Chapter

Large Animal Models in Cardiovascular Research

Hiroaki Osada, Kozue Murata and Hidetoshi Masumoto

Abstract

Studies of not only preclinical cardiovascular research but also those of life science, medical, and pharmacological fields commonly utilize small animal models. However, for the advancement of cardiovascular medicine, researches using large animal models are important step for preclinical validation of therapeutic efficacy and safety by virtue of having models with a body and heart size comparable with that of a human, providing clinically relevant experiments without the concern of over- or under-estimating therapeutic effects and risks. In particular, pigs are considered as a suitable animal model for research in cardiovascular medicine because of the similarities in physiology, metabolism, genomics, and proteomics to those in humans. Another advantage of pigs is the availability of various heart disease models such as myocardial infarction and genetically established cardiomyopathy. The present review updates the contributions of large animal model-based research to the development of cardiovascular medicine, especially focusing on the utility of pig models.

Keywords: large animal models, cardiovascular research, translational research, pig models, disease models

1. Introduction

Studies of not only preclinical cardiovascular research but also those of life science, medical, and pharmacological fields commonly utilize small animal models such as rodents. However, the translation of the rodent-based results to clinical trials does not always provide relevant results in humans possibly due to anatomical differences between humans and small animals, or variations in physiological characteristics and mechanisms of the disease development [1–3]. To confirm safety and efficacy in clinical studies, the transition from small-to-large animal studies, which comprise anatomical, biological, and physiological features similar to humans, is anticipated. Therefore, the selection of animal species and preparation of suitable disease models are crucial to obtain clinically relevant results leading to a better translation to human clinical practices [4, 5].

Among traditionally used large animal models in research like non-human primates, horses, bovines, sheep, goats, dogs, cats, and pigs, the pig model is considered to be a desirable experimental model because of its similarity to humans in terms of body and heart size to humans, enabling the researcher to prepare clinically relevant disease models such as a myocardial infarction model [6]. Other advantages of the pig model are its similarities in physiology, metabolism, genomics, and proteomics
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to humans [7–10]. Genetically modified pig disease models, such as the genetic cardiomyopathy model, have also been developed [11]. The current review updates the contributions of large animal models for research of cardiovascular medicine, especially focusing on the utility of pig models.

2. Advantages of large animal models for cardiovascular research

2.1 Clinical relevance by virtue of the proximity in size to humans

To develop therapeutic materials and procedures in cardiovascular medicine, preclinical validation of therapeutic efficacy and safety of medical materials using large animal models is an important step considering the comparable body and heart size with those in humans, which provides clinically relevant experimental procedures (Figure 1).

Since Gibbon first described cardiac surgery using cardiopulmonary bypass more than a half century ago [12], it is no overstatement to say that the history of cardiac surgery is the history of cardiopulmonary bypass development. Large animal models such as sheep and baboons have been used for the development of cardiopulmonary bypass and perioperative management, including anesthesia management models [13–17]. In the recent development of left ventricular assist devices (LVADs) for severe heart failure, large animal models such as bovine, dog, goat, pig, and sheep have been used. The device itself and cannula design, the surgical technique, performance, and the integration within the cardiovascular system must translate from these large animals to human patients [18]. Bovines, such as calves, are considered the most useful large animal model for this study [19].

On the other hand, Stephenson et al. reported the feasibility of the usage of Holstein calves in developing a robotically assisted microsurgical system to perform coronary artery anastomoses [20]. In another review, studies on the pathophysiology

![Figure 1. Advantages of the usage of large animal models.](image)

Advertisements of the usage of large animal models
of chronic thromboembolic pulmonary hypertension using large animal models such as dogs and pigs using either indwelling or Swan Ganz catheters are summarized [21]. An important translational feature of pig models is the possibility of percutaneous coronary intervention using human clinical equipment, and the procedures using metallic stents or angioplasty balloons [22, 23]. These previous reports indicate the importance of using large animal models with similar cardiovascular system sizes to that of humans, enabling human-like experiments.

Additionally, in primary screening tests of drug discovery and toxicology studies, rodents such as mice and rats are mainly used [5]. However, the risk of under- or overestimation of the therapeutic efficacy or side effects remains in studies using animal models where the size of the body and the organs differ so much from humans. Recently, large animals are increasingly taking place as an alternative to rodents [9, 10]. Especially, mini-pigs have been largely utilized as they are easier to handle and suitable for drug discovery and toxicology researches. In addition to their anatomical and physiological similarities to humans, mini-pigs can be used for all routes of drug administration, such as the dietary, continuous intravenous infusion, dermal, or inhalation routes. Furthermore, compared to other laboratory animals, mini-pigs have a much closer metabolism of chemicals to humans [24–26].

Large animal models have been recently introduced not only in pharmaceutical toxicology evaluations but also in cardiovascular regenerative medicine using cell-free materials such as exosomes, microRNAs, proteins such as growth factors, and extracellular matrix components [27]. Therefore, it would be possible that the validation of therapeutical dosage in cardiovascular regenerative medicine would also be tested in preclinical efficacy and safety tests using large animal models [28].

2.2 Advantage of pigs as a large animal model

Pigs are considered a suitable model for cardiovascular research because of the similarities in anatomy, physiology, metabolism, genomics, and proteomics to those in humans (Figure 2). Compared with other animal models, pigs acquire early sexual maturity, sizeable litter size, and have a quick reproduction time. They also breed year-round, which makes them highly suitable for biomedical research programs [29]. On the other hand, like other animals, there is moderate genetic variation between breeds (such as the human population) and within breeds that makes variations in the occurrence of abiogenetic diseases [30].

There are several advantages of pigs as a model animal for research in cardiovascular medicine as listed below:

i. The heart size of pigs and its relative weight to body weight is similar to those of the human heart, therefore similar to human patients. In this context, multiple and longitudinal measurements using imaging modalities (echocardiography, computed tomography, magnetic resonance imaging, etc.) and access to biopsies and postmortem samples are possible.

ii. Resemblance to human cardiac physiology, such as ventricular performance and electrophysiology: there is a functional equivalence of various diseases in humans and pigs.

iii. Pigs have negligible collateral circulation, so each coronary artery supplies a specific cardiac region, unlike other laboratory animals [5, 31, 32].
Furthermore, the porcine cardiovascular system shares many similarities with those of humans, not only in the anatomical structure but also in the lipid profiles and lipoprotein metabolism, and is known to develop spontaneous lesions in the vasculature and cardiac valves [33]. Likewise, pigs show greater similarity to humans as neutrophils are also the predominant circulating blood cell population [34].

Based on these advantages, pig models are widely used in preclinical models in toxicology evaluations or developing medical materials, taking advantage of their anatomical characteristics. Their natural characteristics have also been widely used in the research of aortic valve stenosis, vascular calcification, and atherosclerosis [35–37]. In transplantation medicine, pigs have also been proposed as xenotransplantation donors. Due to the donor shortage, these procedures might enable the future xenotransplantation of porcine organs into humans as the main approach for transplantation medicine [38, 39].

Large animal models are also advantageous allowing much more precise disease model preparations compared to those in small animal models, which enables to create predictable injury sizes at a preferred region of the myocardium [40]. In this context, pig modes are largely used to create myocardial infarction models for stem-cell-based regenerative medicine research [6, 41]. Munz et al. reported a surgical myocardial infarction through permanent coronary ligation that provided a reproducible and standardized pig myocardial infarction model. They showed that the optimal occlusion site in terms of morbidity, mortality, and lesion extent was the midpoint of the left anterior descending artery [31]. On the other hand, ameroid constrictors have been used to create a gradual coronary artery occlusion that might avoid lethal arrhythmia during surgical ischemia induction [42, 43]. Catheter-based coronary occlusion models are reported as well [44, 45].

### Figure 2.
Significance of the pig model.

<table>
<thead>
<tr>
<th>Usefulness of the pig model</th>
<th>Applications of the pig model</th>
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<tr>
<td>Many similarities to humans</td>
<td>Preclinical models in toxicology evaluations or developing medical materials</td>
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<td>The heart size relative to body weight</td>
<td>In transplantation medicine, as xenotransplantation donor</td>
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<td>Ventricular performance and electrophysiology</td>
<td>Tailored large animal models by gene-editing technologies</td>
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<td>Negligible collateral circulation</td>
<td>Application of pig proteome library and transcriptome analyses to humans</td>
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<td>Lipid profiles and lipoprotein metabolism</td>
<td>Allogeneic transplant models using pluripotent stem cells</td>
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<td>Neutrophils are the predominant circulating blood cell population</td>
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**Significance of pig model**

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3. Genetic modification to create disease models

3.1 Genetically modified clinically relevant disease large animal models

Precise and efficient gene-editing technologies enable the generation of tailored large animal models of human diseases that could contribute to the development of new diagnostic tests and therapeutic procedures [35, 46]. Developmental engineering technology has been used to create large animal models. In 1985, Hammer et al. reported genetically engineered livestock animals [47]. In 1997, Petters et al. presented the first transgenic livestock animal disease model, a pig model of retinitis pigmentosa, which expressed a mutated rhodopsin gene [48]. Recent advances in innovative methods, such as intracytoplasmic sperm injection-mediated gene transfer and somatic cell nuclear transfer (SCNT), enabled design-specific animal models, such as “Dolly,” a cloned sheep [49]. Since then, many genetically engineered models of human disease have been generated through large animal models. By integrating SCNT technology and recently developed gene-editing platforms, including TALENs and the CRISPR/Cas9 system, even more diverse modifications of the genomes of livestock species will be seen in the future [50–52]. The commonly used SCNT approach allows concurrent production of transgenic animals expressing a marker gene and non-transgenic clone siblings from the same nuclear donor cells. The cloned animals would provide a useful syngeneic transplantation model [53].

3.2 Specific disease models in pigs

The pig genome has been extensively sequenced and gene-editing technology has been applied to multiple pig strains so far [5, 54]. These techniques have been already largely applied to pigs to create a model for several diseases, such as cystic fibrosis, diabetes mellitus, and neuromuscular disorder [50].

Regarding a model for cardiovascular diseases, Matsunari et al. recently established a genetically modified pig cardiomyopathy model in which they knocked out the δ-sarcoglycan (δ-SG) gene (SGCD) of domestic pigs by the combination of efficient de novo gene editing and SCNT. SGCD-/- cloned pigs exhibit systolic dysfunction similar to that found in human dilated cardiomyopathy and are expected to be highly applicable for the exploration of the feasibility, safety, and efficacy of therapeutic strategies, as well as for elucidating the underlying mechanisms of new treatments for genetic cardiomyopathy [11, 53].

Blutke et al. established a comprehensive biobank of long-term diabetic INSC94Y transgenic pigs, a model of mutant INS gene-induced diabetes of youth (MIDY), which is a model of poorly controlled diabetes mellitus. It is designed to help diabetes researchers discover the molecules and mechanisms involved in the long-term complications of the disease [7, 55]. Furthermore, it would be possible to create human-like atherosclerotic disease models using this severe diabetes mellitus model in the future. Klymiuk et al. established a tailored pig model of Duchenne muscular dystrophy (DMD) by deleting DMD exon 52 in male pig cells and showed the similarity of the transcriptome in dystrophin-deficient pigs with patients with DMD [8]. This technology might pave the way to establish a DMD-related cardiomyopathy model.
3.3 Genetic labeling of somatic cells for the investigations of therapeutic mechanisms

Genetically modified animal models with fluorescent marker genes by SCNT are highly useful as they enable a more efficient monitoring method of cell survival and cellular kinetics in vivo after cell product transplantation [53, 56]. Matsunari et al. produced transgenic-cloned pigs carrying the humanized Kusabira-Orange (huKO) gene, yielding an orange-red fluorescence in its dimeric form. The clear red fluorescence of the huKO protein is maintained in paraffin-embedded tissue sections. The pigs express fluorescent protein not only in various organs but also in pig stem cells or progenitor cells [57].

In hepatocytes or liver organoid transplantation experiments, donor cells/organoids were derived from transgenic KO-expressing pigs and transplanted KO-negative littermate, which showed the distribution and survival of transplanted materials [58, 59]. These techniques are also used in allograft ligament construction to analyze intrinsic and extrinsic cellular dynamics during graft healing [60]. It would also be applicable in research of cardiac regenerative medicine using stem cells in which the tracing of survived cells after transplantation and the mechanisms of graft and host interaction have been investigated by 3-dimensional (3D) imaging of heart tissue after tissue clearing using light-sheet microscopy [61, 62]. Upon distinguishing recipient and donor cells in transplanted grafts, KO-expressing pigs may become sufficient genetic modification models for the 3D posttransplantation analysis of vascular network formation inside of the graft.

4. Future directions of large animal model-based cardiovascular research

4.1 Pig proteomics and the extrapolation to humans

Recent efforts have been focused on the characterization of experimental animals at the molecular level such as genomics, transcriptomics, and proteomics [35] which revealed the close similarity of proteomics in pigs and humans. Since matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was first reported in the 1980s, the technologies have been successfully used in genome, proteome, metabolome, and clinical diagnostic research [63]. It is useful in qualitative and quantitative analyses of disease biomarkers in various specimens that lead to not only diagnosis but also risk stratification and guidance in the selection of therapeutic modalities [64]. Linscheid et al. recently performed systematic analyses of cardiac proteomes across cardiac chambers in humans, pigs, and four commonly used animal models and identified and quantified approximately 7,000 proteins, comparing them with respect to cardiac function and mechanisms of diseases [65]. Tamiyakul et al. performed a proteome analysis on the myocardium of the DMD pig model that showed an altered abundance of several proteins such as reduction of myosin-6, which is directly involved in muscle contraction [66]. Müller et al. reported a pig cardiac transcriptome analysis. They assembled 15,926 transcripts, stratified them, and validated the results by complementary mass spectrometry [67]. These attempts to analyze the proteome and transcriptome in animal models and even in humans will play an important role in the future of cardiovascular regenerative medicine. The knowledge of novel heart failure biomarkers may allow a more personalized medicine in the future [68].
4.2 Allogeneic transplantation models for cardiovascular regenerative medicine

In pluripotent stem cell-based cardiovascular regenerative medicine, the advantage of induced pluripotent stem cells (iPSCs) over embryonic stem cells (ESCs) is the availability of autologous cells for the treatment. When iPSC products are generated autologously, better engraftment free from the risk of immune rejection after the transplantation of cell products is theoretically anticipated [69]. Human iPSCs are also expected to mitigate immune rejection after cell/tissue transplantation in human leucocyte antigen (HLA)-controlled allogeneic use, which is investigated in various animal allogeneic transplantation models [70, 71]. The allogeneic use of iPSCs is expected to avoid disadvantages of autologous iPSCs transplantation, such as the cost and time required for quality control of each individual cell line [72]. Furthermore, autologous cell products from patients with genetic disorders such as genetic cardiomyopathy may take over its diseased phenotype that would hamper the therapeutic effects of the products.

Medicetty et al. introduced allogeneic bone marrow-derived cell transplantation to a pig myocardial infarction model in which cells are delivered by catheter directly to the coronary artery. They showed a significant positive modulation of left ventricular function and remodeling [73]. Regarding iPSC-based cardiovascular regenerative medicine, Shiba et al. reported an allogeneic transplantation experiment using cynomolgus monkeys (Macaca fascicularis). iPSC-derived cardiomyocytes from major histocompatibility complex (MHC)-homozygous animals were transplanted into MHC-matched monkeys by direct intra-myocardial injection. Transplanted cardiomyocytes showed electrical coupling to the recipient’s heart tissue and survived without immune rejection in monkeys treated with clinically relevant doses of immunosuppressants, whereas the transplantation of cardiomyocytes to MHC-mismatched monkeys even treated with immunosuppressants exhibited immune rejection of grafted cardiomyocytes with severe infiltration of T lymphocytes [74]. Kawamura et al. reported a cynomolgus monkey-based allogeneic transplantation experiment using cell sheets prepared from iPSC-derived cardiomyocytes. In the experiments, the monkeys with immunosuppressants could show fair engraftment of iPSC-derived cardiomyocyte sheets regardless of MHC matching, whereas even MHC-matched iPSC-derived cardiomyocyte sheets could not be sufficiently engrafted without immunosuppressants, indicating the requirement of immunosuppressants even in MHC-matched transplantation, which may prevent minor antigen-triggered immune rejection [75]. These results may indicate that the significance of MHC matching would be attenuated in iPSC-based cardiac regenerative therapy [76]. The establishment of a swine leukocyte antigen (SLA)-identified allogeneic transplantation pig model [77] and investigations of histological and molecular mechanisms of immune rejection attributed by cell transplantation would contribute to develop the strategy to avoid immune rejection associated with allogeneic human iPSC therapies as well as therapeutic mechanisms of the allogeneic transplantation.

Additionally, researchers have been struggling to establish pig somatic stem cell lines such as bone marrow-derived stem cells or live progenitor cells using somatic cell cloning technology, which may realize a syngeneic donor-recipient system in pigs [53]. Furthermore, attempts are being made to establish pluripotent stem cells from pigs such as pig iPSCs or ESCs. Xu et al. reported pig iPSCs generated by infecting pig pericytes and embryonic fibroblasts with a retroviral vector encoding Oct4, Sox2, Klf4, and c-Myc. The pig iPSCs could be differentiated into cell derivatives of all three primary germ layers in vitro [78]. Chakritbudsabong et al. also reported the
generation of pig embryonic fibroblast-derived iPSCs and their differentiation ability into cardiomyocytes [79]. Choi et al. reported the generation of pluripotent pig ESCs derived from in vitro-fertilized and parthenogenetic embryos [80]. Although iPSC generation from various animal species has been attempted and criticized [81], it will largely contribute to the autologous/allogeneic transplantation experiments in the future, which may further promote stem cell-based cardiovascular regenerative medicine.

5. Conclusion

The usefulness of large animal models in cardiovascular medicine, especially pigs, was outlined on the basis of literature. Although the advantages and disadvantages of large animals should be further evaluated, there is a possibility that large animal-based approaches may contribute to the investigations in cardiovascular medicine. On the other hand, it is also recognized that there are opinions not to welcome large animal models for research purpose use and accurate regulation is indispensable. The number of animals used should be reduced to a minimum following the three R’s principle (to reduce, refine, and replace animal models). Experiments using animals should be optimized and standardized considering their translatability and the welfare of the animals [82].

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Conflict of interest

H.O declares that he has no conflict of interest. K.M. declares that she has no conflict of interest. H.M. has received research grants from Japan Agency for Medical Research and Development (AMED).
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