability, confirmed by GFP+ staining. They also exhibited mature cardiomyogenic cTnI and vWF expression, confirming vasculogenesis throughout the patch [72]. All patch groups showed increased LVEF, but no significant difference was found between cell-patch groups to cell-only groups. Functional integration was not observed in this study, which warrants future research to integrate structural and functional analysis in vivo. However, these results showed improved cell viability in long-term implantation, which had been a problem in the past. The patch also provided growth factor-rich islands to promote the paracrine effect in the heart. The application of a 4D adaptable engineered

cardiac patch is recommended in larger animal models as they recapitulate the human heart better.

Limitations of Regenerative Therapy in Cardiac In Vivo Models

While reports showed favourable results in small animal models, there are still a few limitations that need to be resolved. In vivo tissue engineering studies are frequently met with challenges such as inefficient delivery methods, poor cellular engraftment and survival, and low cellular differentiation, with the physiological disparity between animal models and human subjects [64]. The arrhythmogenic potential of implanted cardiomyocytes may be masked by the rapid heart rate of rats; therefore, larger animal models with similar heart rates would be better to explore this circumstance [64]. Arrhythmia is a serious side effect of implanted cardiomyocytes which may be caused by immature electrophysiologic activity of newly differentiated hiPSC-CMs, varying cardiomyocyte subtypes on the implanted patch, and an unstandardised number of implanted cells. It is still unclear how many days differentiated hiPSC-CMs are best for a cardiac patch; younger hiPSC-CMs have a higher proliferative activity which is essential to help survive culture and transplant conditions but at a higher risk of arrhythmia. Fan et al. reported that hiPSC-CMs on the infarct zone were indeed less mature [67]. Additionally, there is currently no clear guideline on the optimum number of delivered cells, which is important to consider, as a low cellular count is at risk of being washed away or undergoing apoptosis; meanwhile, higher number of cells potentially induces arrhythmia. Also, if transplanted cells are of allogeneic origin, do we need immunosuppressive therapy to minimise cell rejection, and if we do, how will it affect the overall impact of therapy? The therapeutic potency of cell therapy may be hindered by these factors; therefore, solving these problems is vital in the development of cellular therapy.

Multiple studies have proven the presence of proangiogenic and anti-fibrotic growth factors in in vitro and in vivo models of cardiac patches. However, in our reported studies, it is still unclear whether it was these factors alone that benefited the injured myocardium or in combination with implanted cells. Further studies should aim to investigate to which extent paracrine factors affect anatomic and functional healing of infarcted hearts and compare those with hiPSC-CMs or other stem cell lines. With respect to study methods, it is essential to compare how long until treatment is delivered since the onset of injury. All studies reported here delivered treatment at the same time point; therefore, it is unknown whether cardiac patches improve cardiac function in all or only up to a certain degree of injury after a certain time point. This analysis translates roughly to the door-to-needle time in a real-world setting. Reported models were mostly of acute conditions (myocardial infarction) that produce significant injury at the acute onset, but these results should be compared to cases of heart failures undergoing a slower, more permanent disease progression [68]. It is imperative to identify at which degree of fibrosis or at which time points these cardiac patches will still be beneficial. Results were also limited to a short study time, meaning that it is unknown whether functional improvement is permanent, whether repeated treatment is needed, or if in a certain amount of time, the heart will start to decline. Future studies should be able to consider current limitations to better translate their results for future human subjects.

Future Directions in Cardiac Tissue Engineering

While it is still mainly impossible to generate all cell lines in the native heart, it is essential to find key structures and properties to recreate the vital component in in vivo, so that cardiac models can still be representative and reproducible. Complex cardiac models are costly, challenging to reproduce, and possibly low throughput, which may cost researchers their consistency and quality [73]. Despite that, hiPSC-CMs still possess valuable knowledge for multiple uses. Existing knowledge can be developed to further personalised biomedicine, especially for diseases that require regenerative therapy. Personalised medicine might be the bridge to recovery rather than delaying transplantation in cardiac diseases that lead to heart failure and transplant. These may include specific growth factor inhibitors, cardiac patches, altering pathophysiology, and even altering organogenesis in a prenatal diagnosis of congenital heart disease. Current animal models have sufficiently provided data that guide researchers to ameliorate cardiac tissue characteristics in vivo suitable for regenerative therapy.

Growth factors offer the potential to guide tissue-specific actions to promote healing, such as cellular regeneration, angiogenesis, and attenuation of fibrosis. While its usage had been impeded by the rapid degradation, sustained release particles offer the key to regulating the spatiotemporal release of GF to sustain long-term effects in vivo [35]. This method allows simultaneous GF administration which is all essential in the complex tissue interaction of the healing process. It should be noted that optimal dose, chemical and enzymatic interactions, and its safety should first be explored before proceeding to clinical trials. Furthermore, appropriate biomaterials should be explored that elicit minimal toxicity with maximum advantages to deliver suitable growth factors.

hiPSC-CMs have recently been used to analyse drug mechanisms in long QT syndrome (LQTS). In the context of therapy, hiPSCs also offer genetic engineering approach using Cas9/clustered regularly interspaced short palindromic repeat (CRISPR) system. This method uses hiPSC-CMs to carry targeted gene knockouts, offering the chance to modify cells that carry a pathological component and alter it to the preferred phenotypes as had been done in hypertrophic cardiomyopathy [74]. It is not long until we can use this system to modify in vivo characteristics of a diseased organ. hiPSC-CMs have provided preclinical data for phase I drug testing. Future uses can include pharmacokinetic and pharmacodynamic modelling to establish in vivo conditions or assess beneficial side effects other than the intended mechanism of the drug. We can start comparing clinical information from patients and find a correlation to in vitro disease models that carry genetic mutation. Hopefully, this may identify novel pathologic genetic mutation and capture variable phenotypes to answer a specific hypothesis that leads to well-understood pathophysiology and the discovery of novel therapies.

Conclusion

Tissue engineering and stem cell technology are highly valuable assets in the future of medicine. They cover a multitude of uses in cardiac research, whether in drug screening, disease modelling, or constructing autologous cardiac patches for in vivo implantation. Even though current hiPSC-CM models do not embody maturation levels like the native heart, this offers possibly new areas of research as partially undifferentiated cells have the possibility to induce better tissue engraftment and cardiomyocyte regeneration. Available 2D and 3D technologies, including cardiac tissue, EHT, Biowire II, and heart-in-a-jar, brought us closer to recreating the human heart ex vivo. Recent studies have also shown that the combination of engineering techniques and the application of cardiac tissue in vivo successfully restore tissue functionality in small and large animal models without major cardiac adverse events. This step is highly critical, as it is of utmost importance to confidently determine the safety, feasibility, and tissue characteristics preclinically before proceeding to human trials. Tissue engineering requires significant collaborative action to mimic the adult human myocardium better, whether for in vitro research or as a novel cell-based therapy as an alternative to pharmacological treatment or transplantation.

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Declarations

Competing Interests The authors declare no competing interests.

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