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Title: Engineered human cardiac tissues for modeling heart diseases

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ABSTRACT

Heart disease is one of the major life-threatening diseases with high mortality and incidence worldwide. Several model systems, such as primary cells and animals, have been used to understand heart diseases and establish appropriate treatments. However, they have limitations in accuracy and reproducibility in recapitulating disease pathophysiology and evaluating drug responses. In recent years, three-dimensional (3D) cardiac tissue models produced using tissue engineering technology and human cells have outperformed conventional models. In particular, the integration of cell reprogramming techniques with bioengineering platforms (*e.g.*, microfluidics, scaffolds, bioprinting, and biophysical stimuli) has facilitated the development of heart-on-a-chip, cardiac spheroid/organoid, and engineered heart tissue (EHT) to recapitulate the structural and functional features of the native human heart. These cardiac models have improved heart disease modeling and toxicological evaluation. In this review, we summarize the cell types for the fabrication of cardiac tissue models, introduce diverse 3D human cardiac tissue models, and discuss the strategies to enhance their complexity and maturity. Finally, recent studies in the modeling of various heart diseases are reviewed.

INTRODUCTION

The heart is a vital organ that maintains every other organ in the human body through the pumping and circulation of blood. Therefore, heart problems pose a great threat to human morbidity and mortality. In this context, cardiovascular disease, including ischemic heart disease, cardiomyopathy, and hypertensive heart disease, is one of the leading causes of death for mankind, and the global death toll and consequential burdens have continued to increase (1). To overcome these diseases, it is necessary to identify the pathological factors of disease progression and their mechanism, and to further develop effective treatments suitable for each disease. For this purpose, diverse three-dimensional (3D) human cardiac tissue models that mimic the human heart have been actively developed. These tissue models are free from the ethical issues which usually surround conventional animal models and can also eliminate gaps present between different species. Depending on which cardiac cells and engineering platforms are used, cardiac tissue models showing various shapes and characteristics can be developed (Fig. 1). Moreover, some biophysical cues such as mechanical and electrical stimulation have been applied to cardiac tissue models to further generate functionally matured ones.

The developed cardiac tissue models can be utilized for the modeling of various heart diseases. By using patient-derived induced pluripotent stem cells (iPSCs), cardiac tissue models expressing disease-associated genes can be developed (2). This model helps us to understand the differences in characteristics and phenotypes caused by a particular gene. Recently, such models can also be produced by gene editing methods, such as the CRISPR-Cas9 system, which manipulate specific target genes, and the role of genes can be investigated through comparison with normal models (3). Finally, heart disease models can be established by induction of the causative pathway of disease or by exposure to

unfavorable environments (*e.g.*, hypoxia, inflammation). In this review, we describe the different approaches to generating human cardiac tissue models using cardiac cells and engineering platforms, and then introduce several heart disease models produced with engineered cardiac tissue models.

CELL SOURCES FOR CARDIAC TISSUE MODELS

Cardiomyocytes for cardiac tissue models

Cardiomyocytes (CMs) are the basic beating units of the heart, and there are several types such as pacemaker cells, ventricular CMs, and atrial CMs. For decades, fully differentiated primary CMs extracted from the heart have been used as *in vitro* models. However, there are limitations as they are difficult to use due to their low viability and loss of maturity during culture (4). As an alternative, obtaining CMs using cellular reprogramming, which changes the fate of cells, is currently in the spotlight.

There are two methods for acquiring CMs, the differentiation method from human iPSCs and the direct reprogramming technique. The differentiation method from iPSCs first generates mesoderm cells, and then CMs are generated through cardiac progenitor cells. This process mimics normal embryonic development, for which several signaling pathways, such as Wnt/β-catenin and Activin A/BMP4 signaling, are regulated at each stage (5). With this method, CMs can be obtained without burden to the patient and an unlimited supply may be possible if iPSCs are cultured adequately. However, the differentiation method still has limitations such as yield, purity, variability, reproducibility, and cost-effectiveness (6). Direct reprogramming, which enables the acquisition of CMs without going through pluripotent stem cells, is gaining attention as an alternative to produce cardiac cells (7). Directly induced CMs were first produced using three

transcription factors (*Gata4*, *Mef2c*, and *Tbx5*) in 2010, and many studies have been conducted to produce induced CMs more simply and efficiently by adding small molecules or microRNAs (8-10). Although the iPSC technology is superior in terms of expandability and efficiency, direct reprogramming can provide cardiac lineage cells with less tumorigenicity via relatively simple process (11).

Non-myocytes for cardiac tissue models

Since cardiac tissue models, composed only of CMs, lack maturity compared to the native heart, the cell composition of cardiac heart tissue models is currently being highlighted (12). An adult human heart contains many types of non-myocytes, including endothelial cells (ECs), cardiac fibroblast (CFs), and leukocytes, and their amount is even greater than that of CMs (13). Each plays a different role in the human heart and affects the maturity of CMs, which will eventually be important in creating disease models (14).

The ECs distributed inside the vessel form the major population of the heart, and serve to transport oxygen and nutrients to maintain the heart. For implementation of vascularized networks in cardiac tissue models, human umbilical vein endothelial cells (HUVECs), human cardiac microvascular endothelial cells (HMVECs), and human adipose-derived stem/stromal cells (hASCs) have widely been used (15-17). Although vascular structures can be produced using these cells, studies on the differentiation of human iPSCs to ECs are also actively underway, due to the strengths of reprogramming techniques, such as the possibility of mass production, development of patient-specific models, and simulation of interaction in developmental stages. ECs can be differentiated simultaneously with CMs from the cardiac mesoderm, and such differentiated ECs are advantageous for reconstituting vascular networks in heart (18). In recent years, more

efficient EC differentiation methods have continued to be developed (19, 20). For example, the method of generating functional ECs with high purity from cardiogenic and hemogenic mesoderm was developed (20). For the same reason, the CF differentiation methods from human iPSC have also been developed (21, 22). Until now, CMs, ECs, and CFs have been recognized as the three major cell types for cardiac tissue models, and other non-myocytes, such as smooth muscle cells, have been occasionally added (23). Furthermore, as the immune response is a critical factor for cardiac homeostasis and disease pathophysiology, it is necessary to implement immune cells in 3D cardiac models (24). For example, macrophages in heart are known to interact with CMs via connexin 43 for improved electrical conduction (25).

Cellular interactions for cardiac tissue models

CMs and non-myocytes play important roles in the transmission of signals through interactions with other cells, and these interactions are also involved in disease progression and normal development. The ECs contribute to cardiac development, remodeling, and regeneration by interacting with CMs (26). They not only secrete several paracrine factors, such as nitric oxide (NO), neuregulin-1 (NRG-1), and apelin (APLN), to improve function and contractility of CMs during normal development, as well as to enhance the cardio-protection in a disease environment (26). ECs actively communicate with other non-myocytes. ECs resident in endocardium contribute to CF generation through endothelial-to-mesenchymal transition during normal embryonic development (27). ECs also participate in immune responses, helping immune cells migrate, by secreting cytokines, and even acting as an antigen presenter (28). The main role of CFs, generally located between CMs, is to construct the extracellular matrix (ECM)

environment and maintain the homeostasis of ECM in the heart. In normal conditions, they communicate with CMs via gap junctions, membrane nanotubes, and paracrine signaling, and ultimately enhance the structural maturation and electrophysiological function of CMs (22, 29). However, excess activation and accumulation of CFs could be one of the phenotypes in cardiac diseases, such as myocardial infarction, cardiac fibrosis, and hypertensive heart disease. From this point of view, the implementation of CFs is important in establishing both the functionally advanced normal cardiac models and the heart disease models. The interactions between CFs and other non-myocytes have been examined in previous studies. When tested in the co-culture model, CFs assisted the proliferation of ECs and their sprouting (30). IL-1β expression in CFs, under a disease condition of myocarditis, has been shown to recruit leukocytes and induce the inflammatory process (31). Therefore, the incorporation of non-myocytes to cardiac tissue models is essential to replicate their interactions in a human heart tissue and predict the disease environment more precisely. In addition, it is required to develop culture platforms that can realize their interactions, including the secretion and absorption of cytokines, while each cell type can be maintained in optimal conditions.

ENGINEERED CARDIAC TISSUE MODELS

Cardiac spheroids

Various types of cardiac tissue models, possessing different characteristics and merits, can be produced depending on technologies used for fabrication. The simplest form of the model is a cardiac spheroid, which can be mainly prepared using devices that enable the collection of single cells, including a microwell, hanging drop plate, and V-bottom plate (22, 32, 33). The multicellular cardiac spheroids are fabricated by combination of

CMs, ECs, and CFs. For instance, multicellular spheroids comprising human iPSC-derived CMs (70%), ECs (15%), and CFs (15%) showed enhanced electrical maturation and contractile phenotypes, compared with multicellular spheroids generated with other fibroblasts (*e.g.*, skin fibroblasts) (22). In another study, spheroid models produced with different cell compositions (50% CMs, 25% ECs, and 25% CFs) were used to investigate the crosstalk between three cell types in doxorubicin-induced cardiotoxicity mediated by nitric oxide synthase (32). Since these methods can easily produce a large amount of uniform-sized 3D tissue models mixed with various cells, it is suitable for high-throughput drug screening (34). Therefore, cardiac spheroids are likely to be used as a screening platform for determining the efficacy and cardiotoxicity of the drug in pharmaceutical industry.

Engineered heart tissues

Engineered heart tissue (EHT) models, first established by Zimmerman *et al*., can form the desired shape by using a mold and contraction of the hydrogel (35). Depending on the structure of the mold, EHTs of various shapes such as ring, rod, and patch, have been fabricated. The EHT model can recapitulate the environment of *in vivo* CMs in a simple way. The alignment of CMs can be naturally developed by contraction of hydrogel, which eventually enhances electrical integration and synchronized contraction of the tissue models (36). The use of hydrogel enables the implementation of cell-ECM interaction and the encapsulation of bioactive proteins or functional materials (*e.g.*, carbon nanotubes, gold nanostructure, and graphene oxide). Moreover, incorporation of additional devices, such as solid/elastic pillars with micromanipulator, allows the application of mechanical stimulation or the measurement of contractile force (37, 38).

Heart-on-a-chip

Organ-on-a-chip technology has been applied to develop *in vitro* models of various organs over a number of years. Cardiac tissue models can be manufactured using a microfluidic platform and cardiac cells, called a heart-on-a-chip (39). Microfluidic heart-on-a-chip shows unique advantages such as supply of well-controlled flow or stimulation, and the ability to analyze physiological functions by integrating biosensors (40-42). Since chips are generally fabricated using elastomer polydimethylsiloxane (PDMS), they have elastic mechanical properties suitable for stretching the entire device (39). Another advantage of the chip system is compartmentalization, which facilitates the co-culture of various cell types including CMs, ECs, and CFs in different chambers and channels. This is particularly helpful in establishing the vascularization of tissue models and, observing cell type-dependent drug-induced responses, such as contractile function of CMs and permeability change of ECs (40). To create a 3D cell structure on a chip, beyond the limitations of the conventional 2D cell layer, the EHT was integrated with microfluidic chips, leading to cardiac tissue models with the merits of each system. For example, the EHT formed in the chip could be electrically stimulated by 3D electrodes integrated in the chip, and endothelialization could also be induced through separate channels (43). Moreover, EHT fabricated using human iPSC-derived CMs (80%) and human iPSC-derived stromal cells (20%) within microfluidic chip showed an increase in cellular alignment and ECM production, and further matured under medium condition enriched with fatty acids (44).

Cardiac organoids

In recent years, the development of cardiac organoids, which mimic early heart development in the human body, has been demonstrated. The first developed cardiac organoids derived from aggregates of human iPSCs recapitulated the cardiomyogenesis of embryonic development and the expression of other surrounding regions and cells, such as mesenchymal cells and ECs (45). As cardiac organoids are generated via intrinsic development program, they can represent the *in vivo* human heart better than other cardiac tissue models. More recently, self-organizing cardiac organoids have been developed to contain the chamber with vascularization (46, 47). However, current cardiac organoids still remain in the early developmental stage (48). Therefore, efforts are required to produce more complex and mature cardiac organoids through long-term incubation in bioreactors and microfluidic chips or by incorporating additional cell types that do not develop naturally along with organoids (*e.g.*, immune cells). In addition, unlike other cardiac models fabricated with defined cell numbers and controlled stages of cell differentiation, cardiac organoids may encounter the issues on batch variation and heterogeneity, which need to be addressed for standardization.

Cardiac tissues with functional hydrogels

Establishing ECM environments artificially in cardiac tissue models has been conducted using hydrogels prepared from synthetic materials (*e.g.*, poly(ethylene glycol)) and natural materials (*e.g.*, fibrin, collagen, and Matrigel) (49). The hydrogels play a role in anchoring cells at certain locations, and influence cell differentiation and tissue formation through cell-matrix interactions. Hydrogels prepared from fibrin and collagen have been used to fabricate EHT models, and Matrigel has been used to embed cardiac organoids. Hydrogels made of synthetic materials have a low variability due to their chemically

defined composition, and are easy to control mechanical and physical properties (e.g., modulus, stiffness). On the other hand, natural hydrogels exhibit superb biocompatibility and similarity to the native tissue environment (50). Decellularization technique allows removal of cellular components from native organs and tissues, resulting in fabrication of biological scaffolds containing tissue-specific ECM. Therefore, decellularized tissue-derived matrix can reconstitute the complex extracellular environments of the tissues (51-53). Decellularized tissue matrix has been manufactured as hydrogel and integrated into tissue models (54, 55). For instance, EHT model with heart ECM was fabricated using neonatal rat cardiomyocytes and 3D bioprinting (56). Decellularized heart-derived ECM hydrogel was used as a bioink with other supporting materials (*e.g.*, poly(ethylene/vinyl acetate)), which contributed to the increased alignment and differentiation of CMs in the EHT model (56). In another study, EHT model was established using human iPSC-CMs encapsulated in decellularized heart ECM hydrogel with reduced graphene oxide to implement tissue-specific biochemical cues and electrical conductivity (57). This hybrid scaffold enhanced contraction force, conduction velocity, calcium handling, and action potential duration of the EHT model (57).

Cardiac tissues stimulated with electrical/mechanical cues

Cardiac tissue models currently tend to be manufactured using human iPSCs. However, the maturity and functionality of these tissue models are still limited when compared to the *in vivo* human heart. To solve this problem, many efforts to create advanced models have proceeded in the direction of recapitulating the microenvironment and signaling of the native heart (58). Thus, in addition to incorporation of non-myocytes or a chip system, as previously mentioned, the stimulation of cardiac tissue models with an electrical pulse

and cyclic stretch, has been tested for cardiac maturation. Electrical signals, which play a critical role in inducing the conduction of heartbeat, are associated with the functional phenotypes of CMs such as electrical interconnectivity and synchronized contraction (59, 60). Accordingly, the malfunction of electrical signal often leads to heart rhythm problems such as arrhythmia (59). When electrical stimulation was applied to the cardiac tissue models through bioreactors and biowire devices, structural and functional maturity could be substantially enhanced (Fig. 2A) (61, 62). Similarly, application of cyclic stretch using elastic scaffolds and stretchable bioreactors promoted the maturation of EHT models through the formation of aligned structure and increased electrical functionality (Fig. 2B, C) (63, 64). Another study reported that maturation of EHT model fabricated with early-stage human iPSC-CMs was enhanced by gradually increasing the frequency of electrical and mechanical stimulation (from 2 Hz to 6 Hz by 0.33 Hz per day) (65). Maturity of this EHT model was similar to that of the adult heart, which was confirmed through various analyses, such as ultrastructure, oxidative metabolism, frequency-dependent acceleration of relaxation, positive force-frequency relationship, and calcium handling (65).

HEART DISEASE MODELING

Cardiomyopathy

Cardiomyopathy is a heart disease indicated by enlarged, thickened, or stiffened heart muscle, which is associated with several intrinsic and extrinsic factors, but has been mainly recognized as an inherited disorder (66). Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), characterized by a thickening of the left ventricle and dilated left ventricle, respectively, are representative examples among the several types of cardiomyopathies (67). HCM is caused, in the majority of cases, by mutations of

genes that encode the sarcomere proteins, while DCM can be caused by more diverse factors such as mutation of sarcomere and cytoskeletal proteins, systemic pathology, maternal mitochondrial DNA, and X-linked mutations (68).

For cardiomyopathies caused by genetic mutations, disease models can be produced using either patient-derived iPSCs or gene editing systems like CRISPR/Cas9 if the genetic information is known. In 2015, EHT was developed for DCM modeling using patient-derived iPSC-CMs which have mutations in the *TTN* gene encoding titin, one of the filamentous proteins contained in sarcomeres (69). Interestingly, contractile deficits were not significantly observed in single cells, but they were expressed in the developed DCM EHT model (69). These results may be attributed to the increased CM alignment and enhanced contractile proteins in the EHT models. The *TTN* gene-deficient EHT models were manufactured via the CRISPR/Cas9 gene editing, which was verified by confirming reduced contraction. Barth syndrome (BTHS), caused by mutations in the *TAZ* gene encoding tafazzin, is a disease characterized by the combinational symptoms of cardiomyopathies such as HCM and DCM (70). The BTHS heart-on-chip model, developed using patient-derived iPSCs or the CRISPR/Cas9 gene editing system, showed a diminished contractile stress. This impaired phenotype was restored by treatment with TAZ nucleoside-modified messenger RNA (modRNA) or linoleic acid (71). Ma *et al*. constructed a myosin-binding protein C (*MYBPC3*) deficient human iPSC line using TALEN-mediated gene editing. Cardiac microtissues were then generated using *MYBPC3*-deficient human iPSC-CMs and synthetic filamentous matrices fabricated via two-photon polymerization (72). They found that the lack of a *MYBPC3* ($MYBPC3^{-/-}$) gene in a mechanically overloaded environment driven by filamentous matrices caused impaired contractile phenotypes and calcium dynamics, as seen in HCM and DCM (72).

The effect of heterozygous gene deficiency (*MYBPC3*$^{+/-}$) was also investigated using cardiac microtissue fabricated with micro-heart muscle arrays (73). In another study, the association of HCM with mutation in the *ACTN2* gene, which encodes α-actinin 2, was investigated using an EHT model (Fig. 3A) (74). The *ACTN2* mutant EHT model, fabricated with patient-derived iPSC-CMs and CRISPR/Cas9-mediated gene editing, possessed HCM phenotypes such as a longer action potential and the prolonged relaxation of contraction (74). As such, cardiac tissue models can be used to identify novel genes related to cardiomyopathy and investigate their roles in cardiomyopathy occurrence.

Cardiomyopathies occur often without genetic mutation and family history, such as nonfamilial HCM. These cases can be modeled through the provision of external factors or stimuli. Zhao *et al*. fabricated an EHT model using patient-derived iPSCs and a biowire chip, and then induced a left ventricular hypertrophy by chronic electrical stimulation for a period of up to 6 months (75). The left ventricular hypertrophy EHT model showed different mRNA profiles and contractile functions, when compared to a normal model (75). In a separate study, non-genetic cardiomyopathy was induced by treating angiotensin II in an EHT model that was constructed using human iPSC-CMs and human ventricular cardiac fibroblasts in a biowire chip (76). The EHT model of angiotensin II-induced progressive cardiomyopathy showed a gradually decreased contractile force and electrical activity, and was used for evaluation of the toxicity and efficacy of the drugs (losartan, relaxin, and saracatinib) (76).

Arrhythmia

Arrhythmia is a common disease in which an irregular heartbeat appears for various reasons. Arrhythmia is caused predominantly by problems in the electrical circuit in the

heart (77). One of the major inherited arrhythmic disorders is long QT syndrome (LQTS), which is caused by mutation of cardiac ion channels. LQTS is named due to its clinical feature of a prolonged QT interval, and there are several types depending on which ion channel is affected (78). Thus, LQTS cardiac tissue models could be developed by incorporation of LQTS patient-derived cardiac cells or by the treatment of drugs that block specific cardiac ion channels. For instance, ring-shaped EHT models were generated with LQT2 patient-derived iPSC-CMs (79). These models exhibited a prolonged action potential duration, an abnormal calcium transient, and arrhythmic responses (79). The same research group demonstrated ring-shaped atrial arrhythmia in the EHT models and tested therapeutic strategies of electrical stimulation or anti-arrhythmic drugs (Fig. 3B) (80). QT prolongation in the heart could also occur due to the adverse action of the drugs (81). Previous study demonstrated the development of two types of LQTS EHT models (LQT1 and LQT2) through the treatment of drugs which inhibit each ion channel (HMR-1556 and E-4031 for blocking I_{Ks} and I_{Kr}, respectively) (82). Torsade de Pointes (TdP) means “twisting of the points” of heartbeat, which is one of the representative characteristics of life-threatening arrhythmia (81). As the occurrence of TdP, along with QT prolongation, contributes to the withdrawal of new drugs, drug safety screening systems have been focused on these features (83, 84). To model TdP *in vitro*, E-4031, which prolongs the field potential duration, was treated to cardiac tissue models, and consequently, spiral wave re-entry and TdP-like arrhythmic responses were observed (84). In another study, anatomical defects were induced to produce a structural arrhythmia disease model (85). After constructing ventricle models with neonatal rat ventricular myocytes and nanofiber spinning system, an artificial hole injury was generated to induce an arrhythmia with an abnormal flow of contraction (85).

Myocardial ischemia and myocardial infarction

Myocardial ischemia is characterized by a limited oxygen supply due to a blood vessel occlusion, which leads to the deterioration of heart function. Myocardial infarction is a life-threatening disease in which necrosis of the heart occurs due to extended ischemia or an acute blockage of the coronary artery (86). Models to simulate these events can be developed through culture in hypoxic conditions. For example, a previous study cultivated a cardiac tissue model fabricated using human iPSC-CMs in three types of hydrogels with different stiffness (0.8, 8, and 30 kPa) under conditions of a 1% oxygen concentration and investigated the effects of cell age and tissue stiffness on viability and reactive oxygen species (ROS) production (87). In another study, a myocardial infarction model was constructed by gradually decreasing the oxygen concentration towards the inside of the multicellular spheroids comprising 50% human iPSC-CMs and 50% non-myocytes (CFs, HUVECs, and hASCs) under conditions of a 10% oxygen concentration (88). This spherical disease model exhibited several pathological phenotypes, such as a fibrotic response and impaired calcium handling, and was used to test anti-fibrotic drugs and to identify the exacerbation of cardiotoxicity by drugs (88).

Reperfusion therapy is widely used to treat myocardial infarction, however, it can cause additional injury, called ischemia-reperfusion injury (IRI). IRI cardiac tissue models were first developed in the form of EHT in 2019 via a method of restoring oxygenation from ischemic conditions (89). These IRI models can be used for testing several therapeutic approaches targeting ischemic preconditioning, intracellular pH, the opening of the mitochondrial permeability transition pore (MPTP), and oxidative stress (89). Spherical IRI cardiac tissue models were also developed in another study using a

similar inducing method, and these models showed some hallmarks of disease phenotypes such as cell death and increased secretion of cytokines associated with inflammation, angiogenesis, and cell migration (90). The angiogenic effect of IRI was confirmed through enhanced tube formation of HUVECs cultured with conditioned medium from spherical IRI tissue models (90). ECs are known to be an important mediator in myocardial dysfunction after IRI (91). To accurately predict pathological responses in IRI, it is necessary to implement the interaction between ECs and CMs. In this context, the cardioprotective roles of endothelial extracellular vesicles and their mechanism were investigated using an IRI heart-on-a-chip model (92). In a separate study, an IRI chip model was constructed through co-culture of human iPSC-CMs and iPSC-ECs in microfluidic chip, and changes in TSG101 and CD63 subunit expression of exosomes secreted in chip models were detected after ischemic injury and IRI, respectively (93).

Cardiac fibrosis

Severe heart defects caused by myocardial infarction and ischemia evoke cardiac fibrosis, which eventually leads to heart failure. CF, known to maintain the homeostasis of ECM, is a key cell type in cardiac fibrosis characterized by excessive ECM production and disrupted balance of ECM homeostasis (94). In this regard, cardiac tissue models based on CFs have been developed for modeling cardiac fibrosis, and various fibrosis induction methods have been used. To simulate a fibrotic response in cardiac tissue models, the application of biomechanical cues could be used. In many organs including the heart, overexpression of transforming growth factor-β (TGF-β) signaling is known to accelerate fibrosis after injury by activating fibroblasts, remodeling ECM, and promoting myofibroblast conversion (95). Accordingly, activation of TGF-β signaling resulted in a

fibrotic response of the cardiac tissue model with CMs and CFs encapsulated in gelatin methacryloyl (GelMA) hydrogels (96). Moreover, cyclic mechanical compression was applied to a microdevice containing CFs encapsulated with GelMA hydrogels, and this triggered a phenotypic remodeling of CFs to myofibroblasts (97). In another study, cardiac fibrosis-on-a-chip was designed and fabricated in the form of EHT containing human iPSC-CMs and CFs, and fibrosis was induced by activation of TGF-β signaling (98). This fibrosis EHT model in chip showed hallmarks of fibrosis such as collagen deposition and increased stiffness, and therapeutic efficacy of anti-fibrotic drugs like pirfenidone was examined (98).

The fibrotic cardiac tissue model could be optimized by tuning the ratio of CMs and CFs. Wang *et al.* used a biowire chip for generating EHT models, and a 3:1 and 1:3 ratio of CMs and CFs was incorporated for a normal EHT model and cardiac fibrosis EHT model, respectively (99). The resultant fibrotic EHT model showed numerous disease phenotypes such as collagen deposition, diminished contractile function, abnormal calcium transient, and impaired electrophysiological properties (99). The drug effect was investigated using p-guanidinomethyl-phenylacetyl-Arg-Val-Arg-4-amidinobenzylamide (PCI) in fibrotic EHT models at early and late time points, and the changes in passive tension, active force, and collagen deposition were compared with those of normal EHT model (99). Early treatment of PCI reduced passive tension, active force, and collagen deposition in the fibrotic EHT model, whereas only passive tension was significantly decreased by the treatment of PCI at late time points (99). In another study, Daly *et al.* developed a cardiac fibrosis model by adjusting the ratio of CFs in a cardiac tissue model manufactured by assembling spheroids using 3D bioprinting (Fig. 3C) (100). In this study, a total of 20% of CFs were used for the healthy spheroids and 80% of CFs were used for

scarred spheroids. The developed fibrotic spheroids had a severely damaged contraction profiles (100). With the advantage of bioprinting, it was possible to create a cardiac tissue model that was locally scarred in a specific area, and the delay of the contraction due to scarred region was spatially demonstrated (100). When the cholesterol modified miR-302b/c was applied for cardiac repair in the fibrotic model, recovery of contractile phenotype and activation delay was observed (100).

CONCLUSION

Owing to the development of stem cell and reprogramming technologies and the inaccuracy and ethical issues in animal experiments, 3D cardiac model production is in the spotlight. Numerous cardiac tissue models, each having their own advantages, have been developed and shown the benefits of modeling heart diseases. The cardiac tissue models that incorporate genetic diseases have been realized by using reprogramming and gene editing techniques. Modeling heart diseases with various risk factors in addition to genetic factors, such as myocardial infarction and cardiac fibrosis, has been achieved via creation of disease-specific microenvironments. Despite significant improvement in heart disease modeling, the complexity and maturity of the cardiac tissue models are still insufficient to simulate the complicated features and pathophysiology of heart diseases. Therefore, application and incorporation of tissue-engineering platforms (*e.g.*, functional biomaterial scaffolds, microfluidic chip, bioprinting, and electroconductive materials, *etc.*) need to be further expanded for precise recapitulation of the cardiac microenvironments, induction of apparent disease phenotypes, and high-throughput culture and analysis.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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FIGURE & FIGURE LEGENDS

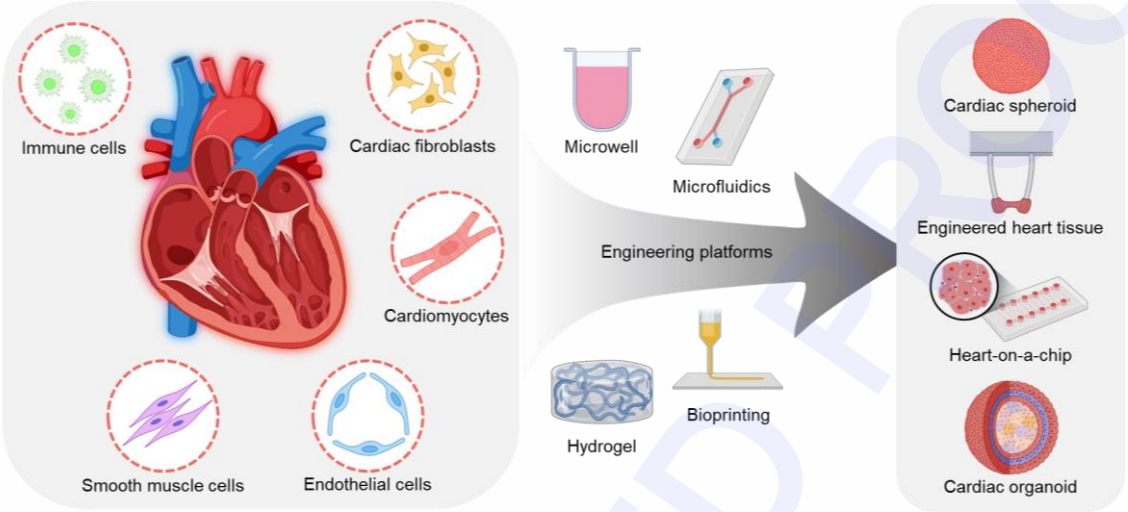

Figure 1. Cell components and engineering platforms for the development of cardiac tissue models. Several types of cardiac tissue models have been developed, to include various cardiac cells, using engineering platforms such as microwells, microfluidic devices, functional hydrogel, and bioprinting. This figure was created with BioRender.com.

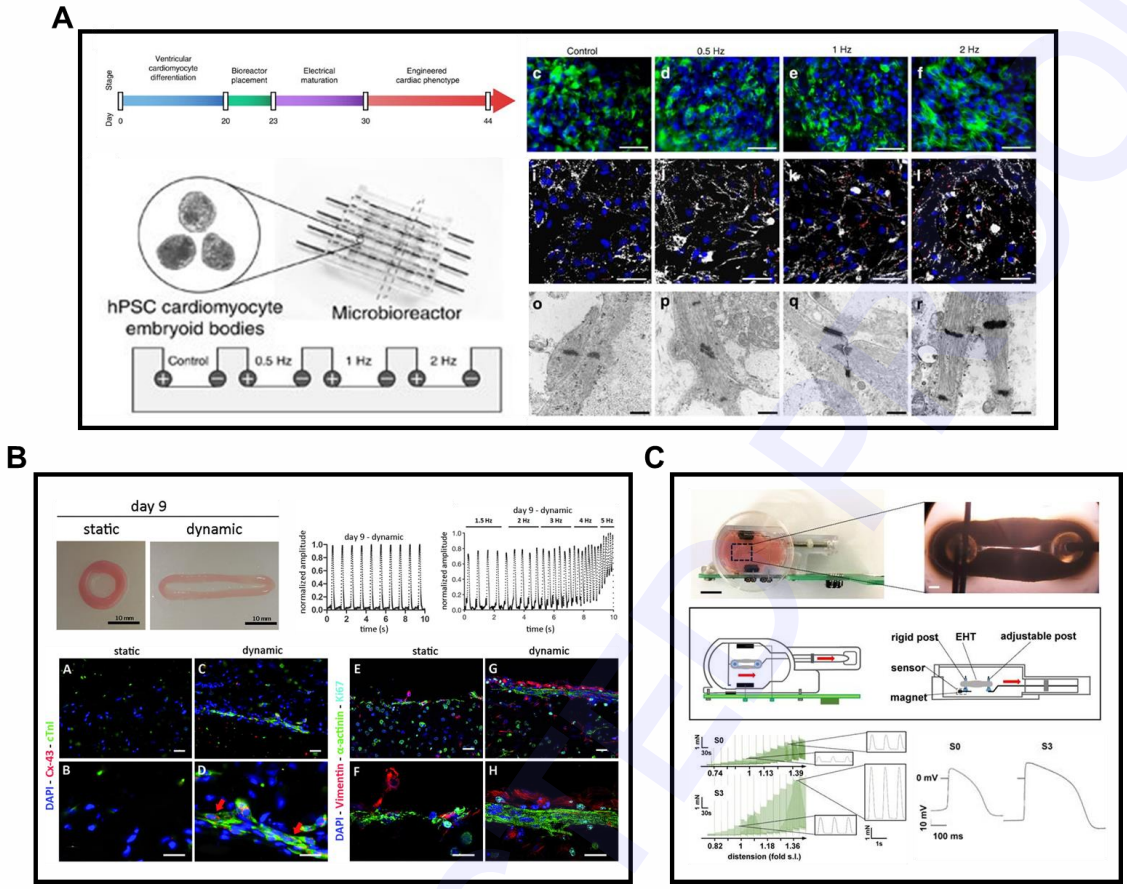

Figure 2. Biophysical stimulation to improve the cardiac tissue models. (A) Cardiac spheroids cultured in microbioreactors and subjected to electrical stimulation at various frequencies. Electrical signals increased expression of gap junction and sarcomere thickness in cardiac spheroids. Adapted from Eng *et al*. (61) (CC BY 4.0 license) Copyright 2016, The Authors, published by Springer Nature. (B) EHT models stimulated with uniaxial cyclic stretch (10% strain, 1 Hz) using a bioreactor. The expression of gap junction and sarcomere structure and electrical responses were enhanced by mechanical stimulation. Adapted from Massai *et al*. (63) (CC BY 4.0 license) Copyright 2020, The Authors, published by Frontiers Editorial Office. (C) Ring-shaped EHT models with electrical pulses and stretch conditioning, which showed improved biomechanical properties and electrical coupling. Adapted from Lu *et al*. (64) (CC BY 4.0 license) Copyright 2021, The Authors, published by Ivyspring International Publisher.

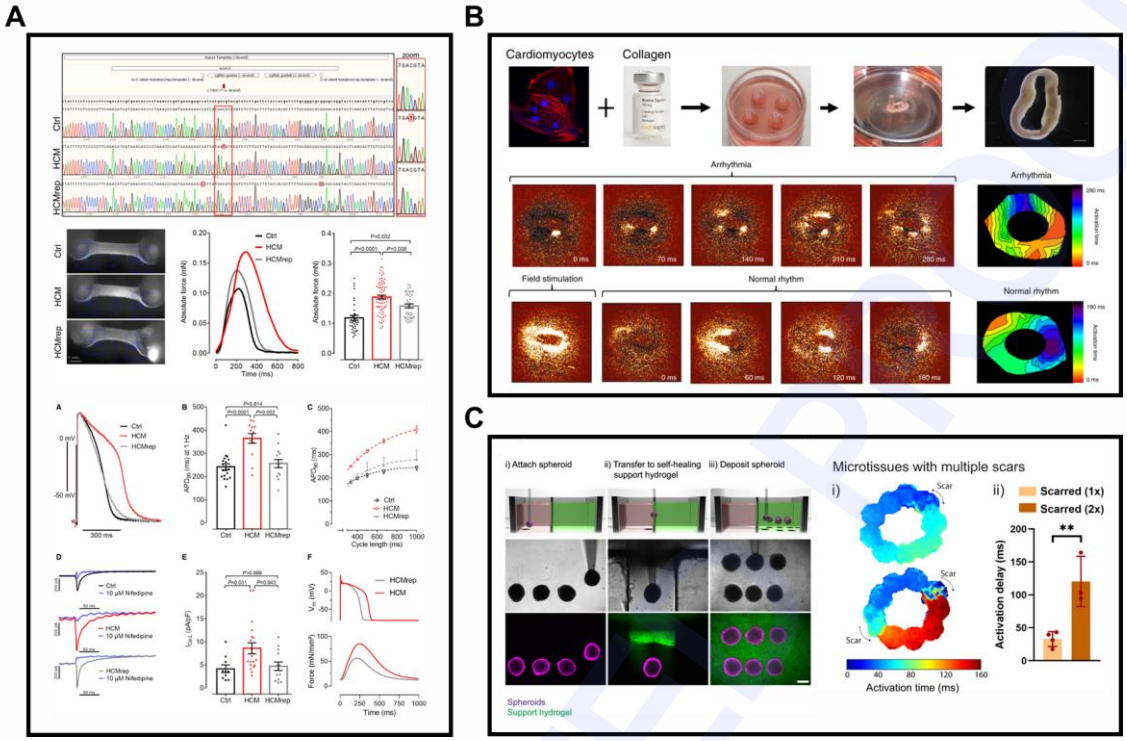

Figure 3. Modeling various heart diseases using cardiac tissue models. (A) Cardiomyopathy modeling using EHT with genetic defects of *ACTN2*. Adapted from Prondzynski *et al.* (74) (CC BY 4.0 license) Copyright 2019, The Authors, published by John Wiley and Sons. (B) Atrial arrhythmia modeling using ring-shaped EHT. Adapted from Goldfracht *et al.* (80) (CC BY 4.0 license) Copyright 2020, The Authors, published by Springer Nature. (C) Cardiac fibrosis modeling by assembling spheroids using 3D bioprinting. Adapted from Daly *et al.* (100) (CC BY 4.0 license) Copyright 2021, The Authors, published by Springer Nature.