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Cardiovascular disease models: A game changing paradigm in drug discovery and screening

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Abstract

Cardiovascular disease is the leading cause of death worldwide. Although investment in drug discovery and development has been sky-rocketing, the number of approved drugs has been declining. Cardiovascular toxicity due to therapeutic drug use claims the highest incidence and severity of adverse drug reactions in late-stage clinical development. Therefore, to address this issue, new, additional, replacement and combinatorial approaches are needed to fill the gap in effective drug discovery and screening. The motivation for developing accurate, predictive models is twofold: first, to study and discover new treatments for cardiac pathologies which are leading in worldwide morbidity and mortality rates; and second, to screen for adverse drug reactions on the heart, a primary risk in drug development. In addition to *in vivo* animal models, *in vitro* and *in silico* models have been recently proposed to mimic the physiological conditions of heart and vasculature. Here, we describe current *in vitro*, *in vivo*, and *in silico* platforms for modelling healthy and pathological cardiac tissues and their advantages and disadvantages for drug screening and discovery applications. We review the pathophysiology and the underlying pathways of different cardiac diseases, as well as the new tools being developed to facilitate their study. We finally suggest a roadmap for employing these non-animal platforms in assessing drug cardiotoxicity and safety.

Keywords

Cardiovascular diseases; *In vitro* disease models; *In silico* disease models; *In vivo* disease models; Drug discovery; Human induced pluripotent stem cells; Organ-on-a-chip; Cardiomyocyte

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1. Introduction

The cardiovascular system, with its broad interdependence with circulation, blood vessels and blood constituents, as well as renal and nervous systems, is especially complex to model [1]. The myriad of possible signaling pathways involved in both normal function and in pathogenesis makes it a challenge to track drug interactions, limiting our ability to make predictions about a drug's effectiveness, safety, and global effects on the body. The motivation for developing accurate, predictive cardiac models is twofold: first, to study and discover new treatments for cardiac pathologies which are leading in worldwide morbidity and mortality rates; and second, to screen for adverse drug reactions on the heart, which is a primary risk in drug development.

Cardiovascular toxicity claims the highest incidence and severity of adverse drug reactions in late-stage clinical development [1]. For example, Vioxx (Rofecoxib), originally designed to treat pain related to osteoarthritis and approved by the Food and Drug Administration (FDA) in 1999, was linked to over 27,000 cardiovascular-related deaths and myocardial infarctions (MI). It was withdrawn from the market in 2004, although later relicensed for more specific indications, with implementation of regulatory and transparency safeguards [2]. In preliminary clinical investigations, the drug showed effectiveness in its target treatment and adverse events were not significant. It was not until four years of long-term clinical studies that it became evident that the risk of heart attack and stroke was actually two-fold higher with Vioxx compared to the control group [3]. Some other compounds, such as Micturin (Terodiline, for urinary incontince), Fen-phen (Fenfluramine/phentermine, anti-obesity treatment), Seldane (Terfenadine, allergy medication), Zelnorm (Tegaserod, for irritable bowel syndrome), Meridia (Sibutramine, appetite suppressant), and Darvon/Darvocet (Propoxyphene, analgesic drug), have all had a similar record in terms of adverse cardiovascular effects [4–6].

While up to 70% of human toxicity may be approximated in the preclinical stage, there are other subtler and higher-risk cardiovascular events that only emerge when drugs are administered to humans for longer periods of time and in larger populations [7]. Even though a step closer toward representing the human population than preclinical studies, clinical trials are also challenging. Clinical trials have relatively small sample sizes, narrow subgroups of patient demographics, and require non-invasive endpoints for study monitoring [8]. As a result, rare events may not be detected until after clinical trials, when the drug is already in the clinic. Similarly, the small patient sample sizes may not recapitulate the genetic and environmental variations that a target population may experience. There is also the issue of determining toxicity endpoints in a clinical trial. Although non-invasive, biomarkers assessed via biochemical assays, genomic markers, antibody-based methods and imaging techniques [9], often yield information that reflects already serious and acute toxicities, with limited insight available for long-term damage [8]. Identifying biomarkers that can signal both acute and long-term toxicities before irreversible organ damage is incurred would be ideal, but how to do this with current technologies remains a challenge, suggesting a need for new technologies.

Using the current drug screening and safety testing paradigm, it takes approximately 12 years and over $2 billion before a single compound may reach patients [10]. Even so, late-stage attritions are still frequent [11]. Novel approaches are needed to make drug discovery affordable and effective. In this review, we describe current platforms for *in vitro*, *in vivo*, and *in silico* modelling of healthy and pathological cardiac tissues as well as their pros and cons for drug screening and discovery applications. To this end, we first briefly review the pathophysiology of different cardiovascular diseases (CVDs) and current knowledge about their underlying pathways, as well as the new tools being developed to facilitate their study. We finally suggest a roadmap for disease understanding and employing these platforms in assessing drug cardiotoxicity and safety.

2. Pathophysiology of cardiovascular diseases (CVDs)

Cardiovascular diseases (CVDs) including heart attack, stroke and heart failure (HF) are a leading cause of morbidity and mortality, contributing to an estimated 17 million deaths annually, around the world [12]. There are many underlying pathologies that lead to CVDs. Here, we will elucidate the pathophysiological pathways that play key roles in the development of different CVDs.

2.1. Atherosclerosis and myocardial infarction (MI)

2.1.1. Main players in atherosclerosis—Atherosclerosis is defined by cholesterol deposition in large- and medium-sized arteries. This accumulation leads to the proliferation of certain types of cells, such as macrophages and smooth muscle cells (SMCs), within the arterial wall. Enlargement of atherosclerotic plaques can gradually impinge vessels and block blood flow. Atherosclerosis is the most likely cause of MI and stroke [12–15].

High levels of cholesterol and hypertriglyceridemia are risk factors for atherosclerosis. Both cholesterol and triglycerides (TG) can have endogenous or dietary sources and their metabolism is interrelated. Cholesterols are mainly carried in the blood by various lipoproteins. Chylomicron which carries dietary lipids from the intestines, is made up of a large portion of TG and a small portion of cholesterol and apolipoprotein(apo)B48 molecules that is secreted by the intestines and liver (Fig. 1). On its way to the liver, chylomicron undergoes lipolysis in peripheral tissues losing some TGs and gaining some other lipoproteins and becoming chylomicron remnant (Ch remnant) (Fig. 1). In the liver, Ch remnant can be converted to the very-low-density-lipoprotein (VLDL), a lipoprotein with higher cholesterol content and density than Ch, and with apoB100 instead of apoB48 (Fig. 1). Lipoprotein lipase (LPL), found in the circulation, converts lipoproteins to higher density particles by reducing free fatty acids from the lipoprotein, thereby converting it into intermediate density lipoprotein (IDL). IDL is taken up by the liver. In the liver, IDL is converted to LDL by LPL and TG lipase (Fig. 1) [16,17]. The LDL cholesterol level in circulation is regulated by the liver, via the LDL receptor. A protein, named proprotein convertase subtilisin/kexin type 9 (PCSK9), which is mainly expressed in the liver, controls LDL receptor degradation in the liver. The LDL receptor number on the surface of liver cells regulates the LDL cholesterol in circulation [5].

Atherosclerosis normally starts in arteries at points with high turbulent shear stress. Turbulent flow decreases nitric oxide (NO) generation, a mechanism for vascular relaxation, by enhancing endothelial nitric oxide synthase (eNOS) uncoupling and promoting reactive oxygen species generation. Moreover, uptake of lipoprotein and its modified forms in endothelial cells (ECs) perturbs NO bioavailability [18]. In contrast to high turbulent shear stress, low shear stress initiates endoplasmic reticulum (ER)-stress induced apoptosis of ECs [19]. Regeneration of endothelium by recruitment of blood-borne endothelial progenitor cells and by proliferation of adjacent ECs is not always perfect.

In healthy endothelium, lipid and inflammatory cells may enter into sub-endothelial spaces, through both intra- and trans-cellular mechanisms which are not completely understood, as reviewed in detail in other publications [20,21]. Damaged endothelium provides gaps in cell junctions which facilitate lipid and inflammatory cell migration to the sub-endothelial space, thereby promoting atherosclerosis. Reduced NO levels and perforation of blood vessels further promotes deposition of apoB containing lipids (LDL, VLDL, lipoprotein(a) and remnant lipoproteins), through receptor mediated endocytosis and transcytosis in sub-endothelial spaces [18,22,23]. ApoB containing lipoprotein in subendothelial space is considered to be atherogenic. The concentration of atherogenic lipoproteins in the circulation, their charge, particle size and cholesterol content, all govern their entry and retention in the sub-endothelial spaces [24]. In the sub-endothelium, positively charged apoB interacts with negatively charged extracellular matrix (ECM) proteoglycans [25], enhancing lipoprotein modification [26]. *In vivo*, LDL can be oxidized by enzymatic (e.g. 12/15 lipoxygenase) or non-enzymatic (e.g. free or heme-associated iron) pathways [27]. The occupation of sub-endothelial space with a high concentration of modified lipoprotein leads to lipoprotein spillover into resident ECs, SMCs and macrophages, and as a result a series of inflammatory reactions are initiated. Lipid uptake by resident cells is not a LDL receptor mediated process, instead sets of receptors with redundancy in function, that are called scavenger receptors, such as SRA, CD36 and LOX-1 or lipoprotein pinocytosis, are responsible for lipid uptake [27–29]. Macrophages are the most crucial cells in atherosclerosis [15,30]. Their phenotype is elastic in the plaque microenvironment, partly due to cholesterol deposition in the cells, creating a phenotype called foam cells. Foam cell formation is the result of an imbalance between lipoprotein uptake and cholesterol efflux. Irrespective of the uptake pathway, inside the cells in the late endosome and lysosome compartments, acidic cholesterol esterase generates free cholesterol from lipoproteins [31]. In the absence of adequate cholesterol efflux to prevent cholesterol-induced cytotoxicity, cholesterol is re-esterified by Acetyl-CoA acetyltransferase (ACAT-1) in the endoplasmic reticulum. To efflux cholesterol esters from intracellular lipid droplets, cholesterol esters need to be hydrolyzed to free cholesterol by a cholesterol ester hydrolase, associated with lipid droplets [31]. In another mechanism, when lipid droplets and autophagosomes are fused, the acid lipase involved in autophagy generates free cholesterol [32]. Several transporters in macrophages, including ATP-binding cassette (ABC) transporters, mediate efflux against cholesterol gradients. ABCA1, the most important transporter in cholesterol efflux, transfers cellular cholesterol to lipid-poor apoA-I and apoE to form pre-βHDL [33,34]. Other ABC transporters, such as ABCG1 and ABCG4 load more cholesterol on pre-HDL to form mature HDL [35].

HDL is formed in peripheral organs to help excrete cholesterol from the body in the form of bile acids. ApoA-I synthesized by the liver and intestines is a main component of most HDLs, but they may also contain apoE and apoC, which have distinct functions. ApoE, similar to ApoA1 in HDL is important in cholesterol efflux. As it is shown in Fig. 1, lipoproteins also carry triglyceride and apoC is regulating triglyceride homeostasis in HDL [36]. HDL has a number of anti-atherosclerotic effects other than cholesterol excretion, such as anti-inflammatory effects [37].

Besides macrophages, ECs and SMCs also take up modified lipoproteins and undergo phenotypic and metabolic changes leading to atherosclerotic plaque accumulation. These cells attract additional inflammatory cells with chemokines, cytokine secretion, and expression of adhesion molecules, thereby further amplifying atherosclerosis progression [38].

Complications arising from atherosclerosis mainly include narrowing of blood vessels and thrombosis. Thrombosis happens due to unstable plaque rupture, erosion, or calcified nodules in lesions, and may lead to infarction. Unresolved inflammation in atherosclerosis, together with lipid deposition and non-regulated lipid uptake in cells, leads to ER-induced stress and other mechanisms that trigger apoptosis in all stages of atherosclerosis. Impaired efferocytosis (i.e. clearance of apoptotic bodies) at later stages of atherosclerosis leads to secondary necrosis and the development of a necrotic core. Plaques with a large necrotic core and thin cap are considered unstable. The increased release of pro-inflammatory proteases in the necrotic milieu thins the fibrotic cap and degrades ECM, further enhancing plaque instability. Release of tissue factors into the circulation following plaque rupture leads to activation of coagulation pathways and promotes thrombosis [23,24]. Fig. 1 shows the schematic image of lipoprotein metabolism and conversions in body

2.1.2. Myocardial infarction—Unstable atherosclerotic plaque erosion leads to acute *myocardial infarction* (MI) (Fig. 2), where oxygen supply to myocytes becomes restricted. Myocyte contraction requires oxygen for energy supply, thus the limited oxygen supply following infarction results in cardiac damage and cell death. Reperfusion after ischemia restores blood flow; but over-oxygenation after ischemia, with the induction of reactive oxygen species (ROS), induces further damage to cardiac tissue by necrosis, apoptosis, autophagy, and secondary necrosis [40]. The extent of damage is related to the time of ischemia and reperfusion. Damaged tissue releases damage-associated molecular patterns (DAMPs), a set of danger molecules which signal for the infiltration of inflammatory cells, and for the clean-up of the damaged tissues. DAMPs bind to pattern recognition receptors (PRRs) of the innate immune system, on surrounding parenchymal cells and on infiltrating leukocytes, to recruit more inflammatory cells [41]. Border zone cardiomyocytes (CMs) also sense the ROS-inflicted damage through toll-like receptor (TLR) ligands and interleukins (IL), and respond by increasing their expression of cytokines, chemokines and adhesion molecules, to promote recruitment of more inflammatory cells [42,43]. ECs, which are abundant in cardiac tissue, also detect DAMPs and increase adhesion molecule expression, promoting neutrophil infiltration. Fibroblasts, similar to other resident cells in the heart, will also secrete inflammatory cytokines and chemokines in response to DAMPs, ROS and IL-1 signals [44].

In the process of fibrotic repair, fibroblasts convert to myofibroblasts (myoFB). There is conflicting evidence about the source of fibroblasts participating in fibrotic repair. They may come from resident fibroblasts, bone marrow progenitor cells, ECs undergoing mesenchymal trans-differentiation, pericytes or epicardial epithelial cells [44]. MyoFBs develop stress fibers and express contractile proteins, such as the embryonic isoform of smooth muscle myosin and α-smooth muscle actin, which allows myoFBs to migrate and contract. MyoFBs also have large ER compartments which allow them to secrete elevated levels of ECM. MyoFBs secrete interstitial collagens (initially type III, then during infarction healing, type I). Collagen deposition increases tensile strength and prevents ventricular wall rupture. MyoFBs also generate more fibronectin and various matricellular proteins, such as thrombospondin which help myoFB migration and the healing response [45]. However, extensive cardiac fibrosis causes electro-mechanical disturbances in the heart and decreases the cardiac contractile reserve, which results in an attenuated ability of the heart to maintain cellular perfusion under normal cardiac filling pressure, eventually leading to HF [46]. Microvascular networks in the infarct area are formed by angiogenic signaling that causes EC infiltration and proliferation. Microvessels are crucial in supplying oxygen and nutrients during the repair process, thus motivating extensive tissue engineering efforts focused on revascularization [47].

2.2. Arrhythmia

Through excitation-contraction coupling, cardiac action potentials (AP) cause a coordinated contraction of CMs that pump the blood forward to peripheral tissues. AP is the result of a series of highly ordered opening and closing of channels in the CM cell membrane, thereby conducting the AP signal from one myocyte to another. Differential expression of channels in CMs of various regions of the heart results in a unidirectional electrical waves. However, disruption of the unidirectional ordered electrical wave, caused by physical obstacles like dead or ischemic tissues, fibrosis and inflammation, can cause *arrhythmia* [48]. Other causes of arrhythmia beyond physical obstacles include mutations in connecting molecules such as connexin 40, 45 and 43, as well as disturbances in parasympathetic innervation, hypovolemia and electrolyte disturbance [49]. An imbalance between outward and inward channel activity, as observed with mutated channels, can also trigger arrhythmia. Physical and phenotypic changes can alter the expression of ion channels. These changes affect AP duration and restitution, leading to arrhythmia and resulting in a heart that beats improperly [48].

2.3. Cardiomyopathy

Cardiomyopathy is a general term encompassing a variety of symptoms including heart muscle enlargement, thickening and rigidity. Cardiomyopathy of various phenotypes, resulting from known or unknown causes, leads to weakening of the heart and modification of its ejection fraction (EF) [50]. Common categories of cardiomyopathy include hypertrophic, dilated, arrhythmogenic, and left ventricular hypertrabeculation cardiomyopathies.

Hypertrophic cardiomyopathy (HCM) is characterized by cardiac hypertrophy, a non-dilated left ventricle (LV), and a normal or increased EF. The hypertrophy is usually asymmetrical,

deriving most commonly from the basal interventricular septum adjacent to the aortic valve. At the cellular level, CMs are hypertrophied, disorganized, and separated by areas of interstitial fibrosis. This type of hypertrophy occurs due to many genetic or non-inheritable causes, leading to a wide range of phenotypes from asymptomatic to fibrillation, HF, and sudden cardiac death [51,52].

Dilated cardiomyopathy (DCM) is defined by an enlarged and poorly contractile LV. Genetic or non-genetic causes, including hypertension and valve diseases, may lead to dilated cardiomyopathy. This type of heart disease is the major cause of HF [51,53].

Arrhythmogenic cardiomyopathy (AC) is an inherited heart muscle disorder which causes sudden cardiac death, mostly in young patients and athletes. Loss of myocytes and replacement of right ventricular myocytes with fat deposition in fibroblasts are pathological features of this disease. Genetic abnormalities of cardiac desmosomes, leading to disconnection of myocytes and alteration of intracellular signal transduction are associated with arrhythmogenic cardiomyopathy [51,54].

Left ventricular hypertrabeculation (LVHT) or non-compaction, is a myocardial abnormality mostly associated with monogenic disorders or chromosomal defects, especially in neuromuscular genes. Presence of a thin, compacted, epicardial layer and a thick, noncompacted, spongy bi-layered myocardium in the endocardial layer is a common diagnosis for LVHT [55].

2.4. Cardiac fibrosis

Cardiac fibrosis is the scarring process that is characterized by cardiac fibroblast (CF) over-proliferation, myoFB activation, and increased deposition of fibrous ECM proteins [56]. It often naturally occurs following MI, dilated cardiomyopathy, and hypertension, and is a leading pathogenic factor in HF [57].

In healthy heart tissue, CFs are responsible for maintaining the ECM structure and forming an anisotropic syncytium [58]. In response to cardiac injury, fibroblasts often differentiate into myoFBs, which have a higher synthetic ability to produce ECM proteins, especially collagen [59]. Increased collagen deposition subsequently reduces compliance and increases stiffness of the affected tissue [59]. While the onset of fibrotic tissue formation may protect the heart from rupture by strengthening the injured site, continuous expansion of cardiac fibrosis will lead to progressive deterioration of cardiac contractile force and eventually result in the impairment of both systolic and diastolic functions of the heart [57].

Moreover, cardiac fibrosis is often associated with arrhythmogenicity by affecting electric signal propagation and causing rhythm disturbance [60]. Fibrotic myocardium displays distinctly altered electrophysiological properties that contribute to cardiac dysfunction. Previous studies showed that CFs could cause increased action potential duration (APD) and calcium transient duration (CTD) [61]. Despite the fact that they have multiple ion channels, fibroblasts and myoFBs are electrically non-excitable and cannot maintain AP propagation on their own. Thus, they often create conduction barriers by physically isolating myocytes,

expanding the distance between neighboring myocyte membranes, and disrupting myocyte-to-myocyte coupling through gap junctions [60].

The mechanisms associated with fibrosis have been extensively studied over the past decade. A variety of signals such as cytokines, growth factors (GFs), and hormones contribute to the pathogenesis of cardiac fibrosis [62]. Effector hormones of the renin-angiotensin-aldosterone system (RAAS) and cytokines such as TGF-β have relatively well-identified profibrotic roles in the heart [63]. TGF-β is the critical mediator of pathological fibrosis after myocardial injury [64]. TGF-β signaling facilitates persistence of CFs and activation of myoFBs through Smad-mediated pathways [65]. The RAAS is also closely linked to the onset and progression of cardiac fibrosis. It is thought that angiotensin II (Ang II) is the predominant mediator of RAAS-associated cardiac remodelling. Ang II is often elevated post injury, causing CF proliferation and collagen overexpression [66]. However, numerous upstream and downstream factors of the pro-fibrotic cascades are still not fully understood [67]. Further studies of the mechanism of cardiac fibrosis will lead to a deeper understanding of fibrosis-associated arrhythmia and HF, and open new doors for potential therapy.

Table 1 summarizes the above-mentioned CVDs and their causes, symptoms, associated complications, prevalence and mortality rates.

3. Quest for CVD models in the drug development pipeline

CVD models including laboratory animal models, *in vitro* disease platforms, and *in silico*/computational models have been used to assess the efficacy and safety of new drugs earlier in the drug development pipeline. Here, we summarize the advantages and limitations of each platform in the context of CVD modelling for the development of safer and more efficient drugs.

3.1. In vivo models

Various animal models have historically been used to further our knowledge on the etiology, pathogenesis, pathophysiology, progression, and underlying mechanisms of CVDs. They offer valuable tools in disease modelling, drug discovery and therapeutic interventions [76,77]. However, differences between the human condition and experimentally-induced pathology in animals in aspects such as cardiovascular physiology and pathophysiology, along with genetic and environmental factors, have made it rather difficult to recapitulate the complexities of CVD conditions with just a single experimental model [78]. The choice of an appropriate animal model to efficiently and reliably study any disease (e.g. CVD) is a challenging process, especially since the outcome would ultimately need to be translated to humans [11,79–81]. To determine the most appropriate animal model several factors, including research questions, number of animals, quality of the anticipated results, and the relevance of the outcomes to complications, need to be meticulously considered [82]. More importantly, it is also vital for the scientific community to reduce animal use from animal welfare and research ethics standpoints, and to comply with the 3Rs principles (replacement, reduction, and refinement) [83]. In this section, we review commonly used animal models

(i.e. small and large animal models) in cardiac research and discuss their advantages and limitations in terms of translational applicability to humans.

3.1.1. Small animal models—Rodent models are widely used in cardiovascular research due to their easy handling and housing, short gestation time, low maintenance costs, and more importantly ability for genetic manipulation. These advantages make them a more appropriate choice for high-throughput studies than large animal models [84]. However, the main disadvantage of these small animal models (i.e. mice, rats, rabbits, and cats) is the relevance of the obtained results to humans due to the distinct physiological differences between these species and humans [84–86]. Ligation-induced MI in mice and rats is one of the most commonly used and well-established models to investigate cardiac regeneration capacity which illuminate the cellular and molecular mechanisms of cardiac regeneration. In addition to this commonly used technique, electrocautery and infarction caused by cryoprobes has also been utilized to induce lesions on the epicardial surface and throughout the ventricular wall, respectively. Despite its widespread use in cardiac research, difficulties in standardizing the injury size of the left anterior descending artery (LAD) ligation model is one of the limitations of this model [88].

Cryogenic injury through open thoracotomy (e.g. LV lesion) or abdominal incision through a transverse laparotomy (e.g. RV lesion) are models which induce confluent necrosis in the heart in order to investigate its regeneration and remodelling [89]. It has been observed that macrophages residing in the lesion site could contribute to regeneration of the extensive lesion after cryoinjury of the LV myocardium in adult mice [89]. Doxorubicin (DOX)-induced HF is a suitable model to perform mechanistic evaluation of non-ischemic CVD. It mimics severe non-ischemic human cardiomyopathy and LV dysfunction. It is achieved by giving a single dose of DOX (20 mg/kg) to the animal, causing clinical symptoms reminiscent of non-ischemic HF [90].

Moreover, transgenic lines can be produced by manipulating genes in animals. Due to the short gestation time and lower cost of the mouse models compared to larger species, these models have gained great attention in cardiovascular research [91,92]. For example, genetically modified mice have been extensively used for feasibility and proof-of-concept studies. However, translational aspects need to be more carefully addressed due to the enormous differences in the heart function and size between mice and humans [93]. Among others, two strains of mice have been engineered by deletion of two of the main proteins such as actin-associated cytoskeletal protein (e.g. MLP), and calcium-sequestration (e.g. CQS) which are involved in formation of actin filaments [94] and myocardial relaxation [95], respectively. These changes simulate the development, progression, and relapse of DCM in humans. The former shows the disease phenotype (i.e. contractile dysfunction) as early as 4–6 months due to hypertrophy leading to HF with a progressive increase in the amount of the connective tissue and decreased myocardial mechanical compliance, whereas the latter contributes to a rapid-onset phenotype leading to animal death by 9–14 weeks [96,97]. Two other transgenic strains have also been developed to study HCM: one by ablating cMyBP-C (e.g. cMyBP-$C^{-/-}$ mice) and another by overexpressing human myotrophin gene (i.e. Tg mice). They have both shown the development of hypertrophy that progressively led to HF, with highly compromised function [98,99]. Although these

genetically modified models could capture some of the features of the CVD phenotype in humans, they typically do not resemble all aspects of CVDs in humans [86,87].

Models of autoimmune cardiomyopathy (AICM) have been engineered by crossing two different transgenic mice: the DQ8 transgenic non-obese diabetic (NOD) mouse with an NOD Major Histocompatibility Complex (MHC) class II β-chain knockout (KO) line, which leads to premature death through development of progressive DCM and HF [100,101]. Duchenne muscular dystrophy (DMD) is a neuromuscular disorder initiated by a mutation in the dystrophin gene [102]. The double knock-out dystrophin/utrophin mouse model caused severe cardiac dysfunction at 8 weeks of age, which resulted in mortality due to respiratory issues or HF [103]. The atrial fibrillation (AF) mouse model has been generated with CM-specific liver kinase B1 (LKB1) KO mice. Among others, this model has shown bi-atrial enlargement along with AF, LV hypertrophy, and cardiac dysfunction between 4 and 12 weeks of age [104].

In addition to these models in mice, several other models have also been generated in rats which show numerous similarities to cardiac abnormalities in humans, and represent the most widely used models for investigating the role and mechanisms of therapeutic strategies, such as stem cell (SC) therapy. Examples of these rat models include ligation-induced MI [105], overload-induced cardiac hypertrophy (e.g. ascending aortic banding) [106], diabetic cardiomyopathy (DbCM) [107], transgenic strains (e.g. hypertensive rats, type II diabetic rats (Goto-Kakizaki (GK) rats)) [108], and DMD rats. The spontaneously hypertensive rat (SHR), a transgenic line of hypertensive rats, have been the animal of choice for screening antihypertensive compounds [109]. In addition, Dahl salt-sensitive rats have been generated by administering animals with a high salt diet [110]. Other hypertension rat models can be categorized as: environmental, pharmacological, renal and stress-induced hypertension, activated sympathetic nervous system, NO-deficient models, and one/two-kidney(s) one/two-clip(s) hypertension models, among others [111]. It is noteworthy that the surgical procedure and invasive hemodynamic assessments are more straightforward to perform on rats than on mice. In addition, due to the larger size of rat myocardium, the number of post-mortem histological and molecular biological analyses that may be obtained are much higher for rats than for mice.

Experimental models have also been generated in animals to study atherosclerosis and thrombosis. Although the use of the high-fat diet in animals (e.g. atherosclerosis models) has been a well-established method to generate these models, not all experimental models (i.e. mice and rats) are acceptable for atherosclerosis due to intrinsic genetic differences, their higher resistance to atherogenesis and the high cholesterol diet [112,113]. This occurs due to the differences in human versus murine lipid processing (i.e. LDL-based versus HDL-based, respectively) [113]. To overcome these deficiencies, as well as to reduce the use of non-human primates and large animal models, genetically modified murine models have been produced. For example, genetically modified ApoE- and Ldlr-deficient mice have higher plasma cholesterol levels (5 times and 2–3 times higher than controls, respectively) [114]. Aside from these, several other transgenic models have also been introduced as suitable experimental animals for studies of plaque deposition and formation, arterial calcification, ulceration, hemorrhage, plaque rapture, thrombosis, and stenosis [115]. For example, Fatty

Zucker rats [116], cholesteryl ester transfer protein (CETP) transgenic rats, LDL receptor-knockout (KO) mice [117], and db/db mice [118] are a few of the genetically modified models developed for this purpose. However, the transgenic lines are relatively costly, limiting researchers from using optimal numbers of subjects in their studies [119]. Therefore, ovariectomized adult female Sprague-Dawley rats with suppressed resistance to atherosclerosis were developed by putting the animal on a 15% w/w of heated vegetable oil diet. The same diet over a longer period (16–24 weeks) promoted atherosclerosis in male subjects [120,121]. In addition, it has been shown that vitamin D3 administration may accelerate atherogenic processes.

Rabbits have been used as intermediate-size models, due to the fact that rabbit myocardium shares more similarities with humans than what small rodents do [122]. The spontaneous Watanabe heritable hyperlipidemic MI (WHHL-MI) model has been developed in rabbits for cardiac research purposes [122]. Although the cost of acquiring and housing rabbits is high, it is still much lower than for other larger species.

3.1.2. Large animal models—Small animal models provide significant insight about CVD phenotypes and pathways. However, there are key differences in physiology and pathophysiology of the heart and cardiovascular system of small animals (e.g. mice) and humans. For example, human heart architecture, heart rates, oxygen consumption, contractility, protein expression, and stem cell populations are different from those of rodents [123]. Therefore, large animal models (e.g. dogs, pigs, and sheep), which more faithfully resemble human physiology, function, and structural features, have been employed as experimental models to translate the cellular and biological findings from murine models into the clinic [123]. For example, the canine model has been used to study post-MI remodelling mechanisms of LV and infarct expansion, e.g. MI, ischemic cardiomyopathy, left-sided coronary artery microembolization, and pressure overload models, as well as to study the impact of therapeutic agents [124]. However, one of the main drawbacks of canine models is the inconsistency and heterogeneity in myocardial lesions and responses, due to the collateral coronary circulation which makes the comparison of the outcomes difficult post-injury [125]. In addition, there are growing efforts to minimize the use of canine models due to ethical concerns. Due to these complexities, alternative animal species such as porcine and ovine have been used for translational purposes, due to their greater anatomical similarity to humans, and the ability to make lesions of the same size [126]. For example, the arterial anatomy and collateral coronary circulation in pigs can better mimic those of humans. In addition, infarct size in porcine models can be precisely predicted [84]. Among porcine models, balloon occlusion of LAD coronary artery is the most commonly used model. In this model, infarction is induced by balloon inflation by means of a catheter through the femoral artery and placing an angioplasty balloon in the artery. For example, to quantify progressive infarct expansion over a 2-month period in adult porcine models, MI induction was tracked by radio-opaque markers. This model has shown promise for studying pharmacological effects on infarct expansion [127]. The described models have also been used to investigate the impact of stem cell transplantation on LV contractile function [128] as well as delivery of angiogenic and arteriogenic GFs [129]. Transplanted stem cells and delivered GF were able to preserve contractile function [128], as well as to restore stable

collateral networks and enhance myocardial perfusion and function in the infarcted myocardium [129], respectively. More recently, our team also reported a minimally invasive delivery of human cell-derived polymeric cardiac patches to the epicardium in a porcine model [126]. Despite the keyhole access, this model still requires surgical facilities, specialized equipment, and expert personnel, limiting the feasibility of the procedure [130]. Table 2 summarize the pros and cons of each model.

Although large animal models are more physiologically relevant for studying disease phenotypes, they are costly and impose several ethical restrictions. Therefore, the bioengineering and tissue engineering communities have been implementing extensive efforts to develop *in vitro* and *in silico* CVD models for more physiologically and clinically relevant readouts of CVDs.

3.2. Cardiac tissue engineering approaches

3.2.1. Cell sources—Finding an appropriate cardiac cell source is the starting point of any attempt to engineer *in vitro* platforms. Generating high quality cells, that can retain their phenotype and resemble native heart tissue without losing their biological functions is critical [131]. The sources of suitable cardiac cells include primary cells, cell lines, and undifferentiated human pluripotent SCs [131].

3.2.1.1. Primary cells.: Primary cells are cells that are directly harvested from the tissue; these cells have a limited lifespan as they are not genetically or virally transformed, in contrast to transformed, immortalized cell lines. Primary cells retain relatively similar qualities to their *in vivo* phenotype [132]. For example, CMs isolated from embryonic chicken and neonatal rats/mice, among other animal species, have been mainly used as *in vitro* cardiac models [133]. The usage of human primary cells is restricted by some important limitations [134]. For example, primary cells derived from different donors can behave differently depending on age, sex, and genetics. Moreover, in addition to their low proliferation rate, limited expansion capacity, and finite lifespan, it is difficult to use primary cells more broadly in drug discovery, but the advent of commercially available, fully authenticated sources of primary cells is starting to address this issue [135]. There are also no abundant sources of viable primary human CMs, as they can only be isolated from small cardiac biopsies, which are only performed rarely in cardiac disease. At the fundamental level, primary human CMs have an extremely limited ability to proliferate, thus they cannot be expanded to appreciable numbers from cardiac biopsies.

3.2.1.2. Cell lines.: Most *in vitro* models use cell lines due to their availability and unlimited expansion capacity [136]. Common cell lines include fibroblast, SMC and EC lines. Their widespread, standardised use is one great advantage of the inclusion of authenticated cell lines in engineering of vasculature *in vitro* or in a model of cardiac tissue regeneration [132]. However, there are some issues associated with the use of cell lines *in vitro*. For instance, cell responses may be altered at different passage numbers. Similarly, cellular behavior can depend on culture medium and serum as well as the genetic manipulation required for immortalisation, impacting cellular phenotype, function and behavior. In addition, cell lines are not similar to primary cells (i.e. *in vivo* cells) in several

important aspects [136]. For example, many cell lines are derived from tumors affecting their functional properties [136].

Primary CMs naturally cannot proliferate; however, there are a few cell lines such as AC16 [137] and HL-1 [138] which can. AC16 is an immortalized human ventricular cell line, developed by fusing primary ventricular CMs with a simian virus 40 transformed fibroblast cell line. It was reported that these cells can proliferate, express cardiac-specific markers and differentiate under specific culture conditions [137]. The HL-1 cardiac cell line, derived from atrial sarcoma, exhibits a well-organized sarcomere structure and the ability to maintain contractile function after passaging, similar gene expression to the adult CMs, spontaneous depolarization and good ion channel expression [138,139].

Progenitor cells are relatively immature and partially specialized cells that are precursors to entirely differentiated cells of the same tissue type. Some researchers believe that there is no significant difference between progenitors and SCs. They are typically formed by SCs and are able to differentiate to one or more types of cells, but are not capable of generating all cell types [140,141]. Various types of cardiac progenitor cells (such as c-Kit$^+$, SSEA-1$^+$, Scar-1$^+$ and cardiosphere cells) have been used for *in vitro* and *in vivo* experiments and revealed some potential for therapeutic purposes [142–145]. Although these cells are relatively easy to identify in rodent models, it is considerably more difficult to demonstrate their relevance in primates and humans.

3.2.1.3. Stem cells.: Limitations of primary cells and cell lines have stimulated scientists to focus on SCs for modelling of tissues and organs. SCs are defined as undifferentiated cells with the ability of self-renewal and differentiation into various types of specialized cells [135]. This facilitates their applications for replacing lost tissues and curing devastating diseases. Controlling SC fate in order to generate differentiated cells of a desired lineage is still a challenge. Their proliferation and differentiation depends on a variety of factors, such as GFs, amino acids, proteins, active ions, and co-culturing with relevant cells or tissue types [146].

CMs can reliably be derived from human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) using protocols that involve timed application of GFs or small molecules, that are designed to recapitulate developmental pathways. The timed application of biomolecules is designed to drive the cells from the undifferentiated state, to mesoderm, to cardiovascular progenitors, and finally to CMs [147–151]. In general, these protocols can be applied to embryoid bodies or monolayer culture.

Since directed differentiation yields a heterogeneous cell population with purity of CMs between 50 and 90% on average, it is necessary to enrich CMs for some applications. This can be achieved by centrifugation [152], lactate switch [153] or cell sorting with an antibody against SIRPA [154]. iPSCs have also been demonstrated to differentiate into various cell types, overcoming the ethical issues associated with the use of human ESCs [155]. As they can be patient derived, iPSCs hold a great promise in providing patients with a significant number of cells, without immune rejection as well as providing improved models for disease modelling. This makes them suitable substrates for drug discovery [156]. Recent advances in

iPSC technology [157] have tackled limitations related to low reprogramming efficiency, lengthy differentiation processes, high cost, and variability among iPSC lines [158], enabling the use of these cells for *in vitro* disease platforms. For example, iPSCs derived from patients with Barth syndrome (BTHS) were successfully differentiated to CMs to develop an *in vitro* microfabricated disease platform to model cardiomyopathy in patients with BTHS [159].

3.2.2. Maturation of stem cell derived CMs—*In vitro* engineered cardiac tissues were introduced with the ultimate goal of resembling human heart morphology and function for various purposes including disease modelling, compound testing, and patient specific drug screening. During embryonic development of the heart, from early stages to adult phenotype, not only do CMs go through many structural changes, but they also get exposed to various factors such as mechanical, biochemical, electrical, topographical, cell-cell and cell-ECM signals [160].

iPSC-CMs have become the most appropriate cell source for fabricating functional cardiac tissues [161]. However, due to the lack of a global standard to define and evaluate CM and cardiac tissue maturity, scientists have been comparing engineered cardiac tissue characteristics with adult CMs in regard to different parameters such as morphology [162], structural properties, gene expression [163] and electrophysiology [162].

From the structural point of view, whereas rod-like adult CMs have an aspect ratio between 7:1 to 9.5:1 [164], heterogeneous hiPSC-CMs are much smaller with lower aspect ratios (2–3:1) [165,166]. SCs maintain their proliferation capacity, although this capacity decreases during differentiation [167], but mature CMs are known to have no proliferation capacity [168]. The average length for adult human- and hiPSCs-CMs' sarcomeres is about 2.0–2.2 μm and 1.6–1.7 μm, respectively [169].

Moreover, hiPSC-CMs generally exhibit no T-tubules, they largely have a single nucleus and microscopic analysis can detect mainly their Z-discs and I-bands. On the other hand, adult CMs have prominent T-tubules, they are multi-nuclear cells and transmission electron microscopy clearly shows their H-, A-bands, and M-lines in addition to Z-discs and I-bands [166]. In addition to their structural differences in comparison to the adult human CMs, iPSC-CMs have major gene expression and cell function diversity. Researchers utilized various methods to induce maturation in hiPSC-CMs to achieve morphology and function similar to the adult CMs.

One of the common approaches is adding different biochemical factors to the culture medium, such as triiodothyronine (T_3) [170], ascorbic acid [171] and neuregulin-1β [172]. Among these, T_3 is known to have an essential role in cardiac development. For example, hiPSC-CMs were incubated with T_3 for a week and exhibited an increase in CM sarcomere length, size and anisotropy ratio, besides increase in consumption rate of oxygen and contractile force [170].

Electrical stimulation is among the most important biophysical cues that induce CM growth and maturation [173–175]. In an excitation-contraction coupling mechanism, an electrical

stimulus is converted to contraction of CMs. In a recent study, early initiation of electrical stimulation (day 12 after differentiation) was compared to a late-stage initiation (day 28 after differentiation) in hiPSC-CMs based cardiac tissues [175]. The tissues were subjected to a constant stimulation frequency, or the intensity training by electrical stimulation of ramping frequency. In this study, early initiation (day 12 after differentiation) of intensity training exhibited a remarkable maturation efficiency of the tissue, resulting in sarcomeric structures indistinguishable from those of the adult human heart. hiPSC-CMs were reported to have a sarcomere length of 2.2 μm, 30% mitochondrial density, the presence of T-tubules and highly organized ultrastructure. While functional properties (such as resting membrane potential (−70.0 mV) and conduction velocity (25.0 cm/s)) showed considerable improvement, a less mature phenotype was observed compare to adult CMs [175].

Since CMs experience different mechanical stress such as cyclic stretch (from hemodynamic load), static stretch (cell-ECM interaction), and shear stress (laminar blood flow), scientists mimicked these mechanical cues *in vitro* to induce CM maturation [176]. Mihic et al. seeded hESC-CMs into gelatin sponge and utilized mechanical stretch to enhance cellular maturation [177]. The structure experienced uniaxial cyclic stretch for 3 days. This sample exhibited an increase in the size, number and elongation of cells compared to the unstretched sample. Moreover, the stretched sample showed a lower calcium cycle duration, higher rate of contraction and increase in the expression of ion channels and gap junctional proteins [177].

Tailoring surface topography is another strategy to induce CM alignment [178–192]. Myofiber orientation in the native heart, especially in the LV, shows highly aligned myocardial strands. It has been shown that CM alignment has a significant impact on electromechanical coupling and production of contractile force [193,194]. It was demonstrated that sarcomere alignment and organization affect APD, contractile stress, and kinetics of Ca^{2+} transients (e.g CTD) [195,196]. Various surface topographies such as microgrooves [187,197], nanopatterns [180,198] and microposts/pillars [179,185] have been investigated to guide CM alignment. For instance, our team reported the role of microgrooves/micropatterns and hydrogel stiffness on the alignment and elongation of cardiac cells [188].

Furthermore, some other effective methods have been reported to induce CM maturity, including long term culture of CMs *in vitro* [169], genetic manipulation [199], and modulation of microRNAs [200]. Recently, the combinations of mentioned strategies have been used to improve CM maturity [173,201].

3.3. In vitro models in CVD research

3.3.1. 2 D in vitro models—Pharmaceutical companies have been utilizing 2D cardiac *in vitro* models to assess functional properties and test cardiotoxicity in preclinical stages for decades. For example, electrophysiology and rhythm disorders are among the main parameters that have been measured in 2D models [202]. Fabrication of 2D tissues with aligned CMs have been extensively used in order to engineer native-like cardiac monolayers and to model various diseases such as conduction disorders [203,204]. Alignment of CMs is critical for maintenance of high conduction velocity throughout the native myocardium

[205]. Various techniques have been proposed to meet this requirment in the 2D environment, including ECM protein micro-contact printing [206], coverslip microabrasion [207], and soft substrate micro-molding [202].

Ion channels play an important role in rhythmic and effective cardiac contraction [208]. Many cardiac diseases, such as long QT syndrome (LQTS) [209], are due to ion channel dysfunction. Various high throughput assays including ligand binding assays, voltage-sensitive dye assays, flux-based assays, fluorescence-based assays, and automated electrophysiological assays (e.g. patch-clamping), have been conducted to investigate ion channel activity [210]. hiPSC-CMs from patients with specific inherited cardiac arrhythmias are used as a promising cell source to model these disorders [211–213]. LEOPARD syndrome [214], familial dilated cardiomyopathy [215], Timothy syndrome [216], familial hypertrophic cardiomyopathy [217], aldehyde dehydrogenase 2 genetic polymorphism [218] and long QT [211,212] are some examples of patient-specific iPSC-derived models. For instance, an *in vitro* model of congenital long QT syndrome was developed [212] by reprogramming dermal fibroblasts from a 28-year-old patient with type 2-long QT syndrome. Recorded data from patch-clamp and extracellular electrodes showed prolonged APD compared with healthy hiPSC-CMs, and more than a 60% decline in the cardiac potassium current (I_{Kr}) of long QT cells indicating appropriate phenotypic characteristic [212].

Another important tool used in investigating electrophysiological properties is the microelectrode array (MEA), which enables researchers to measure impulse propagation of CMs seeded on top of a substrate with micro-electrodes. For example, MEA recordings of cardiac ectopic activity detected cardiac arrhythmogenic activities in 38% of patient-specific samples compared to 6% of healthy ones [212].

Although using 2D models enables researchers to investigate cardiotoxicity at the cellular level, these models suffer from lack of suitable environmental factors including 3D ECM-cell and cell-cell interactions. To overcome these challenges and provide a more realistic microenvironment similar to the native tissue, 3D models have been developed and investigated in depth [219].

3.3.2. 3 D in vitro models—To mimic the physiological and anatomical structure of the native heart, researchers have used various techniques to build up more complex 3D microenvironments [220]. To this end, four main approaches were used to fabricate 3D scaffolds *in vitro* including encapsulating cells inside hydrogels [221,222], seeding cells into prefabricated structures [223], utilizing decellularized ECM of the native heart tissue [224], and overlaying 2D cell sheets on top of each other [225].

Among others, cell encapsulation inside a hydrogel is currently a main approach for creating engineered heart tissue (EHT) (Fig. 3) [226]. Development of the first EHTs goes back to 1997 when embryonic chick CMs were cultured in a collagen matrix, and resultant tissue exhibited a direct relationship between increase of applied electrical pacing frequency (from 0.8 to 2.0 Hz) and force generation [226]. Each EHT platform requires three components: 1) isolated heart cells (these might be from chicken, mouse, rat, hiPSC or hESC) [221,227]; 2)

a hydrogel that is able to form suitable ECM for cells after gelation [228]; and 3) a chamber to provide an aseptic environment for further culture of the cell-containing hydrogel (i.e. bioreactor) [229]. These EHTs offer a platform with a high-level of monitoring capacity and a suitable approach toward enhancing the maturity of hiPSC-CMs [229]. A modified version of the EHT platform has been placed in a 24-well plate, to facilitate the need for repeated measurements of different functions of the tissues. Using elastic silicon posts, this modified EHT has the capacity to apply mechanical load without requiring any extra stretching devices. Moreover, the cell density per EHT was 15% lower than the original EHTs [222,230–232]. Myriamed and ETH-Technologies have commercialized the EHT platforms.

3D models have several advantages compared to 2D models such as cell-cell, cell-ECM interactions, physiological cues, more complex microenvironments and higher similarity to native tissue. In addition to these general advantages, EHTs provide the opportunity to measure contractile function, Frank-Starling mechanism, and investigate the effects of mechanical and electrical stimulation [233,234]. Moreover, patient-specific and various disease models [235] can be designed using EHT platforms which allow one to assess their functions for a long period of time. For example, Eschenhagen and Zimmermann have developed EHTs in a two-post system that allows for easy tissue formation and contractile force evaluation [236,237].

These models have been adapted for different studies in the fields of cardiac and skeletal muscle tissue engineering [238–241]. Although EHTs show a great promise in cardiac research, one of the limitations includes the need for a large number of cells per EHT (0.4–2.5 million CMs) [227,232]. In a recent study, Shadrin et al. [242] combined hydrogel molding [243,244] and dynamic culture [245] techniques to develop a platform for culturing and maturing hiPSC-CMs to a clinically relevant size (from 7 mm × 7 mm–40 mm × 40 mm). Their 50 μm-thick platform was grown without any exogenous stimulation and exhibited mature functional properties including conduction velocity of 25.1 cm/s [242].

Motivated by a native cardiac niche, we developed Biowire [246], by seeding cardiac cells encapsulated in a hydrogel into a PDMS microwell, where the cardiac cell suspension self-organized around a suture template situated in the middle of the microwell. Since CMs were aligned toward the suture, the overall intercellular organization significantly improved compared to the age-matched embryoid bodies. Although the platform did not allow for contractile force measurement, the incorporation of electrical stimulation of increasing ramping frequency improved the maturation level of Biowire significantly in terms of both electrophysiological and calcium evaluations. Because most drug candidates target adult human tissues, Biowire, with a more matured phenotype and a closer recapitulation of the adult native cardiac tissue, is considered to have more predictive power for cardiotoxicity screening [247]. In addition, since this model lacks vasculature the tissue diameter was minimized on purpose to ~600 μm to allow for appropriate oxygen and nutrient diffusion [248]. Biowire is being commercialized by TARA Biosystems which enables the maturation of cardiac tissue *in vitro* by electrical stimulation [173,249]. Matured cardiac tissue is an essential factor for translation and commercialization. Table 3 summarizes the pros and cons of each 3D *in vitro* model.

3.3.3. Vasculature-on-a-chip—As already discussed, large 3D organs cannot be fully represented by a cell monolayer which lacks the sophisticated, highly organized structures that are integral to organ function. One of the main missing components is the vasculature, which is responsible for adequate nutrient and oxygen delivery to maintain cellular activity [254]. For example, after oral or intravenous injection, drugs circulate through the body in the bloodstream and enter tissue after penetrating the capillary endothelium. The interaction between drugs and endothelium may release paracrine signals, such as NO, which indirectly influences cellular behavior [255]. To tackle these challenges, the vasculature-on-a-chip platform may be an ideal tool to recapitulate *in vivo* drug-tissue interactions but in the *in vitro* setup. For example, drug candidates that can induce vascular injury, which typically manifest in preclinical animal studies through inflammation and changes in vascular tone [256], may be identified prior to animal testing with vasculature-on-a-chip platforms, reducing unnecessary expenses and casualties.

One of the challenges for vasculature-on-a-chip is to fabricate functional open lumen structures that can withstand continuous perfusion after cell seeding. ECs were exposed to molecules, such as VEGF [257] or thymosin β−4 [258] to create self-assembled or guided vasculature. However, these structures were not stable enough, making the direct perfusion challenging and necessitating the use of microfabrication to create a more stable vascular structure. Microfabrication enables the use of biomaterials to 1) support long-lasting open EC lumens for perfusion; 2) define sophisticated capillary networks to mimic the hierarchy of native vasculature; 3) guide and support EC growth and maintain their cellular functions [259–263]. Many fabrication methods have been recently explored. Zheng et al. [259] used the soft lithography technique to create a patterned collagen I hydrogel and obtain a fully endothelialized lumen network with physiologically relevant geometry. The network was simple but was capable of sprouting angiogenesis with GFs and adequate platelet responses after interaction with whole blood. Kolesky et al. [260,261,264] developed a 3D printing procedure that realized a more complex lumen network with multiple materials/cell mixtures. The printing method was able to give rise to a thick human tissue (> 1 cm) that integrates parenchymal, stromal cells and ECs. It was possible to cultivate the final construct up to 6 weeks. Miller et al. developed 3D printed rigid fiber networks of carbohydrate glass, which were then used as a sacrificial layer to create vascular networks in the hydrogels. These networks could be perfused with blood under high-pressure pulsatile flow [262]. In another study, Zhu et al. reported a rapid, mask-free digital light processing based bioprinting method to create pre-vascularized tissue structures directly with unique resolution and speed. This emerging technique could overcome challenges such as blocking and overflowing that occur during nozzle-based 3D printing and offer greater resolution, speed, scalability and flexibility [265]. More recently, our team has developed a multidimensional scaffold with a built-in vascular network using a 3D stamping technique (AngioChip). With long-term perfusion under gravity driven flow, the system was able to improve the viability of cells embedded in the parenchymal space. The system also enabled vascular sprouting through the micro-holes of the scaffold into the parenchymal spaces (Fig. 4 A-I). The cells in the parenchymal space demonstrated physiological responses to drugs applied through the vasculature, specifically engineered cardiac tissue perfused with epinephrine increased the spontaneous beating rate, and engineered metabolised terfenadine

to fexofenadine [266]. The successes of these studies demonstrated the physiological relevance of vasculature-on-a-chip and its potential in drug testing.

In addition, recent studies described the development of high throughput vasculature-on-a-chip systems, to facilitate drug testing on various organ types [266–271]. For example, Loskill et al. presented a Lego®-like plug & play system, μOrgano, which enabled both individual culture of single organ-on-a-chip platforms, as well as customization with integration of multi-organ systems [272]. Another vascularized and perfusable system was developed in a conventional 96-well plate format [273]. The platform facilitates perfusion using hydrostatic pressure and was theoretically capable of connecting multiple mini-organs (cardiac, liver, etc.) through the vasculature. Similarly, our team developed a multi-organ system, inVADE platform, with an integrated vasculature that enabled functional readouts, such as the contractile behavior of cardiac tissue (Fig. 4 J(1–6)) [274]. In the inVADE platform, cardiac tissues were formed from hiPSCs-derived CMs encapsulated in fibrin gel. Two cantilevers were also used to determine the frequency and force of tissue contraction in a non-invasive way. We also reported the impact of biochemical (a β-adrenergic agonist epinephrine) and electrical stimulation on cardiac tissue contraction. Immediate increase in tissue contraction frequency was observed after drug perfusion through the microvasculature. Electrical stimulation in this 96-well plate platform showed the gradual increase in tissue contraction under pacing which is known to improve cardiac tissue maturation [275].

These platforms are versatile tools to investigate specific drug effects on individual tissue models or to link multiple different tissue types together via integrated vasculature, and therefore potentially enable modelling of systemic exposure to either a drug and/or its metabolic by-products. Thus, micro-tissues corresponding to various organs would provide valuable information to aid pre-clinical and clinical drug screening. However, it remains a challenge to develop functional evaluations that allow systematic assessment of the risks and efficacy of drug candidates in such platforms.

Several vessel-on-a-chip platforms are currently being commercialized. For examples, Nortis developed a vessel-on-a-chip platform for drug testing [276]. The chip contains a tubular microchannel embedded inside a hydrogel. These channels are endothelialized and surrounded by pericytes. Robust endothelial sprouting and pericyte interaction have been reported [276]. Another example is an artery-on-a-chip device [277,278] by Quorum Technology which enables *ex vivo* probing of structural and functional properties of small diameter blood vessels. Organos Inc. has also commercialized μOrgano platform, which may be used to build a vascularized cardiac tissue [279].

3.3.4. Thrombosis on-a-chip—Fluid shear stresses and recirculation zones at stenosis or vessel bifurcations are critical contributors to atherogenesis [280–282]. The most frequently-used *in vitro* thrombosis models are cone-and-plate, parallel plate flow chamber viscometers and orbital shaker-based flow systems [283,284] (reviewed elsewhere in detail, for example [285,286]). These models can promote our understanding about the pathobiology and pathophysiology of thrombosis by inducing physiological shear stresses on cultured ECs or by recirculating blood flow to study the mechanism of shear stress on

coagulation. However, they are incapable of faithfully mimicking 3D micro-physiological structure and hemodynamics of the vascular, arterial and microvascular networks. Therefore, microfluidic devices (i.e. thrombosis-on-chip devices) are of interest to mimic a range of flows and shear stresses, starting from low (i.e. in veins) to high values (i.e. in arteries) [287–290]. Microfluidic perfusion models that are used to model early stage atherosclerosis and later thrombosis have a significant potential in drug development [291–294]. These devices possess endothelialized lumen that can reliably capture several features of blood vessels such as atherosclerosis (i.e. fatty plaque deposition and formation) and bifurcations (i.e. branches and bends) by changing the channel geometries and flow patterns, ranging from laminar steady flow to pulsatile flow [287–290,295–304]. For example, platelet adhesion and thrombosis have been studied in a microfluidic device fabricated by soft lithography which resembles vascular networks [305]. In this work, endothelialized perfusable microchannels were fabricated with collagen hydrogels to study whole blood interactions with ECs (Fig. 5 A). Significant reduction in platelet adhesion on the lumen of the collagen hydrogel microchannels was observed as compared to a device fabricated from phorbol12-myristate-13-acetate (Fig. 5 B-D). Platelet adhesion to the lumen wall is known to trigger the secretion of von Willebrand factor (vWF) and the subsequent significant increase in platelet recruitment.

In a recent study [296], a 3D-printed thrombosis-on-a-chip model has been developed by using GelMA hydrogel (Fig. 5 E-H). ECs were seeded on the channel walls and perfused with human whole blood to understand the cellular interactions and clinical relevance of this coagulation model. Thus, 3D printing could provide an alternative technology to soft lithography, which is often burdened by high costs, lengthy multistep procedures and difficulty in curvature fabrication. Although microfluidic and 3D-printed platforms can complement the use of *in vivo* models, they may not fully predict biological processes especially in a longer period of time due to the coagulation issue. Long-term studies will inevitably be needed to systematically evaluate thrombotic processes using whole blood. To address this issue, Qiu et al. developed an endothelialized, agarose-gelatin IPN hydrogel-based microvasculature-on-a-chip platform. This platform can be used for longterm real-time visualization, with high spatiotemporal resolution, of microvascular obstruction and endothelial permeability under physiological flow conditions [303].

Some of these chips are now being commercialized and implemented by pharmaceutical companies. For example, Johnson & Johnson is validating the thrombosis-on-chip device by Emulate for the studies of pulmonary thrombosis.

3.3.5. Cardiac fibrosis-on-a-chip—A majority of disease models of cardiac fibrosis are based on 2D coculture and thus lack the intricate microenvironmental properties of the fibrotic ECMs [306,307]. A 3D *in vitro* microenvironment provides a promising non-animal platform to elucidate pathways governing ECM remodelling, electrophysiological properties and contractile functions. A few tissue engineered fibrosis models have also been reported. van Spreeuwel et al. developed an *in vitro* cardiac fibrosis model by systematically manipulating the number of fibroblasts and collagen concentration in the engineered cardiac tissues, to mimic fibrotic myocardial composition [308]. Increased fibroblast number was shown to significantly reduce contractile force and alter beating frequency [308]. In another

study, Spencer et al. generated a reconstituted tissue model of fibrotic cardiomyopathy by modulating CM and myofibroblast volume fractions (Fig. 6 A) [309]. The results demonstrated a significant role of myofibroblast population in reducing impulse conduction velocity in the fibrotic cardiac tissue (Fig. 6 B) [309]. Sadeghi et al. reported a 3D *in vitro* cardiac fibrosis model in a GelMA hydrogel-based platform (Fig. 6 C) [310]. By tuning mechanical properties of GelMA hydrogel and applying subsequent biochemical stimulation by transforming growth factor-β1 (TGF-β1), fibrotic-like tissues with activated myofibroblasts were created. The diseased tissues presented asynchronous beating behavior and increased mechanical stiffness. Figtree et al. also developed vascularized cardiac spheroids as a 3D *in vitro* model to investigate the mechanisms involved in cardiac fibrosis, by coculturing CMs, ECs, and CFs isolated from dissociated neonatal rat hearts in hanging drop cultures. A significant rise in collagen deposition following TGFβ1 treatment in spheroids was observed. In addition, cell death and disturbed vascular networks in spheroids were observed after addition of a cardiotoxic and profibrotic agent (i.e. Doxorubicin). These findings demonstrate that cardiac spheroids can be used to elucidate the underlying pathways as well as therapeutics for preventing and treating cardiac fibrosis *in vitro* [311].

These studies highlighted the opportunity to use engineered cardiac disease models in the studies of fibrosis pathogenesis and molecular pathways underlying cardiac remodelling. However, the aforementioned models were engineered with either rat or mouse CMs. A highfidelity human cardiac tissue model using CMs derived from hiPSCs would be an asset in our progress toward generating human-relevant cardiac disease models enabling personalized medicine and preclinical drug screening.

Moreover, advances in RNA-Sequencing (RNA-Seq) analysis and mass spectrometry-based proteomics have facilitated the examination of gene expression and global protein profile to better understand the transcriptional and translational regulation underlying human cardiac disease. There are several human signaling pathway databases distributing comprehensive datasets from systematic collections of compiled experimental results [67]. They constitute a helpful resource that can guide targeted investigations of future pathway studies. Therefore, combining these technologies with 3D *in vitro* platforms will provide valuable insights into the pathological processes responsible for cardiac fibrosis.

3.4. In silico approaches

Recent developments in statistical/empirical and mechanistic cardiac modelling have opened new avenues improving drug-induced cardiotoxicity screening. *In silico* approaches have been implemented by both regulatory agencies as well as pharmaceutical companies. The paradigm change in cardiotoxicity assessment started with the mechanistic modelling of electrophysiology of cardiac cells (i.e. a modification to Hodgkin–Huxley formulation) [312]. These studies could delineate the contribution of specific ion channels and transporters to normal and disrupted heart rhythm [313–316]. *In silico* modelling also provides a robust tool for demonstrating drug-ion channel interactions and their effects on heart function. For example, one of the potential adverse effects of a drug is the proarrhythmic effect due to the interaction between drug compounds and cardiac membrane ion channels [317]. Several theoretical models have been developed to address these

phenomena. Receptor theory is one of the well-established and important frameworks for computational modelling of pharmacological actions on cardiac ion channels [318]. A receptor is modelled using the known laws of physical chemistry to delineate binding of molecules to cellular receptors [319]. In addition, other modelling frameworks, such as the Markov chain model, were developed for simulating interactions between drugs and Na^{+} and Ca^{+2} channels [320,321]. These mathematical models have been used to explain the effect of β-adrenoceptor on ventricular arrhythmogenesis in association with long QT syndrome [322,323].

Current preclinical screening assays typically involve *in vitro* and *in vivo* models to detect structural, contractile and electrophysiological toxicity, which could lead to myocyte impairment and loss, heart failure and proarrhythmia, respectively. These simple and non-specific assays for the early screening of cardiac toxicity can lead to unjustified attrition of new drug entities due to false-positive findings. This risk can also be augmented by the differences among the effects of compounds in animals and humans. Therefore, more-comprehensive multi-parametric approaches, which combine *in vitro* and *in silico* technologies have been implemented to detect such cardiotoxicities. More recently, this concept has evolved through the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative [324,325]. CiPA provides a mechanistic evaluation of drug effects on ventricular myocyte electrophysiology based on *in silico* reconstructions of human ventricular electrical activity and multiple cardiac ion currents (i.e. delayed repolarization, repolarization instability and early after-depolarizations) to predict the proarrhythmic risk. In the subsequent steps of testing, the results are then validated with hiPSC-CMs platforms [326]. The combination of the human ion channels, cardiac currents, *in silico* reconstructions of human cellular proarrhythmia and hiPSC-CM models to assess contractile and structural cardiotoxicity is expected to lead to significantly diminished drug attrition rates and to the reduced number of animals in pre-clinical drug discovery.

Furthermore, a new quantitative systems pharmacology (QSP) discipline has recently emerged, that uses mathematical computational models to describe biological systems, disease progression and drug pharmacology in a single modelling framework [327–329]. QSP combines elements of translational drug pharmacokinetics (PK), pharmacodynamics (PD) and systems biology to address clinical needs, such as representation of patient variability for dose selection, demonstration of long-term disease progression to find optimal treatment, and facilitation of modelling accessibility for clinicians and pharmaceutical companies in order to understand the diseases [330]. The main goal of the QSP modelling approach is the identification of drug targets in a biological system. QSP is based on dynamic computational modelling that integrates experimental evidences and data from multiple studies. This approach has been used in several cases related to CVDs [330,331]. QSP modelling can also be used to investigate cardiotoxic events. In addition, QSP computational modelling attempts to define the mechanisms underlying the drug-induced arrhythmic events, in order to find the drug's therapeutic window where the occurrence of such events may be avoided [332]. For example, an *in silico* platform was developed by combining a PK model with a cardiac model to simulate AP at data points along the PK profile [321,333]. *In silico* reconstruction of known cardiac biology can be integrated with the observed pre-clinical and clinical data to predict CVD progression in individuals or

populations. In this regard, the physiologically based pharmacokinetic model (PBPK) modelling approaches can also be implemented to predict the concentration time courses in the tissue of interest (e.g. myocardium) and PK of drug-drug interactions. For example, PBPK was integrated with the response model of CM ion channels [334,335] in order to predict the drug-drug interaction of domperidone and ketoconazole on QT prolongation.

Another area that QSP modelling can have significant impact is the development of tyrosine kinase inhibitors (TKIs). TKIs are cancer therapeutics that often exhibit cardiotoxic effects, including LV malfunction and HF. It would be ideal to establish a mechanistic approach first, to assess and evaluate new TKIs efficacy, especially on signaling networks in CMs, as well as to identify new mechanisms of action to minimize accompanying cardiotoxicity. These can be addressed by the QSP approach, where large-scale measurements are combined with mechanism-based mathematical modelling [336].

In addition, data driven quantitative modelling has also been used in the drug screening process. For example, Conant et al. [337] have developed an artificial neural network model to analyze the experimental results in an efficient and unbiased manner to select kinase inhibitors with maximum efficacy and minimum side effects on cardiac cell viability and function. This model helps identify cardiotoxic effects for some available kinase inhibitors that were not detected during clinical trials.

Overall, these examples demonstrate the potential use of mechanistic and data driven modelling in dose selection, studies of drug-drug interaction, and identification of the most efficient therapies [338].

4. Roadmaps for CVD drug screening

CVDs are among the most prevalent diseases worldwide accounting for approximately 30% of deaths annually [12]. Common strategies for prevention and treatment of such complications have been the administration of drugs in early stages, and interventional procedures in later stages of the diseases [339]. Therefore, there is a growing need for new therapeutic agents with improved efficacy and safety. Pharmaceutical companies invest on average 2 billion dollars for each new drug candidate, taking over 12 years to develop an approved drug and place it on the market [10,340,341]. Despite high demand and ongoing R&D efforts, the number of new regulatory-approved therapeutic compounds has been declining over the past decade [10,341]. New drugs must pass strict regulatory standards before entering the market, which are upheld by national and international agencies (e.g. FDA, Health Canada, EMA, etc) [342,343]. However, despite the exhaustive, timely and costly efforts in the early stages of drug development, many of those therapeutic drug candidates cannot enter the market due to safety (e.g. cardiotoxicity or hepatoxicity) or efficacy concerns in the late stages of the drug development pipeline. The attrition rates in Phase II and Phase III development stages have been reported to be as high as 80% [75,344]. In addition, some drugs have also been withdrawn after approval due to their cardiotoxicity [345]. Over 60% of expenditures in the drug development process occur during early stages of development [346]. This fact emphasizes the importance of investing in reliable, economical, safe preclinical assays to screen for effective drug candidates in early stages, in

order to reduce the tremendous development costs as well as replace and optimize the less efficient steps of the drug development process [347].

Lack of the appropriate human disease models has been reported as a key limitation in early stage drug discovery [348]. Even though animal models have conventionally been used to elucidate the disease response, there are vast limitations in terms of predictability and reliability of these models [115]. Although significant efforts have been made in terms of developing alternative animal models to shed light on the mechanisms of human diseases and to advance novel and more effective therapies and diagnostics [349], there are still unmet needs in order to overcome the challenges of moving drugs from the bench to bedside. Current animal models, including genetically modified ones for CVDs, are incapable of fully recapitulating human physiology and pathophysiology, and thus imprecisely predict the biology and mechanisms involved in human cardiac dysfunctions [84]. Therefore, pharmaceutical companies use alternative systems such as the relatively simple 2D monolayer cell cultures of cell lines, and, more recently, CMs derived from iPSCs [350] which are unable to accurately mimic aspects of the 3D microenvironment of tissues and organs of interest [351], where the main functions and physiological features are more complex. Therefore, questions have been raised about their clinical predictability and accuracy [350].

3D tissue models, such as *in vitro* tissue-engineered disease models have shown promise for addressing the current obstacles faced with 2D *in vitro* cultured cells and animal models [352–356]. Engineered 3D *in vitro* disease models that use cells from patients differentiated to the specific cell fate under standard culture conditions, provide a deeper understanding of the breadth and complexity of human disease mechanisms, and can be used for testing the efficacy and safety of emerging therapeutic drugs during preclinical testing [156,357].

During the past decade, some important advances have been reported for mimicking features of native tissue physicochemical microenvironments *in vitro*. These more relevant physiological structures can promote cell phenotype, metabolic activity, maturation, and functionality, leading to more reliable and more predictive models [202].

Recent advances in tissue engineering and microfabrication technologies have contributed to the emergence of organ-on-a-chip models for emulating human systems biology [358]. These novel techniques could be a valid support for the development of 3D biomimetic systems displaying the intricate structural and functional characteristics of human organs [359]. These platforms contain more complex and sophisticated topographical and biomechanical cues for human tissue modelling than a thin layer of cells [360]. However, engineering of disease models still remains a challenge [361].

In parallel, *in silico* modelling and computational medicine are emerging fields focused on developing quantitative approaches to help make decisions, reduce drug discovery costs and enhance the chances of success in therapy [365,366]. *In silico* modelling is a low-cost, ethical and practical way to quickly investigate multiple hypotheses [367]. One of the well-established approaches in computational medicine is mechanistic modelling or knowledge-based method, which translated biological events to mathematical expressions [368–370].

For example, QSP is a powerful tool for developing a dynamic mechanistic model capturing the interactions between drugs and disease networks [371]. These *in silico* drug-disease models have received significant attention in the attempts to find optimal treatment strategies for CVD patients with multiple risk factors [332].

The *in vitro* and *in silico* disease models have so far been used to complement the use of laboratory animal models, however it is foreseen that the combination of these two fast-growing and emerging platforms with less costly, more ethical and accurate outcomes, would be a promising substitute, which can reduce animal model consumption and acquire high-quality results in the future. Fig. 7 represents different steps in the drug development pipeline that are required to receive a regulatory approval.

5. Future perspective

Engineered *in vitro* disease models have already made incredible progress in academic and pharmaceutical R&D settings, thanks to the high-level engineering approaches which enable one to replicate organ-like complexity and functionality *in vitro* [202,372–374]. The development of a multi-functional platform that combines knowledge about pathophysiology and etiology of CVDs, ever-growing engineering technologies (i.e. micro/nanofabrication) and stem cell biology, brings hope to the mandate of reducing the use of experimental animals in preclinical research and improving translation and drug discovery. For example, the ultimate goal would be to develop a multi-organ-on-chip platform which integrates different functional organs to assess efficacy, toxicity, and PK at the same time in a controlled manner [375,376]. Despite these advantages, these platforms currently face some limitations; for example, real-time high-resolution imaging (due to the low transparency and high thickness of the chips) and biomarker detection in the chips are difficult. Current studies are focused on solving these issues by integrating on-chip analytical techniques (e.g. HPLC and mass spectrometer, electrophoresis, etc.) [377–379] and *in situ* imaging tools to improve the suitability of organ-on-a-chip platforms in high-throughput screening and to generate high quality, reliable, results [376,380,381]. However, further improvements and optimization are needed.

Another challenge in these platforms is the incorporation of different cell types (i.e. co-cultured cells) in a single tissue (i.e. heterocellular tissues) to better resemble native tissue environments. This could be a costly and difficult process due to the complexity of the environment and the requirement for tissue stability in a longer period of time [382,383]. In addition, emerging additive manufacturing technology (e.g. 3D printing, electrospinning, biotextiles, etc.) could tackle the issues regarding the timely, costly, multi-step and labor-intensive microfabrication process [384–393]. However, these technologies are still in their infancy and need further improvement and optimization.

As we reported in this review, the majority of cardiac disease research has so far been conducted in animal models, with primary cell cultures, or cell lines. However, animal models are not entirely capable of representing human disease states, and the phenotypes of isolated primary cells often change drastically during culture [115]. On the other hand, human-derived cells are often difficult to obtain [394]. Accessing limited cell populations

such as CMs from living patients is particularly impractical. The advent of iPSC technology represents a landmark breakthrough in regenerative medicine, as it enabled acquisition of hiPSCs without ethical concerns and their targeted differentiation into any cell type [395,396]. For example, various cell types that make up the heart can be produced via directed differentiation. The derivation of CMs can be achieved by controlling the expression of Wnt and BMP at specific times from the mesoderm stage [397,398]. Although few protocols reported differentiation of different subtypes of CMs including the ventricular [399,400], atrial [401,402], and pacemaker cells [172,398,403], they still need standardization and harmonization as they result in heterogeneous cell populations. This will enable more precise modelling of specific diseases in the ventricles, or the atria and will open new insights for differentiating patient-specific bioengineered pacemakers. Moreover, hiPSCs could provide an unlimited source of cells for the construction of diseased tissue. Differentiating iPSCs into disease-relevant mature cells and constructing diseased tissues using these cells combined with pathological cues have important implications for understanding disease mechanisms [404]. On the other hand, the maturation of hiPSCs-derived cells is vital to being able to fully mimic the adult phenotype in humans. This process can be expedited by applying chemical, physical and electro-mechanical cues to the differentiating cells. More studies are needed to better understand paracrine signaling, the possible influence of non-CMs (e.g. fibroblasts), and scaffold stiffness [405,406] on CM differentiation. Although hiPSCs could provide an infinite source of cells, the differentiated cell population (i.e. ventricular-, atrial-, and pacemaker-like cells) is not homogeneous [152]. This issue could be solved by cell sorting techniques such as microfluidic cell sorting [407–409].

It is known that patient-specific genotype can significantly affect disease onset and progression, as well as influence drug responses [211,214,410]. Further, hiPSCs can be generated from patients harboring genetic diseases, thereby providing target cells that will possess relevant pathological genetic backgrounds [411].

Patient-on-chip platforms including engineered *in vitro* tissue models associated with patient derived cells (i.e. individual- and disease-specific hiPSCs) have the potential to revolutionize the drug discovery paradigm, and bring us closer towards the reality of personalized medicine [160,359,412]. Towards this aim, there are efforts to systematically generate iPSCs from hundreds of phenotypically healthy donors, plus several cohorts of donors with inherited genetic diseases, using a standardised experimental pipeline. For example, the International Stem Cell Banking Initiative (ISCBI) and other counterparts in Europe (human induced pluripotent stem cell initiative (HIPSCI)) and around the world have coordinated efforts to regulate the derivation, culture, and characterization methods of hiPSCs [413–418].

Moreover, genome editing technology (e.g. the clustered regularly interspaced short palindromic repeats (CRISPR) technology) promises to be the most important translational perspective in the future [419–421]. Accurate genome editing of mammalian cells using CRISPR technology could change the landscape of personalized medicine. Combining tissue engineering and organ-on-a-chip technology with genome editing/cellular reprogramming to

generate new human cell-based models for CVDs can promote accuracy and predictability of these platforms.

Furthermore, there is an unmet need for high throughput toxicity screening for drug testing in which many compounds can be tested with minimized costs and time. There are many drugs that have received U.S. FDA approval, such as TKI-related drugs for cancer, that were developed based on high-throughput screening technologies. However, development of a suitable platform for high-throughput drug screening with reliable, repeatable results and sufficient physiological function to the native cardiac system is challenging, due to the technical limitations as well as the resultant tissue maturity. To assess *in vitro* cardiotoxicity of a new drug, the FDA has requested companies to investigate inhibition of the cardiac hERG gene that encodes a potassium ion channel of cardiac cells. Inhibition of the hERG channel can be an indicator of the APD prolongation and cardiotoxicity in early stages of drug development [11]. In addition, using patient-specific hiPSC-CMs, researchers are able to model various CVDs such as long QT syndrome, LV non-compaction, hypertrophic cardiomyopathy and dilated cardiomyopathy [217,423,424].

In addition to the recent breakthroughs with engineered *in vitro* models, *in silico* models (i.e. theoretical and computational modelling such as QSP modelling and *in silico* modelling of electrophysiology) have enabled us to capture dynamic systems at variable scales, simulating the impact of drugs and therapeutic strategies used in clinical settings, and testing the validity of current biological understanding and clinical outcomes [325,425]. These computational models are far less costly solutions for making predictions about drug PK, PD and patient population responses [426]. They also provide novel insights into underlying biology which augment our knowledge of diseases [329]. For example, the CiPA initiative has revolutionized the regulatory decision-making paradigm by using a mechanistic assessment to eliminate the risk of a rare and life-threatening arrhythmia which is induced by a newly discovered drug. Thus, the implementation of these new technologies in early stages of drug development (e.g. preclinical and clinical-phase I stages) could help prevent later-stage drug attritions [330]. Similarly, bringing together academic and industrial scientists with regulatory agencies, to make decisions about standardization, regulation and validation of the current platforms to ensure accuracy, specificity, and reproducibility could prevent late-stage drug failures. Furthermore, the synergy between *in vitro* and *in silico* CVD models based on individual genomics, environmental factors and lifestyle choices would lead to more reliable *in vivo* predictions, which would benefit CVD patients who would have access to safer and more effective drugs. The new drug discovery paradigm can also reform the current approach of utilizing animal models in the preclinical stage.

6. Conclusion

In this review, we have reported several types of CVDs that lead to cardiac complications and reviewed current understanding of their underlying pathophysiological pathways. We have also summarized conventional disease models such as experimental animal models and evaluated their relevance towards modelling human disease, as well as translational success and limitations. We also discussed current human-specific tools including *in vitro* and *in*

silico approaches to model human cardiac tissue and vasculature in both healthy and pathological states, along with the their advantages and limitations.

Emergence of human-based engineered cardiovascular platforms has revolutionized preclinical cardiac regulatory assessments. This paradigm change has emerged from our ever-growing knowledge of the basic molecular and cellular pathways underlying CVDs, the advent of hiPSC-CMs, the availability of high-throughput screening platforms and *in silico* models which take into account complex physiological functions. These combined human-based *in vitro* and *in silico* approaches will complement preclinical *in vivo* assessments and can fine-tune decision making steps in drug discovery and development pipelines.

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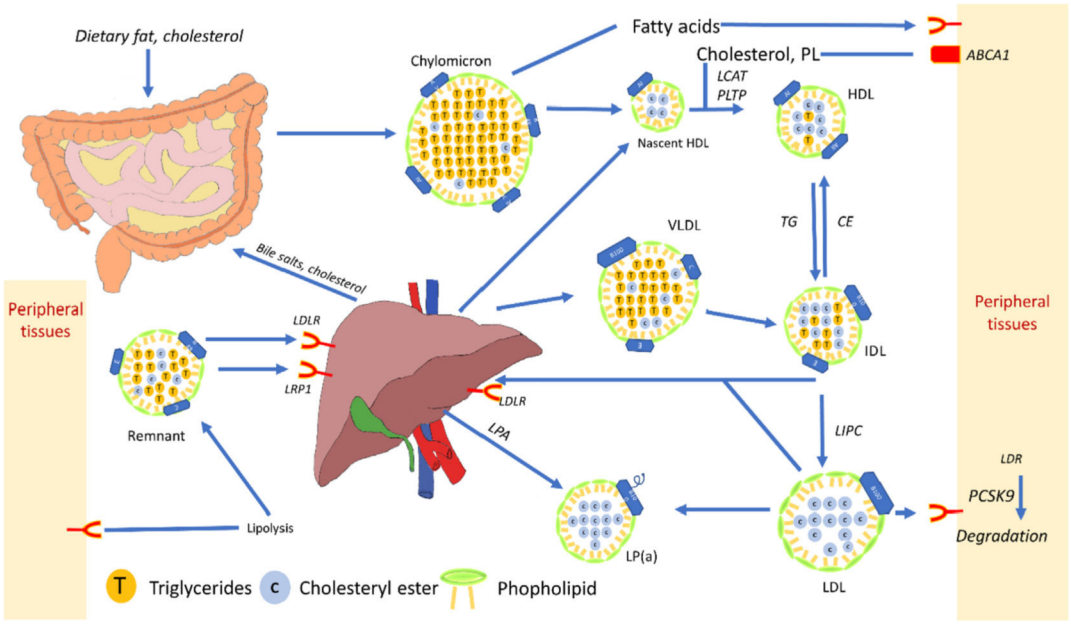

Fig. 1.

Schematic of lipoprotein metabolism and conversion in the body. Dietary lipid and cholesterol, digested from food with the help of cholesterol and bile acids in the intestines, pass through enterocytes and enter into circulation as chylomicrons. The molecules go through a series of lipolysis steps in the peripheral tissue and interact with nascent HDL on their way to the liver. They loose TG and get more cholesterol as they become denser and form remnants. Remnants enter the liver by interacting with LDL receptor-related protein 1 and LDL receptor, being modified to VLDL. VLDL communicates with HDL in circulation by Cholesterylester transfer protein (CETP) enzymatic activity and through lipolysis becomes IDL. Hepatic acid lipase (LIPC) converts IDL to LDL. IDL and LDL can go to the liver or peripheral tissues for further metabolism. In the liver and in circulation, LDL can be modified to form Lp(a). HDL acts as an acceptor of TG in circulation and from peripheral tissues, carrying TG to the liver for further metabolism and excretion. The concept of the figure is adapted from Ref. [39].

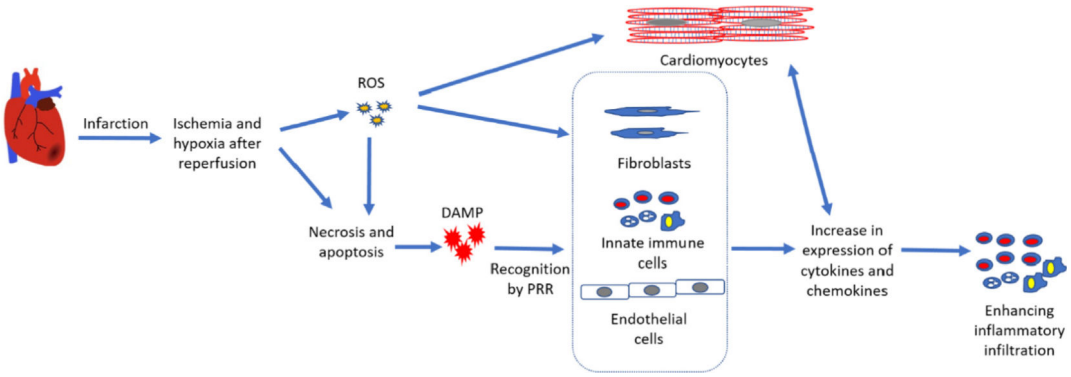

Fig. 2.

Schematic of the consequences of myocardial infarction in the heart at a cellular level. Infarction restricts blood supply to the surrounding cells, leading to apoptosis and necrosis. After clog removal or reperfusion, surrounding live cells, which are at hypoxic states, start to generate ROS. ROS can trigger another phase of apoptosis and necrosis, as well as inflammation. Apoptotic and necrotic bodies release DAMPS. DAMPS, similar to ROS, are recognized by cells inside the heart (ECs, CMs, fibroblasts and immune cells) through a series of receptors (PRRs) and TLRs, triggering the expression of inflammatory cytokines and adhesion molecules. Enhanced inflammatory state is sensed by the surrounding cells, including CMs, thus leading to further expression of inflammatory molecules.

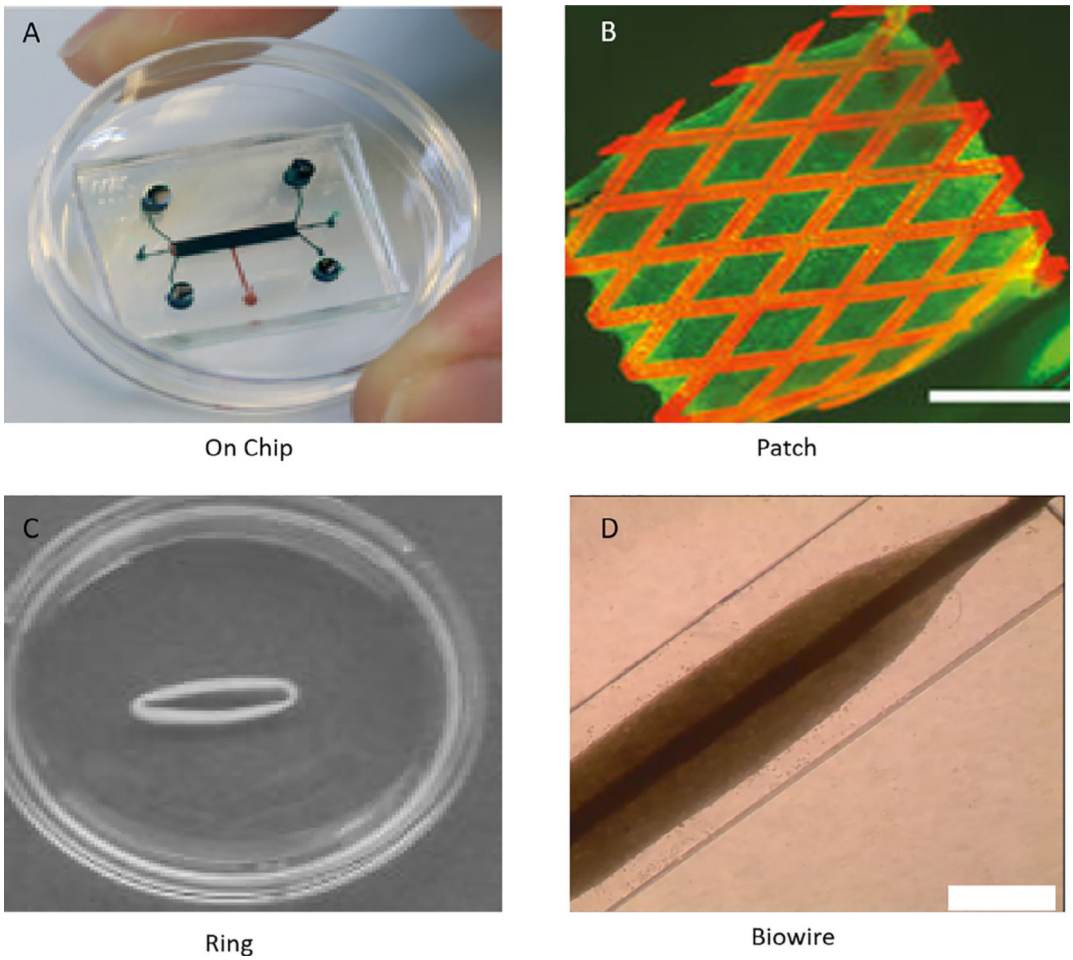

Fig. 3.

Different approaches to fabricate engineered heart/cardiac tissue: A) Organ on chip [250], B) Cardiac patch; scale bar: 2.5 mm [126], C) Circular EHT to apply mechanical stimulation [251], D) Rod-shaped Biowire to apply electrical stimulation; scale bar: 0.5 mm [252]. (Reprinted with permission from Ref. [126,250–252]).

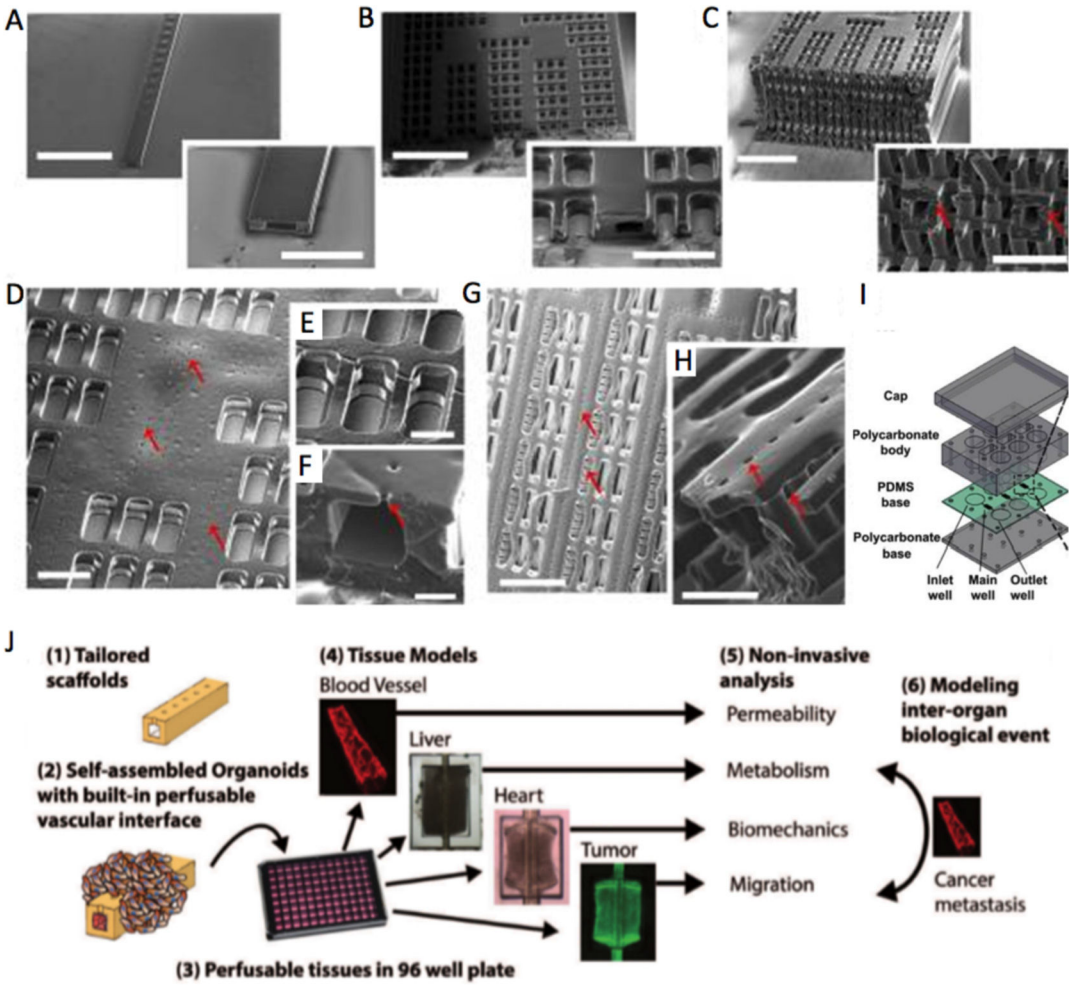

Fig. 4.

Vasculature-on-a-chip (A–C), SEM of lumen networks for A) a 1D tube (scale bar = 1.5 mm and 500 μm), B) a 2D AngioChip scaffold (scale bar = 1 mm and 300 μm) and C) a multi-layer 3D AngioChip scaffold with 20 μm micro-holes (scale bar = 1 mm and 400 μm) created with the 3D stamping technique. SEM of parenchymal spaces of D) an AngioChip scaffold with 10 μm micro-holes on the channel walls (Scale bar = 200 μm); E) the 3D lattice matrix in between the microchannels (scale bar = 100 μm); F) the cross-section of a 10 μm micro-hole on the channel wall (scale bar: 50 μm). Red arrows point to the micro-holes. (G–H) SEM of the parenchymal space of an AngioChip scaffolds with 20 μm micro-holes on the top and side walls of the micro-channels. Red arrows point to the micro-holes on the top and side walls. Scale bar: G) 400μm, and H) 100 μm. I), Schematic of the assembly of the bioreactor showing inlet, main and outlet wells. (Reprinted with permission from Ref. [266]). J) schematic diagram that summarizes the key aspects of the InVADE platform (Reprinted with permission from Ref. [274]).

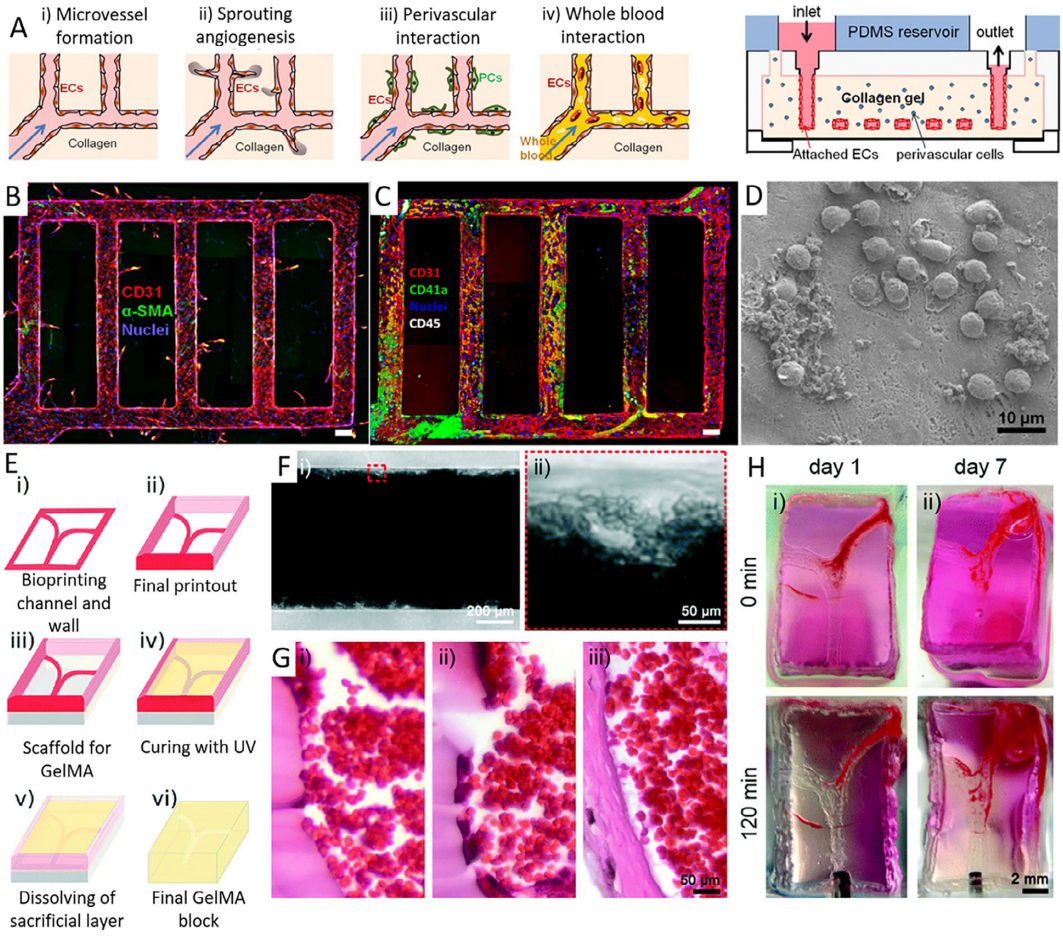

Fig. 5.

Thrombosis on-a-chip. A) Endothelialized perfusable microchannels fabricated with collagen hydrogel to study whole blood interactions with ECs, B) Z-stack confocal image of endothelialized microfluidic vessels showing endothelial sprouting from the walls, Red, CD31; blue, nuclei. (Scale bar: 100 μm), C) Leukocytes and platelet adhesion on stimulated microfluidic vessels after perfusion with of whole blood for 1 h. Red, CD31; green, CD41a; white, CD45; and blue, nuclei. (Scale bar: 100 μm), D) SEM of leukocyte adhesion on and migration through stimulated microfluidic vessels after 1 h of whole blood perfusion (Scale bar: 10 μm) (Reprinted with permission from Ref. [305]), E) Schematic of the bioprinting process: i, ii) bioprinting of a Pluronic mold; iii) assembly of the dried mold on PDMS; iv) filling the mold with GelMA followed by UV crosslinking; v) washing off the sacrificial channels to create vi) the final construct with hollow channels, F) Optical image representing the formed thrombus in a microchannel, where aggregated RBCs were clearly observed (Scale bar: 50 μm), (G) Optical image showing H&E-stained transverse sections of (i) a thrombus in control channel and (ii) a thrombus in endothelialized channel with HUVECs, both after 7 days, and (iii) a thrombus formed *in vivo* at 7 days (Scale bar: 50 μm), (H) Time-lapse photographs showing the thrombolysis of (i) a 1 day clot and (ii) a 7 day clot (Scale bar: 2 mm) (Reprinted with permission from Ref. [296]).

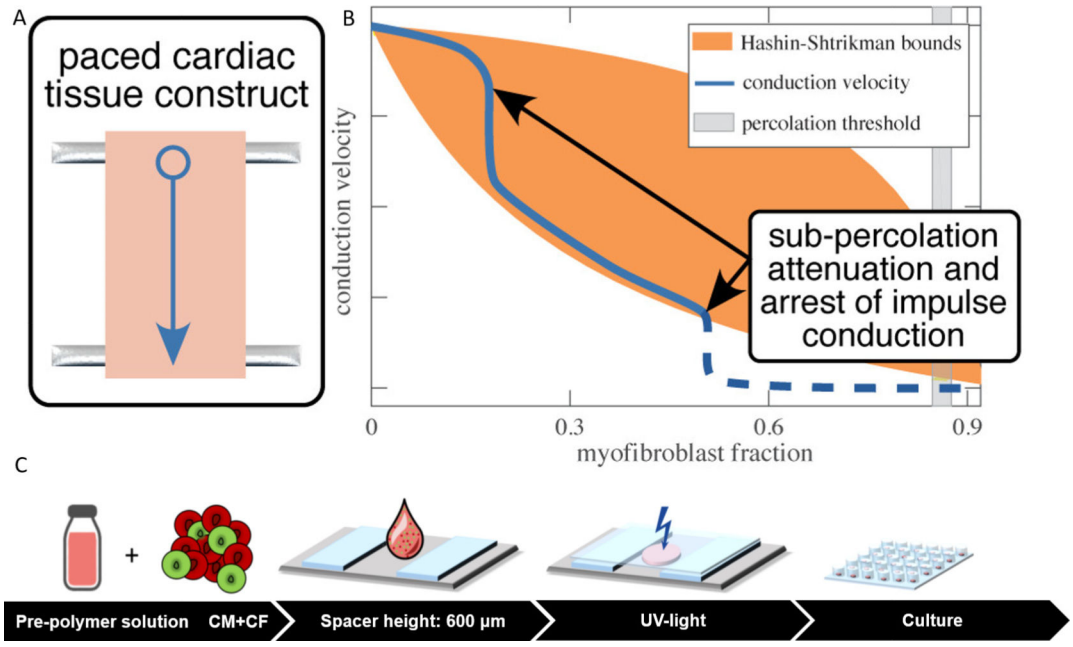

Fig. 6.

Engineered disease models of cardiac fibrosis. A) An engineered cardiac tissue model of fibrotic myocardium based on modulation of CM and myofibroblast volume fractions. The fibrotic EHTs were constructed by encapsulating chicken embryonic cardiomyocytes and myofibroblasts in Type I rat tail collagen. The fibrotic tissue models were generated by replacing cardiomyocytes with myofibroblasts. B) The effects of cellular composition on impulse conduction in this fibrotic model. The CM and myofibroblast volume fractions determined the impulse propagation velocity [309]. C) A simplified 3D hydrogel platform to study cardiac fibrosis. Primary neonatal rat CMs and CFs were encapsulated within a GelMA-based pre-polymer solution to generate *in vitro* EHTs. Cell-laden hydrogel was placed into a customized UV-chamber and subsequently crosslinked by UV light. The fibrotic EHTs were treated by TGF-β1 [310]. (Reprinted with permission from Ref. [309,310]).

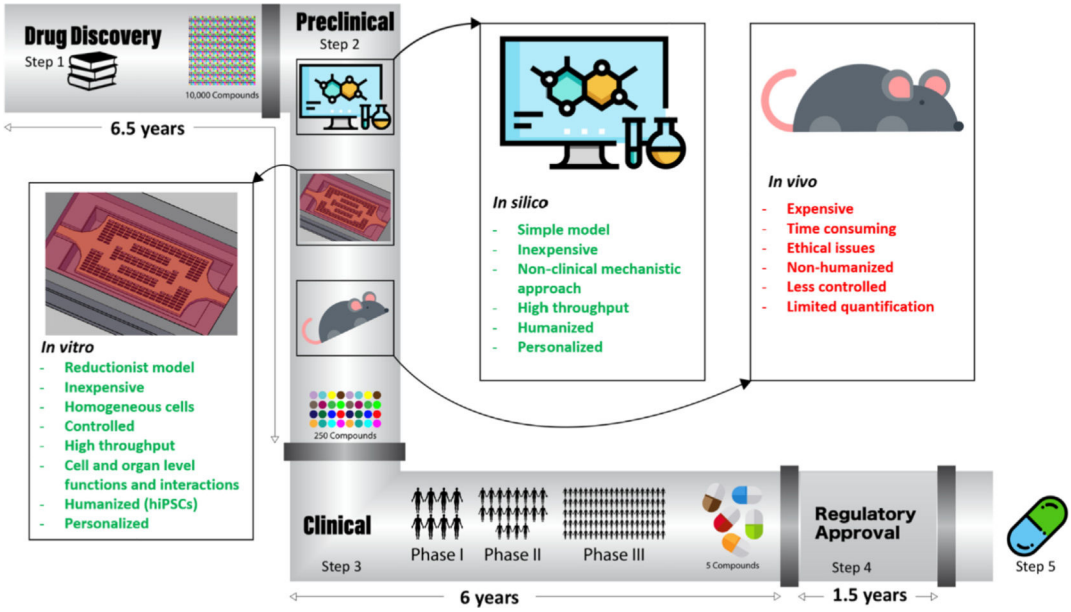

Fig. 7.

Drug development pipeline. *Step 1: Discovery:* Search for a drug candidate starts in the R&D setting; *Step 2: Preclinical Research*: Drugs undergo *in vitro* and *in vivo* testing to address basic concerns about safety; *Step 3: Clinical Research:* Drugs are evaluated on people in terms of their safety and efficacy; *Step 4: FDA Review:* Regulatory teams meticulously evaluate all master files related to a new drug or device and decide on their approval; *Step 5: FDA Post-Market Safety Monitoring:* FDA monitors all device and drug safety once products are marketed.

Table 1

Summary of CVDs and their causes, symptoms and associated complications.

Disease	Cause(s)	Consequence	Prevalence (%)	Annual mortality rate(%)
Atherosclerosis	Cholesterol and lipid deposition in subendothelial space and sustained inflammatory reactions	Arterial blockage, arterial stiffness, calcification, thrombosis and myocardial infarction	30[68]	5–6% (per 100 000 population)[68]
Myocardial infarction	Arterial blockage by atherosclerotic plaques or by thrombotic plaques	Dead myocardial tissue, fibrosis, decrease in ejection fraction and systemic circulation, arrhythmia	5% (age: 40–59)15% (age: 60–79)30% (age > 80)[69]	34%–42% [69]
Arrhythmia	Improper function of cardiac ion channels or cell junctions, physical obstacles (e.g. infarcted tissue) disturbing electrical wave propagation	Atrial and ventricular fibrillation leading to improper blood pumping in heart	15–25% (relative to age)[70]	15[70]
Cardiomyopathy	Genetic predisposition and as yet unknown causes	Various types with ranges of symptoms. CMs can become enlarged, thick or rigid decreasing pumping function	1:200–500(0.2–0.5%)[71]	6–8[71]

Table 2

Pros and cons of animal models of CVD.

	Small Animal Models (Rodents)	Large Animal Models (Swine)
Pros	✓ Easy breading and handling	✓ Closer to human anatomy, better tissue availability and more accurate minimally invasive measurements
	✓ Short reproductive cycle	✓ Closer lipoprotein profile to human except for human HDL subclasses
	✓ Relatively cheap	✓ Moderately atherosclerosis sensitive on normal diet
	✓ Well-defined genome	✓ Similar vascular lesion structure and lesion distribution to humans
	✓ Ease of genetic manipulation	✓ Rare thrombosis due to plaque rupture
	✓ Large litter number	✓Suitable for translational research
Cons	× Resistance to atherosclerosis development in Wild type (need for transgenic model)	× Costly and difficult maintenance and handling
	× Different gross anatomy compared to humans	× No genetic modifications
	× Different lipoprotein profile to humans/high level of lipid	× Limited genetic models available
	× Compromised lesion formation	× Rare thrombosis due to plaque rupture
	× Absence of plaque rupture and thrombosis	× Ethical concerns

Table 3

Summary of current in vitro cardiac models.

Shape	Cell source	Scaffold	Culture time	Cell number	Stimulation method	Pros	Cons	Ref.
tab	Embryonic chick ventricular CMs	Collagen	6–11d	1M	None	-Some structural and functional maturation -Genetic modification in cardiac tissue	-Instability in culture	[253]
Ring	Neonatal rat-CMs	Collagen	14d	1–10M	Cyclic mechanical stretch	- Some structural and functional maturation	-High number of cells -Specialized set-up is needed for mechanical stimulation	[222]
Cylinder	hESC-CMs, hiPSC-CMs	Collagen	1wk	0.5M	Biphasic electrical stimulation	-Lower cell number -Structural and functional maturation	- Inability to measure contraction force	[173]
2D culture	hESC-CMs,hiPSC-CMs	Matrigel coated plate	80–120d	0.175M	None	-Universal culture technique -High level of maturation	- Long culture time -2D shape -Inability to measure contraction force	[169]
Patch	hiPSC-CMs	Matrigel/Fibrinogen	3wks	0.5–1.75 and 8.5 M depending on patch size	None	-Therapeutic approach in animal model	-Relatively thin structure	[242]
Ring	hESC-CMs	Gelatin sponge	5d	6M	Cyclical stretch	-Therapeutic approach in animal model -Biodegradable scaffold	- Relatively limited maturation	[177]
Cylinder	hiPSC-CMs	Fibrin	5–6wks	2M	Biphasic electrical stimulation	-Highly structured mature tissue	-Shape not ideal for animal transplantation	[201]