Chapter 1

Experimental Models of Cardiovascular Diseases: Overview

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Abstract

Cardiovascular disease is one of the most common causes of deaths in clinics. Experimental models of cardiovascular diseases are essential to understand disease mechanism, to provide accurate diagnoses, and to develop new therapies. Large numbers of experimental models have been proposed and replicated by many laboratories in the past. Models with significant advantages are chosen and became more popular. Particularly, feasibility, reproducibility, and human disease resemblance are the common key factors for frequently used cardiovascular disease models. In this chapter, we provide a brief overview of these experimental models used for in vitro, in vivo, and in silico studies of cardiovascular diseases.

Key words Animal model, Myocardial infarction, Cardiomyocyte, Electrophysiology, Modeling, Pulmonary hypertension, Heart failure, Left ventricle, Rodents, Large animals

1 Introduction

Prevalence of cardiovascular disease (CVD) has been increasing worldwide, and the recent report from AHA (Heart Disease and Stroke Statistics—2017 Update) [1] indicates over 90 million US adults have at least one CVD, which is expected to increase more in a rapid pace. Extensive research focusing on prevention, diagnosis, and treatment of CVD has improved outcomes of patients with CVD; however, efforts continue to improve further. Importantly, bench science has been the driving force for this achievement and will continue to play major roles in future research.

Various models of CVD have contributed to new therapeutic discovery and identification of disease pathophysiology. In vitro models allow fast, efficient, and controllable experiments using cells or tissues. In contrast, in vivo models allow evaluation of mechanisms and therapeutic efficacy in more complex biology system. Recent advances in computation and software also enabled reliable in silico modeling of CVDs. To provide an overview of these models that aim at simulating human CVD, this chapter concisely reviews experimental models of CVD. Detailed protocols

Kiyotake Ishikawa (ed.), *Experimental Models of Cardiovascular Diseases: Methods and Protocols*, Methods in Molecular Biology, vol. 1816, https://doi.org/10.1007/978-1-4939-8597-5_1,

to produce these models are provided in the following chapters of the book (Experimental Models of Cardiovascular Diseases), and the relevant chapter numbers are included as references.

2 In Silico Models

Recent advances in computing and technology now enable various approaches to model diseases in silico. By integrating data of key elements obtained from experiments, these models can help understand complex and dynamic biology as a system, offer high-throughput and efficient analysis, and provide novel insights into biological mechanisms. For example, cardiomyocyte action potential has been successfully modeled by integrating data from respective ion channel properties and contributed to understanding the impact of genetic disorders of each ion channels or effect of drugs [2]. These data can be further applied to more complex systems such as tissue or organ level electrophysiology (Chapter 2). The myocardial contraction was modeled using various methods such as finite-element analysis [3] and provided a spectrum of new mechanistic insights. Cardiac contractility as a part of systemic hemodynamics has been modeled using pressure–volume loop concept [4] and is a useful approach to estimate and understand how interventions affect these factors. Furthermore, similar to other areas of research, computational approaches are the main drivers of analyzing mass data and extracting important biological information. As the technologies advance, in silico approaches are expected to become more powerful and may replace many of in vitro and in vivo experiments in the future.

3 In Vitro Models

The key advantages of in vitro modeling systems are the availability of a large number of cells and precise control of experimental conditions that provide ability to efficiently conduct signaling pathway studies, cell-specific mechanistic studies and high-throughput drug screenings. Primary isolated cells and immortalized cell lines have contributed enormously in improving our understandings in the molecular and physiological regulation of cardiovascular system, and recently, human embryonic and induced pluripotent stem cell (ESC and iPSC, respectively) derived cells joined these repertoires, offering new in vitro approaches to study cardiovascular diseases.

3.1 Primary Isolated Cardiomyocytes

Neonatal cardiomyocytes, which are commonly isolated from 1- to 5-day-old rats, are one of the most popular in vitro model systems of cardiac function and disease. These cells are relatively easy to

isolate and culture, while offering feasible manipulation of gene expression profiles [5]. Various types of pathophysiological stimulations can be applied on neonatal cardiomyocytes, all mimicking in vivo cardiac pathology. Cardiac hypertrophy is induced by drugs such as norepinephrine [6], angiotensin II [7], and endothelin-1 [8]. Mechanical stretch can be applied directly on cells to simulate cardiac volume overload associated myocardial stretch [9]. In addition, ischemia–reperfusion is replicated by hypoxia–reoxygenation [10] or oxygen-scavenging compound (e.g., $Na_2S_2O_4$) treatments [11]. These stimulations often lead to cellular responses that closely represent cardiomyocyte changes in vivo setting, such as hypertrophy, apoptosis, autophagy, and fetal gene expression, rendering them a reliable model of cardiac diseases. Notwithstanding, immature morphology [12] and some dissociation in gene expression profiles compared to adult cardiomyocytes [13] are limitations of this cell type.

In contrast, adult cardiomyocytes (Chapter 3) more closely represent morphology as well as the behavior of cells in the intact human heart. Cells can be isolated from animal hearts of different age, sex, and species including human using enzymatic digestion protocols. Additionally, cardiomyocyte isolation from transgenic animals and diseased animals allow for a wide spectrum of experiments focusing on gene function as well as pathological stimuli. Most notably, thanks to the mature sarcomeric structure and ion channels, these cells also bear sophisticated experimental approaches such as patch-clamp [14], contractility measurements (Chapter 7) [15], and Ca^{2+} imaging studies [16, 17]. Technical difficulties in isolation procedures and culture are the limitation of this cell type. However, above invaluable advantages motivate researchers to continue working on these cells, and they remain one of the most frequently used models for in vitro cardiac research.

3.2 Immortalized Cell Lines

To overcome the limited culturing ability of primary cardiomyocytes [18], efforts have been made to establish immortalized cardiac cells. Representative cardiac immortalized cell lines include H9c2 [19], ANT-T-antigen [20], AT-1cells [21], MC29 [22], HL-1 [23], and AC16 [24]. These cells originate from cardiac cells and thus retain similar gene expression profiles and phenotypic characteristics of their origin. For example, H9c2 cells are derived from myoblast cell line, which was isolated from embryonic BDIX rat ventricular tissue, and AC16 cells from human ventricular tissues (by fusion with SV40 transformed human fibroblasts). Cell line-specific features and limitations need to be well recognized when using these cells, and validation in other cardiac models is likely necessary. Nevertheless, feasibility in culture and ability to use cells after freeze-thaw cycles render them a useful in vitro modeling system.

3.3 ESC and iPSC Derived Cardiomyocytes

Cardiomyocyte-like cells can be induced from pluripotent stem cells such as ESCs (Chapters 4 and 5) and iPSCs (Chapter 6). There is a recent surge on use of these cells as tools for modeling cardiac diseases in vitro. The key advantage is that these cells can be obtained from patients without excising the actual heart. This enables application of cardiac precision medicine by taking into account the individual variability of genomic profiles. Examples include mechanistic studies of gene disorders [25] and screening of potentially harmful drugs that can induce QT prolongation or cardiotoxicity [26]. Challenges remain, however, in cost and effort extensive methods to create these cells as well as physiologically and structurally immature cell status after induction. When these limitations are addressed by new approaches, stem cell-derived cardiomyocytes may become the primary choice of cell type for the majority of in vitro research.

3.4 In Vitro Models of Vascular Disease

Vascular endothelial cells (EC)s and smooth muscle cells (SMC)s are the two commonly studied cell types when modeling vascular diseases in vitro. Pathological modifications of these cells in vivo lead to atherosclerosis, restenosis, hypertension, and aneurysm that are all tightly connected to cardiovascular deaths. Key signaling pathways that contribute to these diseases are sought, and at the same time, methods to enhance angiogenesis are studied using ECs and SMCs. Primary cells can be obtained by enzymatic digestion of the vascular tissues from the variety of animals including human. Unlike cardiomyocytes, they easily proliferate on a culture dish and well tolerate freeze-thaw cycles, thus offer easier use of primary cells. Similar to cardiac cell models, ESC and iPSC derived ECs [27], as well as SMCs [28] have also been developed to model vascular diseases. Commonly performed mechanistic studies using these cells include proliferation, migration, contraction, secretion, and angiogenesis assays. To more closely reflect in vitro setting, cells are sometimes cultured in flow conditions or cultured together with other cell types to address cell interactions.

4 Ex Vivo Tissue Models

Although cell-based models offer efficient and fine-tuned experiments focusing on respective cell types, they usually lack in three-dimensional structure and interactions with other cell types. Ex vivo models of CVD can be used to overcome this limitation while maintaining fine-tuned experimental conditions. Majority of previously proposed ex vivo models use fresh organs explanted from animals or humans. Whole-heart perfusion using Langendorff system (Chapter 8) [29] or inter-animal cross-circulation (Chapter 9) [30] allows for precisely controlled physiological studies at the organ level. These approaches played pivotal roles in establishing the basis of our current understandings in cardiac physiology by

studying pressure–volume relationships, cardiac work, and myocardial oxygen consumption. Sophisticated studies on cardiac electrophysiology are also available using Langendorff system [31]. Optical mapping of Ca^{2+} sensitive fluorescence probes allows for detection of cardiac electric activities with high spatial and temporal resolution in the whole heart as well as in a piece of tissue that can be artificially perfused (Chapter 10). By changing the composition of perfusate, tailored experiments with different circulating concentrations of ions or drugs can be examined [32]. In addition, the impact of ischemia, as well as reperfusion injury may be studied in these ex vivo hearts by temporally or permanently ligating the coronary arteries. One limitation of these freshly explanted organ/tissues is difficulties in maintaining their integrities for long-term. Therefore, these models are mainly used for acute experiments. In contrast, bioengineering approaches allow for a chronic culture of tissues for long-term experiments using tissue engineering techniques from cultured cells [33]. Three-dimensional structure offers better replication of in vivo physiology compared to cell cultures. In addition, incorporation of human iPSC-induced cardiomyocytes allows for experiments on patient-specific disorders (Chapter 11). Continued efforts on improving the technique will likely lead to the development of the engineered whole heart in future.

Similar to the heart, protocols for ex vivo perfusion of vasculatures have also been developed [34]. Serial imaging with high spatial resolution is available using this approach, and may lead to new discoveries in atherosclerosis research. Atherosclerotic plaque rupture and thrombosis cause sudden onset disease and can be fatal, requiring efficient preventive approaches. For evaluating the risk of thrombosis events and to test the efficacies of antithrombotic drugs in specific patient, an ex vivo thrombosis model has been developed (Chapter 12).

5 In Vivo Models

Cardiac function and biology in vivo are meticulously regulated by interplays of various stimuli from outside the heart, such as hemodynamic, neurohormonal, and inflammatory signalings [35]. These signals are activated to maintain body homeostasis; however, sustained activation of these signals provokes cardiac pathophysiological responses. Importantly, cardiac dysfunction induces activation of pathological signaling, while these signals deteriorate cardiac function, thus forming a positive feedback loop in both acute and chronic settings. To understand the cardiac disease pathophysiology and effect of therapeutics in the complex biological system, in vivo models are essential. A variety of animal models have been proposed, and we will briefly review commonly used in vivo experimental models in the following sections.

5.1 Small Animal Models

Rodent models play pivotal roles in vivo cardiovascular research for several reasons [36]. These include anatomical similarity to human heart (four chambers) and vasculatures, easy housing and reasonable cost, fast gestation and short lifespan, and less ethical concerns compared to more advanced species. Owing to the established gene manipulation techniques and feasibility, mouse models offer in-depth analysis of gene function and disease mechanisms. High similarity in many genes between mice and human [37], together with established research tools for detecting gene and protein expressions in mice render them the most popular in vivo model system for cardiovascular research. Rat models, on the other hand, have larger heart and vessel sizes that offer easier surgical manipulation to induce diseases and provide a larger amount of tissues compared to mouse models. Rats are also physiologically closer to the human compared to mice.

Apart from transgenic CVD models, the model induction methods are mostly similar in mice and rats. Surgical manipulation is more challenging in mice due to its smaller size. However, once the researchers have become familiar with the necessary skills, higher throughput research is available in mice. Cardiac ischemia models include permanent coronary ligation and ischemic–reperfusion, both using similar techniques (Chapter 13). Location and the length of ligation determine the size of injury as well as survival after model induction. At the chronic stage, the heart remodels with systolic dysfunction similar to human after MI [38]. It also seems that the infarct healing processes are faster in mice than more advanced species [39]. Another popular method to induce heart failure is pressure overload using surgical banding of the aorta (Chapters 14 and 15). In mice, the heart initially becomes hypertrophic followed by cardiac dilation and systolic dysfunction at the later stage [40]. The speed of disease progression depends on the location of the banding (ascending or transverse) and the degree of the stenosis. In contrast, latter changes are not always observed in rats after aortic banding [41]. Drug-induced cardiac disease models are also commonly employed. Osmotic pumps are used to continuously infuse angiotensin or isoproterenol (Chapter 16) [42], while cardiotoxicity drugs such as doxorubicin are injected systemically (Chapter 17) [43]. Injection of monocrotaline can easily induce pulmonary hypertension in rats (Chapter 18) [44]. In contrast, the effect of monocrotaline in mice is not very reliable and hypoxia-induced pulmonary hypertension is more commonly used when using mice (Chapter 19).

For inducing vascular remodeling, wire injury method (Chapter 20) [45], as well as balloon inflation methods [46], are commonly employed. Disruption of endothelium and stretching of the vasculature with high cholesterol diets induce vascular legions at the chronic stage. Artery to venous fistula model is also a useful tool to study venous remodeling (Chapter 21) [47].

5.2 Large Animal Models

Key advantages of large animal models are the similarities in size, anatomy, and physiology to the human heart. These features enable research using clinically applicable imaging devices as well as clinical sized therapeutic devices including the endovascular catheters. Similar physiology and more complex immune system compared to rodents make it easier to predict human responses to new therapeutic approaches. A large amount of tissue samples is available at necropsy and different assays can be conducted in the identical animal, which is sometimes difficult in small rodent hearts. Limitations of large animal experiments include difficulty in enrolling a large number of animals due to the cost and space limitations, higher ethical concerns, and limited research resources such as antibody and primers. Nevertheless, their important roles in bridging the bench science to clinical practice for drug development, testing clinical devices, and evaluating cardiac imaging modalities make them an essential step before clinical application of these approaches.

Variety of large animal models have been proposed using different species including pigs, dogs, sheep, rabbits, and nonhuman primates. To examine any therapeutic or diagnostic approaches in clinically relevant setting, large animal models should exhibit similar disease phenotype as the targeted clinical population. Among the cardiac diseases, ischemia-induced disease models are most commonly used. Myocardial infarction can be induced by both catheter-based (Chapter 22) [48] and surgical (Chapter 23) [49] approaches and provide a reproducible and controllable degree of systolic dysfunction. Acute studies focusing on reducing the initial myocardial injury associated with ischemia and subsequent reperfusion injury, as well as chronic studies focusing on preventing or reversing the progressive cardiac dysfunction and remodeling can be designed using these models. Other ischemic models include coronary embolization models that develop diffuse cardiac dysfunction (Chapter 27) [50], and chronic ischemia models with hibernating myocardium [51]. Presence of mitral regurgitation in post-MI heart is a risk factor for adverse events, and animal models of this condition have been developed in pigs [52] and sheep (Chapter 23) [53, 54].

Nonischemic cardiac disease models can be induced by continuous tachypacing (tachycardia-induced myopathy) (Chapter 24) [55], volume overload by valvular regurgitation (Chapter 25) [56] or artery to venous shunt [57], pressure overload by aortic banding (Chapter 26) [58] or renal wrapping [59], and cardiac toxic drug injection [60]. Pressure overload-induced models somewhat resemble clinically common heart failure phenotype; heart failure with preserved ejection fraction, but the lack of obesity and metabolic diseases, as well as difficulty in using aged animals, make it challenging to replicate clinical phenotype completely. Right heart failure without accompanying pulmonary hypertension can be induced by

pulmonary artery constriction [61]. Pulmonary embolism model mimics clinical pulmonary hypertension patients with chronic thromboembolic pulmonary hypertension (Chapter 28) [62]. Our group has recently established a post-capillary pulmonary hypertension model that accompanies right ventricular dysfunction (Chapter 29) [63]. Together, these models offer options for choosing appropriate pulmonary hypertension models for experiments in large animals.

A rabbit model of atherosclerosis is a popular vascular disease model for studying imaging and therapeutics for atherosclerosis (Chapter 30) [64]. Combination of high-fat diet and balloon injury to induce vascular lesions are also employed in other species. Aortic dissection and aortic aneurysm models have also been developed in large animals [65].

6 Conclusion

All models described above have their own advantages and limitations. It is important for the researchers to design the experiments based on their solid hypothesis and use appropriate models to answer their hypothesis. Detailed protocols described in each chapter for creating these models in this book should allow for successful and reproducible experiments.

Acknowledgments

This work is supported by NIH R01 HL139963 (K.I.), AHA-SDG 17SDG33410873 (K.I.), and AHA 17POST33410877 (J.G.O.).

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