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Experimental models of cardiac physiology and pathology

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Abstract

Experimental models of cardiac disease play a key role in understanding the pathophysiology of the disease and developing new therapies. The features of the experimental models should reflect the clinical phenotype, which can have a wide spectrum of underlying mechanisms. We review characteristics of commonly used experimental models of cardiac physiology and pathophysiology in all translational steps including *in vitro,* small animal, and large animal models. Understanding their characteristics and relevance to clinical disease is the key for successful translation to effective therapies.

Keywords

primary cardiomyocytes; neonatal cardiomyocytes; ischemic; non-ischemic; pressure overload; volume overload

1. Introduction

Heart diseases represented by coronary artery disease and heart failure are increasing worldwide(1). Researchers devote intense efforts to prevent and cure heart diseases in both clinical and experimental research areas. Experimental research has played and will continue to play key roles in discovering disease mechanisms and developing new therapies. In this review, we provide characteristics of commonly used experimental models of cardiac physiology and pathophysiology by covering all translational steps from in vitro to large animal models (Table 1)

2. *In Vitro* models

First, we discuss *in vitro* models of cardiac disease. Although various types of cells compose the heart, cardiomyocytes account for a majority of heart mass and predominantly affect cardiac function(2,3). Since malfunction of cardiomyocytes is a causative mechanism in most heart diseases(4), a large amount of effort was spent to develop multiple *in vitro* cardiomyocyte models. The *in vitro* model system offers more precise control of experimental conditions and manipulations, which provide many advantages over the *in vivo* models in signaling pathway studies or high-throughput drug screenings (Table 2). For more

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than three decades, several cell culture tools, including primary cells, immortalized cell lines, human embryonic- and induced pluripotent stem cell (ESC and iPSC, respectively) derived cardiomyocytes have been developed to study heart disease in various settings. Herein, we discuss different types of cells used to model heart disease and their essential characteristics.

2-1. Neonatal cardiomyocytes

Rodent neonatal cardiomyocytes are the most widely used *in vitro* models in cardiac research. Since the neonatal cardiomyocytes are isolated from 1~5-days old rats/mice, the cells are yet immature in morphology and transcriptional profiles(5–7). Despite this limitation, neonatal cardiomyocytes have been the workhorse of cardiac research, because of their relatively easy isolation, gene expression manipulation capabilities, and reliable physiologic microenvironment(8–10). Harary and Farley(11) first developed techniques for isolation of neonatal cardiomyocytes more than 50 years ago. Fundamental procedures to isolate and culture the cells have been optimized by many researchers over the years. Thanks to these efforts, commercially available kits now offer easy isolation of neonatal cardiomyocytes for researchers without extensive experiences. Rats are commonly used for their advantages over other species, including low cost, higher yields of viable cells and efficient transfection rates for gene manipulation(8–10). Meanwhile, because of a large number of transgenic lines, mice are also employed to study functional roles of specific genes and proteins(12).

Neonatal rat cardiomyocyte (NRCM) model offers great advantages for studying cardiac hypertrophy. In 1982, Simpson et al.(13) demonstrated induction of cardiac hypertrophy in NRCM culture by norepinephrine treatment. This study opened the door for using this model as a platform for studying hypertrophy *in vitro*. Since then, numerous compounds and growth factors have been tested in NRCM culture and number of molecules have been identified to induce pathological hypertrophy, including phenylephrine(14–16), angiotensin II(17,18), endothelin-1(17,19), and the diacylglycerol mimetic phorbol 12-myristate 12-acetate(20). In response to these stimulations, NRCMs increase both volume and cell surface area, promote sarcomeric organization and reactivate fetal gene expression(13,16,20). Therefore, assessment of these profiles is central to evaluate cardiac hypertrophy in the NRCM model. Recent advances in fluorescence-based staining methods and image-analyzing software enable accurate measurements of cell surface area(17). Staining with phalloidin-Rhodamine, cardiac troponin I or α-actinin is a widely-accepted method to assess sarcomeric organization(21,22). Reactivations of a fetal gene program, such as atrial natriuretic factor, brain natriuretic peptide, α-skeletal muscle actin, and β-myosin heavy chain can be evaluated by qRT-PCR(14).

Sustained increase in mechanical cardiac load is an important pathological factor that promotes cardiac diseases. The sustained stretch model using NRCMs enables *in vitro* replication of increased mechanical load in isolated cells. Komuro et al.(23,24) developed deformable culture dishes using silicone membrane to simulate the stretch-induced stress in 1990. Currently, several apparatuses have been developed to provide computer-controlled sustained or cyclic stretch. Sustained stretch induces dynamic changes in gene expression in

time and stretch-dependent manner. These changes subsequently induce apoptosis, autophagy and hypertrophy in NRCMs(25–29). Fluorescence-based staining to detect hypertrophic, apoptotic or autophagic markers and evaluation of gene expression profiles related to these signaling is usually employed to determine stretch-induced pathology(25–29).

The NRCM model is also a useful tool to study ischemia/reperfusion (I/R) injury. In 1977, Acosta and Puckett first reported effects of hypoxic environment on *in vitro* cell culture(30). Nowadays different levels of hypoxic environments can be generated by culturing cells in hypoxic chambers with different concentrations of gas mixtures, including anoxic condition (e.g. 5 % CO_2 and 95 % N_2)(31), and also by the treatment with oxygen-scavenging compounds (e.g., $Na_2S_2O_4$) (32). Accumulation of reactive oxygen species (ROS), lipid peroxidation, increased membrane permeability and apoptosis represent the impacts of I/R injury(33–36). Staining of ROS by chemical indicator (e.g., MitoSOX)(31), measurement of SOD2 and catalase enzymatic activities(31), flow cytometry-based apoptosis detection(37,38) and western blotting with apoptotic markers (e.g., caspase 3, caspase 7, Bcl-2, Bcl-xl)(35) are used for evaluating impact of I/R injury.

Advantages of neonatal cardiomyocytes over adult cardiomyocytes include relatively ease of culture and high transfection efficiency with liposomal transfection methods. Nevertheless, they have some limitations. Most notably, neonatal cardiomyocytes lack a definitive t-tubular subsystem(5). Some components of signaling pathways differ between neonatal and adult myocytes(39), implying less accurate reflection of Ca^{2+} dynamics and signaling compared to adult cardiomyocytes. Non-uniform cell shortening is another limitation for modeling of contractile measurements in these cells. Hence, neonatal cardiomyocytes are most powerful in quickly and easily determining the effects of the gene manipulation and screening lead compounds through high-throughput drug screening assays(40,41). Ideally, the results obtained from neonatal cardiomyocytes should be validated with similarly designed experiments using adult cardiomyocytes.

2-2. Adult cardiomyocytes

Adult cardiomyocytes best recapitulate the morphology and behavior of the cells in intact human heart. These cells are rod-shaped, binucleated, and have well-organized sarcomeres throughout the cell body. In 1895, Oscar Langendorff first developed a protocol to isolate adult cardiomyocytes by the retrograde perfusion of the heart with an enzymatic solution and current researchers follow protocols that are similar to the original in principle(42). The yield and quality of the isolated cardiomyocytes are affected considerably by the heart cannulation and perfusion. Despite the technical difficulty, however, their above and below mentioned advantages make adult cardiomyocytes as one of the most frequently-used models for *in vitro* research.

The key advantage of adult cardiomyocytes for *in vitro* studies may be the wide-applicability of the isolation protocol to various types of animals, in contrast to the neonatal cardiomyocytes. The animals of all ages can be used to study the effects of aging(43). Adult cardiomyocytes isolated from male versus females allow probing the effects of sex difference on cardiac function, which is not possible with neonatal cardiomyocytes or

immortalized cell lines(44). Furthermore, animals with diverse disease conditions can be utilized for this model. For example, the heart after surgical disease induction or cardio-toxic chemicals can be used to isolate cells, and studied for their structural and functional properties at cellular levels(45–48). A large number of genetic models also make this model attractive. Indeed, isolated cardiomyocytes from transgenic mice were utilized to characterize the roles of genetic mutations found in familial dilated cardiomyopathy patients(49–51).

Another advantage of adult cardiomyocyte is a wide-spectrum of applicable experimental assays. Since adult cardiomyocytes have a mature sarcomeric structure and ion channels, different methods including patch-clamp(52), contractility measurements(53), and Ca^{2+} imaging studies(54,55) can be applied. These methods provide in depth analysis of contractility, sarcoplasmic reticulum Ca^{2+} load, diastolic Ca^{2+} levels and myofilament Ca^{2+} sensitivity. For example, Kerr et al.(56) showed disruption of Ca^{2+} signaling using several methods such as measurements of contractility, Ca^{2+} transient, stretch-induced ROS production, stretch-induced Ca^{2+} influx and Ca^{2+} sparks in isolated cardiomyocytes from a mouse model of Duchenne muscular dystrophy (MDX mice). Through this study, the authors elucidated how post-translational modification of α-tubulin affects Ca^{2+} signaling that underlies Duchenne muscular dystrophy pathologies.

Adult cardiomyocytes are also a useful model to study cardiac hypertrophy. Hypertrophy is induced in adult cardiomyocytes in response to many different triggers, including norepinephrine(57), phenylephrine(58), angiotensin II(59) and isoproterenol(60). Similar to neonatal cardiomyocytes, these stimulations activate fetal gene program and increase the abundance of myosin heavy chain protein, the rate of protein synthesis and total protein levels. Neonatal cardiomyocytes show dramatic changes in cell size (~150%) and sarcomeric organization within 48h of treatment of the triggers(13). However, this is not always the case in adult cardiomyocytes, and the shape of cardiomyocytes is closely related to the contractile function and cell condition. Therefore, instead of the image-based morphometric analysis, qRTPCR and western blotting of fetal genes are widely-accepted methods to detect hypertrophic features in adult cardiomyocytes. [^{3}H] phenylalanine incorporation and colorimetric protein quantitation (e.g., Bradford assay, bicinchoninic acid assay) are also employed to verify the changes in the rate of protein synthesis and total protein levels by hypertrophic stimulations(58,61).

Similar to the neonatal cardiomyocytes, adult cardiomyocytes are also able to be utilized for mimicking mechanical overload or I/R injury by the sustained stretch or hypoxic environments. These stresses turn on the similar signaling pathways with neonatal cardiomyocytes including hypertrophic, apoptotic and autophagic responses, but also induce sarcoplasmic reticulum Ca^{2+} leak and cardiac dysfunction(62,63). Overall, adult cardiomyocytes offer very reliable and efficient models in studying cardiac pathologies.

2-3. Immortalized cell lines

As discussed above, primary cells (adult cardiomyocytes, neonatal cardiomyocytes) are the most accepted *in vitro* models for studying cardiac diseases. However, these cells have some practical problems that make them challenging to use. Since the majority of cardiomyocytes

differentiate terminally *in vivo* in the perinatal period, they cannot be passaged in culture. These cells are fragile and difficult to maintain in culture for long periods. Especially, cultures of primary cardiomyocytes maintained for several weeks undergo morphological and functional changes over time and yield a heterogeneous population of cells(64,65). The difficulties to transfect and recover from frozen stocks are also considerable issues in these cells. In addition, animal sacrifice is necessary for the use of primary cells, which leads to practical and ethical limitations. To address these issues, many attempts have been made to establish immortalized cardiac cell lines. Various groups have developed suitable cell culture systems including H9C2(66), ANT-T-antigen(67), AT-1cells(68), MC29(69), HL-1(70), and AC16(71). Since immortalized cell lines retain similar gene expression profiles and phenotypic features of the cardiomyocytes, these cell lines have commonly served as an alternative to primary cells.

2-3a. H9C2—The H9c2 cells, myoblast cell line, were first isolated from embryonic BDIX rat ventricular tissue in 1976(66). At this stage, cells were not sufficiently differentiated into adult cardiomyocytes yet and lacked a clear cardiac phenotype. However, Menard et al.(72) established a protocol to differentiate them from mono-nucleated myoblasts to cardiac-like myotubes by the addition of all-trans retinoic acid (RA) to a 1% serum media. According to previous total genome microarray study, transcriptome alterations during H9c2 cell differentiation promote the cells to express cardiac-specific proteins, including cardiac troponins, calsequestrin, ryanodine receptor and sarco (endo) plasmic reticulum calcium ATPase(73). Therefore, H9c2 cells need to undergo the differentiation procedure for proper use of this cell. H9c2 cells lack the ability to contract but shows many similarities to primary cardiomyocytes, and thus have been used for investigating the molecular and cellular processes involved in cardiac hypertrophy(74), apoptosis(75), I/R injury(76), and toxicology(77). Notably, in the parallel comparison with NRCMs, H9c2 cells showed a more robust increase in cell surface area and similar levels of fetal gene activation to NRCM(74). Moreover, in another comparison study, H9c2 cells showed more similarity to primary cardiomyocytes than HL-1 cells with regard to energy metabolism patterns, such as cellular ATP levels, bioenergetics, metabolism, function and morphology of mitochondria(76). Based on these data, H9c2 cells are widely accepted as a valid *in vitro* system to study cardiac disease.

2-3b. HL-1—The HL-1 cell line originated from AT-1 cardiac myocytes. AT-1 cells are immortalized atrial cardiomyocytes derived from an atrial tumor growing transgenic mice with the simian virus 40 (SV40) large T antigen expression(68). Although AT-1 cells maintained a cardiomyocyte phenotype and ability to contract, they could not be serially passaged and revived from cryopreserved stocks. In 1998, Claycomb et al.(70) established the HL-1cell line to improve the limitations of AT-1 cells. The author reported that HL-1 cells maintain cardiac morphological, biochemical, and electrophysiological properties and solves the problems in AT-1 cells. HL-1 cells express numerous signaling receptors expressed in adult cardiomyocytes, and respond similarly to their agonists in pharmacological studies, which suggest that HL-1 cells exhibit an adult cardiomyocyte-like gene expression profile(70,78,79). Indeed, in the proteomic analysis of HL-1 cells in comparison with patient cardiac samples, HL-1 cells exerted similar alterations in the

proteome to the biopsy samples taken from patients with ischemic cardiomyopathy, including cell death pathways and oxidative stress response(80). Another important feature of HL-1 cells is the ability to spontaneously contract. HL-1 cells contain highly organized sarcomeres and express the necessary ion channels required for generating action potentials characteristic of primary cardiomyocytes(70,81). Sartiani et al.(82) reported that the electrophysiological properties of HL-1 cells are sufficiently similar to those of primary cardiomyocytes for testing pharmacological drugs acting on ion currents. Meanwhile, HL-1 cells need to be maintained in medium containing adenosine, retinoic acid, and norepinephrine to maintain a differentiated phenotype and stimulate beating(70). These requirements may limit their usefulness in developing models of cardiac disease. Moreover, atrial cell-like gene profiles somewhat limit their utility for studying ventricular failure(83). Nevertheless, HL-1 cells have been used in various *in vitro* settings(76,84–86), and are an efficient alternative for primary cells.

2-3c. AC16—AC16 cells are the cell line that is established most recently. Since this cell line is derived from human ventricular tissues by fusion with SV40 transformed human fibroblasts, it is expected to recapitulate human cardiomyocytes more similarly than other cell types. Dividson et al.(71) reported that AC16 cells express cardiomyocyte-specific markers, such as transcription factors, contractile proteins, muscle-specific intermediate filament protein and the cardiomyocyte-specific peptide hormones. Moreover, these cells appear to express functional gap junctions and myofibrils, which indicate high similarity to adult cardiomyocytes. AC16 cells have been tested in many *in vitro* disease studies, including cardiac hypertrophy, I/R injury, oxidative stress and toxicology studies(87–91). Although many studies support the usefulness of AC16 cells as an *in vitro* model system for cardiac research, no study has compared AC16 cells to primary cells yet. Thus, one should use with caution when interpreting data obtained from AC16 cells.

2-4. ESC and iPSC derived cardiomyocytes

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the capacity to self-renew by dividing and pluripotency to differentiate to all cell types. While ESCs are derived from different sources of blastocysts that are already pluripotent(92–94), iPSCs are generated from somatic cells by the introduction of defined transcription factors, such as OCT4, SOX1, KLF4, and c-MYC(95). ESC or iPSC-derived cardiomyocytes are the cells that are induced to differentiate to cardiomyocytes from these pluripotent stem cells. The research using these stem cells has grown dramatically in recent years to develop an efficient *in vitro* assay using human cells for personalized medicine. For example, stem cell-derived cardiomyocytes enable prediction of cardiotoxicity in respective patient to increase the safety of novel drugs. Peters et al.(96) introduced a novel assay to predict embryotoxicity by the automatic record of contraction of ESC-derived cardiomyocytes. Recently, this assay system was applied for screening embryotoxicity of commonly-used compounds in food and cosmetic production(97). Patients with certain genetic backgrounds exhibit increased cardiotoxic sensitivity to treatment with doxorubicin, and this susceptibility was recapitulated in iPSC-derived cardiomyocyte cultures(98,99). However, these stem cell-based models are not well suited for cardiac physiology applications at the moment. Compared to other model systems, the cultures of ESCs or iPSCs are technically challenging

and cost ineffective. The efficiency of proliferation and differentiation remains low and long-term culture as well as several supplements to induce differentiation makes this system technically difficult and expensive. Moreover, although the spontaneously beating ESC or iPSC-derived cardiomyocytes expressed early cardiomyocyte lineage markers, they remained phenotypically immature and were more similar to neonatal cardiomyocytes than adult cardiomyocytes(100–102). In addition, stem cell-derived cardiomyocytes are not able to be maintained as a cell line and hence they are dependent on new isolations, which cause batch-to-batch-variations. For these reasons, the stem cell-based models have not yet replaced animal-based models in cardiac physiology research. Nevertheless, their ability to study patient unique genetic disorder on dish is a significant advantage. ESC and iPSC can be derived from patients suffering specific genetic diseases, and differentiated into cardiomyocytes that retain those disease-specific traits. Unlike other models, genetic manipulations are not required and even the causative mutation does not have to be known for generating a model. For example, a number of genetic mutations behind hypertrophic cardiomyopathy, the most common inheritable heart disease, have been identified using these models. Carvajal-Vergara et al.(103) generated iPSC from patients with LEOPARD syndrome (Lentigines, Electrocardiographic abnormalities, Ocular hypertelorism, Pulmonary valve stenosis, Abnormal genitalia, Retardation of growth and Deafness), a kind of HCM, that have a mutation at Thr468 in the PTPN11 gene. The cells retained the phenotype in patients with LEOPARD syndrome, such as increase in cell size, sarcomeric organization and preferential localization of NFATc4 in the nucleus. Using these cells, the authors discovered RAS-MAPK signal transduction as a novel signaling pathways that promote the disease phenotype. Besides, the stem cell-based model has been applied to study various genetic disorders, including familial dilated cardiomyopathy(104), Duchenne muscular dystrophy(105), Friedreich's ataxia(106) and catecholaminergic polymorphic ventricular tachycardia(107). In summary, stem cell-based models are promising, but some improvements are yet necessary in the proliferation, differentiation, and maturation of the cells. Current use mostly focuses on personalized screening of the drugs and studies on specific gene mutations.

Supplementation of functional cardiomyocytes for dysfunctional hearts by trans-differentiated ESC or iPSC is an attractive therapeutic option. As of 2018, 29 clinical trials involving hESC-derived stem cell products and three trials that involve hiPSC derivatives have been approved(108). Nevertheless, there seem to be several hurdles for these approaches to become a daily clinical practice. Common to both ESC and iPSC are potential risks of teratoma originating from residual pluripotent cells, the occurrence of arrhythmias and alloimmunization. Moreover, the efficiency of cardiomyogenic differentiation remains a challenge for both cell types. The key advantage of iPSC is the potential autologous application, whereas ESC lines are better standardized at the moment. Understanding the detailed characteristics as well as fine-tuning the trans-differentiation of ESC and iPSC cardiomyocytes is of paramount importance for therapeutic application.

2-5. Engineered cardiac tissues

While individual cell models provide important insights in cardiomyocytes pathophysiology, they do not account for cell-to-cell interactions, particularly those through direct contacts. In

vitro tissue culture models incorporate multi-cellular component and three-dimensional structure of the heart muscle in the model system, allowing assessment of more complex interactions between the cells in cardiac tissue. Engineered cardiac tissues are produced by culturing cardiomyocytes together with other cell types. These cultured engineered tissues are attached to microsensors that measure forces generated by the tissue. Effects of drugs or biological substances on cardiac tissue contraction can be evaluated using this system(109,110), and tissue-on-chip tools are developed to enable high-throughput drug screening using engineered cardiac tissues(111). Efforts to create engineered tissues with pumping function are also being made(112,113).

3. *Ex Vivo* models

Assessment of the heart function as a pump system cannot be examined in *in vitro* system at the moment. Explanted heart allows detailed characterizations of cardiac pump function and electrophysiology in a well-controlled manner. Langendorff perfusion is the most common method employed and uses retrograde perfusion of oxygenate physiological buffer(114). Hearts from various species have been studied using this approach including that from humans(115). Pressure and volume of the heart chambers are measured by inserting a sensor inside the cavity and the impact of ischemia and/or drug effects can be evaluated. Detailed electrophysiological properties can be studied by applying electrodes and also by imaging the fluorescent probes that are sensitive to ion fluxes(116). The advantage of this method is that it avoids interference from the autonomic nerve system and inter-organ communications, which allows examination of pure cardiac response against various interventions. Cross-circulation method is another ex-vivo model system(117). By circulating the heart through the blood circulation from another animal, it avoids potential interference from the artificial perfusate, while more closely mimicking the pulsatile perfusion in *in vivo* setting.

4. *In Vivo* models

In contrast to the controlled and cardiac specific *in vitro* and *ex vivo* model systems, *in vivo* models offer studies in more complex biological system. *In vivo* heart function is regulated by multiple inputs including autonomic nervous system, secreted hormones, and immune systems. Thus, to understand the effects of pathological stimuli, genes, and drugs on the heart in a whole biological system, *in vivo* models are essential. Cardiac diseases have been modeled in drosophila(118), zebra fish(119), and xenopus(120) with a focus on gene regulation and development, but these models are beyond the scope of this review. We will review commonly used small and large animal models of cardiac disease in below.

4-1. Rodent models of cardiac diseases

Rodent models play central roles in the experimental cardiac research in the laboratories. They have 4-chamber cardiac anatomy similar to humans with high similarity in genomic sequences(121), and are relatively easy to handle, require smaller space and less costly compared to more advanced species. Mouse models have become the most popular owing to widely available genetically modified lines and established techniques to manipulate expression of genes. Rats are also commonly used in the laboratories and offer better

surgical manipulations and imaging capabilities compared to mice. Genetically modified rat models are also increasingly used(122). Three major approaches to induce cardiac disease in rodents are surgical, pharmacological, and gene manipulation. Here, we summarize these models in below sections.

4-1a. Surgically induced models—Myocardial ischemia induced by surgical ligation or ischemic reperfusion of the coronary artery represents one of the most common surgical methods to model cardiac disease in rodents. Left anterior descending artery is usually targeted and a very thin suture is placed around the coronary artery. The coronary artery is ligated for inducing transmural infarction, whereas it is temporarily ligated and later released for ischemic reperfusion injury(123). Generally, 30 minutes of ischemia is sufficient to induce infarction, but the longer duration of ischemia and longer reperfusion time is associated with larger acute myocardial infarction(124). Successful induction of myocardial ischemia will result in tissue necrosis and apoptosis acutely, and leads to scar formation at the chronic stage. Degree of systolic dysfunction depends on size of the myocardial area perfused by the occluded coronary artery and ligation time until reperfusion(125). Challenges remain in inducing reproducible infarct size and cardiac dysfunction due to the inter-animal differences in coronary anatomy and difficulties in visualizing coronary arteries in some of the animals(126). Another commonly used surgically induced model is the aortic constriction. Aortic constriction increases cardiac afterload and the heart initially develops hypertrophy. Systolic function is usually maintained at this stage(127). However, after this adaptive hypertrophy phase, the heart gradually dilates and systolic function decreases(128). Degree of stenosis and the location of the constriction are the key factors that determine the speed of this process. Ascending aortic constriction generally results in more severe and rapid progression of heart failure, whereas it is slower with transverse aortic constriction. Commonly, 26 to 27 gauge needles are used to set the degree of stenosis for mouse transaortic constriction, while more variety of needle sizes are used for rat aortic constrictions. Type of sutures used can also affect the model phenotype as the sutures that swell (e.g. silk sutures) can expand and tighten the stenosis after surgery. Systolic dysfunction may not always develop in rat aortic constriction model and only part of the animals with severe ascending aortic constriction show decreased ejection fraction(129). Even using same tools and techniques, the speed of disease progression is sometimes operator dependent. Thus, prior characterization of operator-dependent disease progression is one of the key elements for designing the experimental study using this model. For inducing volume-overload heart failure, needle disruption of aortic(130) or mitral valve(131), and creation of aorto-venous fistula(132) are employed and result in dilation of the cardiac chambers with different cardiac remodeling pattern compared to pressure overload models as described later in the large animal model section(133).

4-1b. Pharmacologically induced models—Pharmacological approaches are also common in inducing cardiac diseases in rodents. Cardio toxins such as doxorubicin and trastuzumab are known to induce cardiac dysfunction in dose dependent manner. In addition to decreased cardiac contraction, interstitial fibrosis and increased cell death are found(134). Myocarditis are induced by injection of Coxsackievirus(135)or auto-antibodies against cardiomyocyte structures such as myosine(136) and troponin I(137). Myocarditis leads to

dilated cardiomyopathy at chronic stages in these models(135). Continuous infusion of isoproterenol using implantable osmotic pump can induce hypertrophy and eventually systolic dysfunction(138,139). Angiotensin II infusion using same technique can result in hypertrophy with characteristics similar to heart failure with preserved ejection fraction(140,141), while combination of unilateral nephrectomy and salty diet led to impaired systolic function(142). Monocrotaline is another substance that is used very commonly in rats to induce pulmonary hypertension. Single injection of monocrotaline results in pulmonary vascular remodeling and right ventricular failure that has similar characteristics to clinical pulmonary arterial hypertension. Nevertheless, some of the drugs that have shown efficacy in this model failed to show same benefit in clinical trials, suggesting incomplete representation of human disease. In addition, despite its advantage on simple and reliable induction method, monocrotaline is not very effective in inducing severe pulmonary hypertension in mice. To induce pulmonary hypertension in mice, SU5416 (vascular endothelial growth factor receptor inhibitor) is often used in combination with hypoxia or other pulmonary vascular injury approaches. Although this is more cumbersome than monocrotaline model, it exhibits closer histological features to human pulmonary arterial hypertension(143).

4-1c. Gene manipulation models—Different cardiac phenotypes induced by specific gene knockout or overexpression are not within the scope of this review. There are some commonly used gene manipulated rodent models including salt-sensitive and spontaneously hypertensive mouse(144) and rat(145), obesity and type 2 diabetes(146), and combination of both hypertension and obesity(147). These models exhibit diastolic dysfunction and are important tools to study heart failure with preserved ejection fraction.

4-2. Other small and middle sized animal models

Guinea pigs, cats, and rabbits are also used for cardiac research. Their classifications are somewhat vague and guinea pigs are often included in rodents, and cats and rabbits are sometimes referred to as large animals. They are larger in size compared to mice and rats and offer easier surgical manipulation. Cardiac physiology is more similar to humans, as the heart rate is generally lower in larger species. In addition, guinea pigs present more similarity to human in electrophysiological properties compared to mice and rats(148), including intracellular Ca^{2+} handling, action potential shape, human-like QT alterations and arrhythmias associated with heart failure(149). Aortic banding is commonly performed in guinea pigs to induce hypertrophy(150). Cats with spontaneous hypertrophic cardiomyopathy have been reported(151) and provide opportunities to study this clinically relevant disease(152). Rabbits show similarities to human in myosin heavy chain ratio (β type predominant)(153) and abundance of calcium cycling proteins(154). Specifically, contribution of Na^{+}-Ca^{2+} exchanger in diastolic Ca^{2+}clearance is around 28–29% in rabbits, which is around 4-fold higher than mice and rats, while similar to humans(155). Surgical approaches are commonly used to induce cardiac diseases such as aortic banding, coronary ligation and tachypacing after pacemaker implantation(156). A unique rabbit model of vulnerable atherosclerotic plaque rapture was developed by a combination of vascular injury and high cholesterol diet(157). These middlesized animal models offer experiments in more

physiologically closer conditions to humans while being relatively cost-efficient compared to the larger animal models described in the following section.

4-3. Large animal models of cardiac diseases

Large animals commonly used for cardiac research include pigs, dogs, sheep and non-human primates. Their advantages over smaller animal models are physiological relevance and size similarity to humans, closer molecular characteristics, and availability in testing clinical sized devices and catheters. The disadvantage includes high cost for acquiring and maintaining the animals, requirement of large space and man power, more ethical concerns, and difficulties in obtaining aged or transgenic animals. Nevertheless, large animal experiments are the key step for translating new therapeutic approaches toward clinic by testing the efficacy in clinically relevant species, examining the safety, and defining optimal endpoint for future clinical trials. Several models have been established each mimicking different cardiac disease phenotypes that are found in patients.

4-3a. Ischemia models—Ischemia induced cardiac disease models are most commonly used in large animal cardiac research for its reproducible induction of systolic dysfunction and relatively simple method. Myocardial infarction associated with ischemic reperfusion injury can be developed by temporally blocking the antegrade coronary flow followed by reperfusion. Duration of ischemia determines the transmurality of the infarct, and short ischemia only induces myocardial stunning(158). Hedström et al(159) reported that duration of ischemia to develop 50% of infarction in the area at risk is significantly different between the species. Dogs required significantly longer ischemic time (181 min) compared to pigs (37 min) and rats (41 min) suggesting the importance of coronary collaterals, which is inherently rich in dogs(160). Left anterior descending artery or the left circumflex artery is commonly targeted to induce left heart failure. Occlusion of the proximal part of the left anterior descending artery is associated with larger infarct size and more cardiac remodeling at the chronic stage compared to the proximal left circumflex occlusion in pigs(161). However, some species have large left circumflex artery and the size of infarct follows the size of the area perfused by the occluded coronary artery. Thus, angiogram prior to induction of myocardial infarction can help predict the size of infarction. In contrast to semi-transmural infarction, a complete transmural myocardial infarction can be induced by surgically ligating the coronary artery or occluding it with embolic coils using endovascular technique. Both approaches result in similar infarct size, but the degree of inflammation and vascularization may be more close to clinical MI after endovascular approach(162). Microembolization is another method that has been used to induce ischemic heart failure(163), but this method usually requires multiple injections of microbeads to induce modest cardiac dysfunction. Although cumbersome, advantages of this model are that the degree of dysfunction can be adjusted, and global LV dysfunction can be induced in contrast to the regional dysfunction seen in other MI models. Overdose injection of microbeads can be used to induce an acute cardiogenic shock model.

In patients, ischemic disease does not always develop acutely and slow progression of coronary atherosclerosis can cause chronic total occlusion. The ischemic myocardium is often rescued by collateral flow through small bridging channels, but the myocardium

becomes dysfunctional due to insufficient blood supply. This dysfunctional ischemic myocardium remain viable and is commonly called hibernating myocardium. Models of hibernating myocardium have been developed to study this interesting myocardial phenotype using coronary occluders(164–166) and flow limiting stents(167).

4-3b. Pressure-overload model—In contrast to the frequent use of pressure-overload models in rodent studies, large animal models of pressure-overload are less commonly employed relative to ischemic models. Nevertheless, considering the wide prevalence of hypertension in the clinic, large animal models of pressure-overload is crucial. Similar to rodents, aortic banding can be applied to large animals using surgical techniques. For the ascending aortic banding model, the hemodynamic profile is similar to patients with aortic stenosis except for that coronary arteries are perfused with increased pressure in animal models of ascending aortic banding. Whether this difference influences heart failure phenotype has not been studied well. These models exhibit significant macroscopic and microscopic hypertrophy, but systolic dysfunction seems to progress much slower than rodent models, if it does(168,169). Heart failure with preserved ejection fraction is a common heart failure phenotype found in the clinic and patients often have hypertension, suggesting its association to cardiac pressure-overload. Development of animal models that completely recapitulate heart failure with preserved ejection fraction is challenging, as the disease pathophysiology is likely multifactorial(170,171). Kidney wrapping on aged dogs has been reported to exhibit similar cardiac phenotype to heart failure with preserved ejection fraction and probably is one of the most relevant large animal models of this disease(172). Specifically, the animals exhibit impaired relaxation, increased left ventricular stiffness, and left atrial remodeling together with histological hypertrophy and fibrosis of myocardium(172,173). There are also efforts to create new clinically relevant models by inducing metabolic diseases in large animals(174).

4-3c. Volume-overload model—Valvular regurgitation, left-to-right cardiac or vascular shunt, and anemia can overload the heart with excessive volume and can promote cardiac dysfunction. Increased volume load results in high left ventricular end-diastolic pressure and dilates all cardiac chambers. Generally, systolic function is maintained until the end-stage of heart failure, but the heart dilates and exhibits eccentric hypertrophy(175). Both aortic and mitral valves can be targeted to overload the LV, but mitral valve regurgitation has been more often induced in large animal models. Surgical or percutanous severing of mitral valve cordae tendinae reliably induces mitral regurgitation and leads to development of chronic heart failure, but is accompanied by high mortality as high as 50%(176,177). High mortality is likely related to the diffculty in controlling the degree of regurgitation in this method. A unique method to control the degree of left ventricle to left atrial regurgitation is to implant a graft between the left ventricule and the left atrium(178). Although implantation is technically challenging, clmping of the graft enables easy correction of the regurgitation. For acute induction of mitral regurgitation, placement of inferior vena cava filters has been used and the degree of regurgitation can be controlled by adjusing the expansion of the filter(179). Mitral regurgitation also developes in patients post-myocardial infarction and is a predictor of worse prognosis. This can be reproduced in large animals by creating large infarction in the left ventricular posterior wall in sheep and pigs. These ischemic mitral

regurgitation models generally exhibit progressive deterioration of heart failure(180,181). Artery to venous fistula models are another common method to induce volume-overload heart failure in large animals(182). The degree of the shunt and the proximisity to the heart are the key factors that determine the severity of heart failure.

4-3d. Other phenotypes of heart failure—Tachycardia induced heart failure is a well-established and reproducible large animal model of non-ischemic cardiac failure(183,184). Rapid pacing of the atrium or the ventricle leads to elevation of left ventricular end-diastolic pressure accompanied by systolic dysfunction. Among different species, dog model of pacing induced tachycardiac heart failure has been the best characterized, and it uses a pacing rate of 200–230 bpm for 3–4 weeks. Slower pacing and shorter duration of pacing result in less severe heart failure phenotype(185). In this model, activation of neurohormonal systems and molecular changes relevant to human heart failure has been demonstrated(186). Similar to human tachycardic heart failure, cessation of tachypacing results in recovery of cardiac function, thus the dysfunction is reversible to a certain extent. Cardio-toxic drug injections into the coronary artery result in systolic dysfunction with fibrosis and myofiber atrophy(187,188). However, this approach is associated with high mortality and requires repetitive administration of drugs. In addition to the left heart failure, right heart failure can be induced by pulmonary arterial banding(189), pulmonary vein banding(190,191), pulmonary microbeads injection(192), artery-to-venous shunt(193) and pneumonectomy(194). In contrast to the LV failure models, right ventricular failure models are much less characterized and more research is needed to identify the differences between the right and left heart failures.

5. Conclusion

We reviewed commonly used *in vitro* and *in vivo* models of cardiac diseases. Wide spectrum of models offers various experiments dedicated to examine specific pathways and therapies, but the researchers need to be aware of their characteristics and relevance to clinical cardiac diseases. Development of more easily-induced, reproducible, and clinically relevant models as well as characterization of new and existing models are important for further refinement of experimental cardiac research.

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Table 1.

Comparisons of experimental models

	In vitro	Ex vivo	In vivo
Features	• Relatively low cost • Cardiomyocyte-specific assessments • Minimize animal sacrifice • High-throughput	• Controlled experimental setting • Limited neurological and hormonal influences • Able to assess pump function • Acute studies only	• Incorporates biological complexity • Clinically relevant disease models • High cost and low throughput • Long-term studies possible

Table 2.

Comparisons of *in vitro* models

	Neonatal cardiomyocytes	Adult cardiomoycytes	Cardiac cell lines	ESC/iPSCs
Pros	• Easy primary isolation • Able to culture for a long period • Well-established checmical drugs to induce pathologic conditions • High transfection efficiency	• High similarity to human cells in the morphology and behavior • Able to obtain cells from diseased heart • Able to assess contractility and Ca^{2+} transient	• Easy to culture • Able to culture for a long period • Able to passage and recover from a frozen stock • High transfection efficiency • Minimize animal sacrifices	• Derived from human source • Able to assess Ca^{2+} transient • Able to study human genetic disorder
Cons	• Immaturity • Non-uniform contraction	• Limited gene manupulation methods • Technically difficult to isolate cells	• Lack of cardiac phenotype • Unable to assess contractility	• Technically difficult • High cost • Immaturity • Batch-to-batch variation
Application	• Hypertrophy assessment • High-throughput drug screen	• Contractility measurement • Ca^{2+} imaging • Patch-clamp • Sublocalization study by immunostaining	• High-throughput drug screen • Toxicology	• High-throughput drug screen • Toxicology • Ca^{2+} imaging • Precision medicine