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Animal models for heart valve research and development

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Valvular heart disease is the third-most common cause of heart problems in the United States. Malfunction of the valves can be acquired or congenital and each may lead either to stenosis or regurgitation, or even both in some cases. Heart valve disease is a progressive disease, which is irreversible and may be fatal if left untreated. Medications cannot currently prevent valvular calcification or help repair damaged valves, as valve tissue is unable to regenerate spontaneously. Thus, heart valve replacement/repair is the only current available treatment. Heart valve research and development is currently focused on two parallel paths; first, research that aims to understand the underlying mechanisms for heart valve disease to emerge with an ultimate goal to devise medical treatment; and second, efforts to develop repair and replacement options for a diseased valve. Studies that focus on developmental malformation, including genetic and epigenetic causes, usually employ small animal models that are easy to access for *in vivo* imaging that minimally disturbs their environment during early stages of development. Alternatively, studies that aim to develop novel devices for replacement and repair of diseased valves often employ large animals whose heart size and anatomy closely replicate human's. This paper aims to briefly review the current state-of-the-art animal models, and justification to use an animal model for a particular heart valve related project.

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Introduction

Heart valves are important components of the heart and their major function is to maintain the unidirectional blood flow from chamber to chamber, and from chamber to major blood vessels. Mitral and tricuspid valves direct the blood flow from the atria to ventricles while aortic and pulmonary valves control the flow from ventricles to aorta and pulmonary artery, respectively. Malfunction of the valves can be acquired or congenital and each may lead either to stenosis or regurgitation, or even both in some cases [1]. While degenerative valve disease is more common in the elderly population in industrialized countries, rheumatic valve

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disease is still one of the most common etiologies of valvular disease in developing countries [2]. In spite of that, the underlying mechanisms of valvular malfunction and disease progression in many acquired and congenital diseases is not yet known. Presently, there is no medical treatment to valve disease other than procedures to repair or replace a heart valve.

Heart valve research and development is currently focused on two parallel paths: first, research that aims to understand the underlying mechanisms for heart valve disease with an ultimate goal of devising medical treatment; and second, efforts to develop repair and replacement options for a diseased valve [3–5].

Since acquired heart valve disease is exclusive to the human race, there is no naturally-occurring animal model for acquired heart valve disease. Congenital heart defects, however, have been reported to occur in animals. Studies that focus on developmental malformation, including genetic and epigenetic causes, usually employ small animals such as chicken, mouse, and zebrafish to develop their models. This is because these embryos can be manipulated relatively easy to disrupt their valve formation. Alternatively, studies that aim to develop novel devices for replacement and repair of diseased valves often employ large animals such as sheep and pig whose heart size, anatomy, material degradation and endogenous tissue growth closely replicate human's. The present review is focused on small and large animal models currently used for heart valve research and development.

Small animal models for studying cardiac valve development

Heart formation in vertebrate animals involves a complex progression of finely-orchestrated events. The heart begins as a tubular structure that soon after formation, starts pumping blood flow [6]. During initial tubular stages, cardiac tissues consist of three distinct layers: the myocardium, a layer of contractile myocardial cells; the endocardium, a monolayer of endocardial cells; and the cardiac jelly layer, an extracellular matrix (ECM) layer in between the myocardium and endocardium. Along the tubular heart, distinct cardiac structures can be distinguished: the atrium, the atrio-ventricular (AV) canal, the ventricle, and the outflow tract (OFT). The initially-linear tubular heart then bends and twists forming a looping heart tube [7,8]. Valve formation and chamber septation occur after looping [9–11].

Endocardial cushions form in the AV canal and OFT early during tubular heart stages [12–14]. These cushions are cardiac -wall thickenings, initially composed of ECM that is rich in ground substance and largely devoid of fibrous proteins. They act as primitive valves by closing the lumen upon myocardial contraction. Cells from the endocardium, then, detach from the neighboring endocardial cells, acquire a mesenchymal phenotype and migrate into the cushions during the process of endocardial-mesenchymal-transition (EMT). In the cushions, EMT-derived mesenchymal cells proliferate, secrete fibrous ECM proteins such as type I collagen, and continue to migrate, while remodeling the endocardial cushion tissues [14]. EMT thus increases cell density in the cushions and changes their ECM composition. The AV canal cushions, which give rise to the mitral and the tricuspid valves, are exclusively populated by EMT-derived cells; whereas in the OFT cushions, from which the aortic and pulmonary valves originate, the cell population is mainly from EMT but neural crest and secondary heart field cells also contribute to the cushion cell population prior to valve formation. Cushion formation and the subsequent cushion cellularization and remodeling, determine valve formation [15]. In fact, a common deficit of genetic anomalies and teratogen exposures is the formation of anomalous cushions that would lead to congenital valve disease and heart defect phenotypes [16]. Thus the cushions, and the contribution of different cells to the cushion population, have been the main focus of several studies aiming at understanding valve formation and congenital heart valve diseases.

Small animal models are typically used to determine how cardiac valves are formed. These models include mouse embryos, as mammalian models, as well as zebra fish and chicken embryo models. The main advantage of using mouse embryos is that genetic manipulations are common practice, allowing investigators to study the effects of different genes on cardiac (and valve) formation [17]. Further, mouse embryos can also be employed in lineage tracing studies, from which it is possible to determine the origin of cells that form the heart and the valves [18]. However, mouse embryos are difficult to access inside the *uterus*, precluding non-invasive *in vivo* imaging of cardiac function and blood flow conditions during early embryonic stages. Zebra fish and chicken embryos, on the other hand, are easy to access for *in vivo* imaging that minimally disturbs their environment during early stages of development [19–27], making them ideal models to study embryonic changes that lead to cardiac defects. In particular, zebra fish and chicken embryos are extensively used to study the EMT that occurs prior to valve formation and is critical for proper valve development [19,28–31].

The advantage of studying valve development using zebra fish and chicken embryo models is that their cardiac tissues are almost transparent at early stages of development. This enables high resolution imaging of the heart and vasculature, as well as monitoring of blood flow dynamics (blood flow velocity, blood pressure; e.g. see Fig. 1). Genetic manipulations in zebra fish models further enable researchers to monitor the consequences of gene anomalies over time, and better understand early EMT dysregulation and its effect on valve formation [30,32]. In chicken embryos, surgical interventions that alter blood flow through the heart are relatively easy to perform [33]. This allows studying the effects

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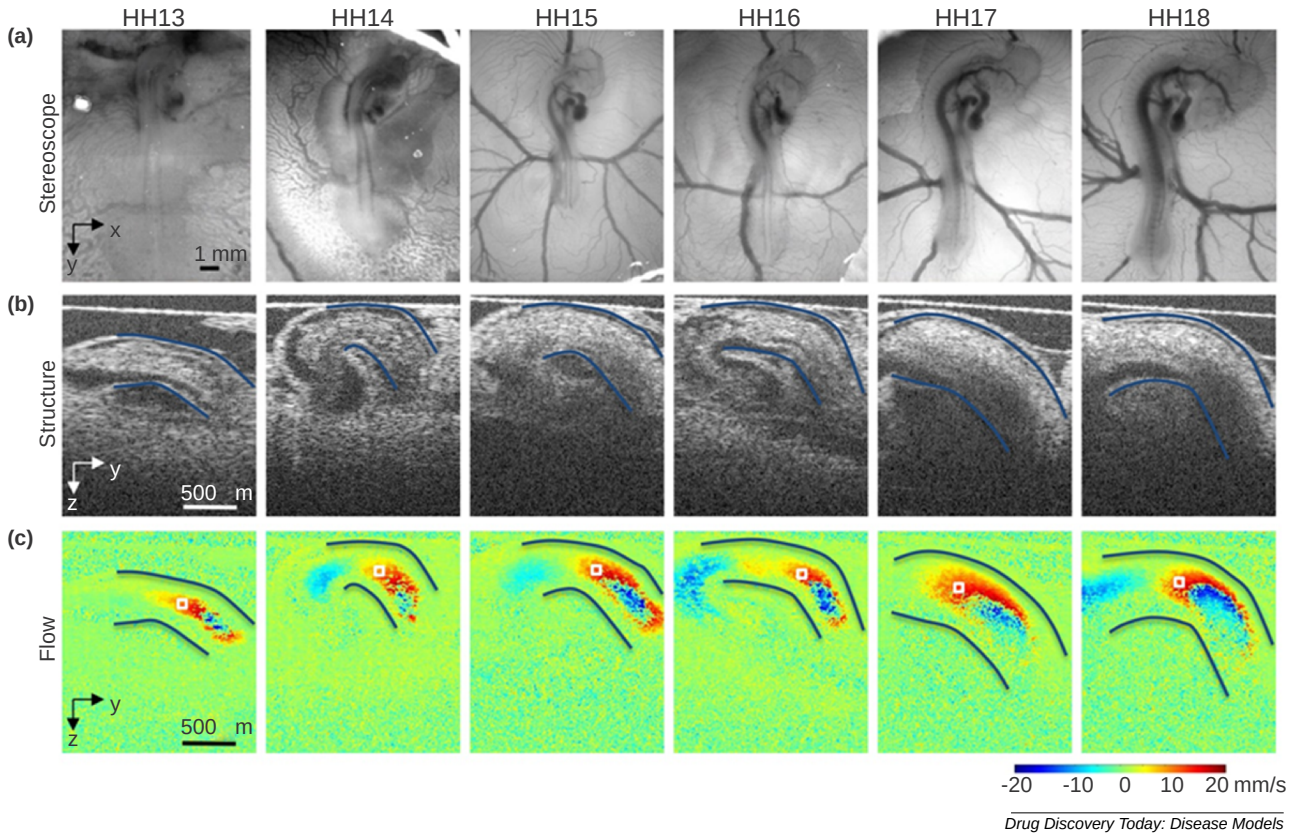

Fig. 1. Images acquired from the hearts of HH13–HH18 chicken embryos, during tubular heart stages. Example (a) optical images of the embryo in ovo on the top of the egg surface, (b) optical coherence tomography (OCT) structural 2D longitudinal images of the heart outflow tract (OFT) and neighboring structures, and (c) corresponding Doppler OCT images, which quantify flow velocity. The OFT myocardial walls are outlined in (b) and (c), and approximately corresponding points to measure blood flow velocity are marked by a box in (c). Endocardial cushions, developing in the OFT at the stages shown, and affected by blood flow, will later give rise to semilunar valves. Reproduced from Midgett et al. [74].

of blood flow dynamics during development of the heart and vasculature. In particular, results from *in vitro* and *in vivo* studies suggest that EMT is modulated by hemodynamic conditions [29,34–36]. Thus valve formation depends on a finely orchestrated sequence of cellular signaling cascades that are modulated by blood flow conditions. Disruption of signaling or blood flow independently or together can lead to valve malformation. While the regulatory function of blood flow on valve formation is widely appreciated, the mechanisms behind this regulation are not yet understood and further studies are needed to elucidate how blood flow influences cardiac and valve formation.

Understanding the factors influencing valve formation is important, not only to understand congenital heart valve disease, but also because developmental programs take place during pathophysiological conditions that lead to valve degradation and malfunction. Therefore, understanding valve formation and malformation, can provide important clues on how to improve treatment and prevention strategies for heart valve disease. Table 1 compares the three small animal models used for valve formation.

Large animal models for research and development of heart valve prosthetics

Preclinical studies to verify the safety and efficacy of new prosthetic heart valves require implantation in proper animal models whose hearts closely mimic human heart. During these studies, functionality, biocompatibility, durability and safety of valve devices are assessed before proceeding to human trials. Traditionally, ovine, swine and canine models have been used for prosthetic heart valve research and development. Although costly and time-consuming, these models help testing the devices' biocompatibility and whether the living tissues are prone to any damage due to the implant in short- and long-term. Overall, the biological responses to implants in these mammalians are fairly similar to human, which can invoke comparable immunological response. However, in case of heart valve tissue engineering research, the mechanisms animal models may affect the degradation rate compared to endogenous tissue formation characteristic should be considered [3,37–40].

Choosing a right animal model for a particular experiment is very important and can significantly affect the cost and the

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Table 1. Comparison among the small animal models for heart valve research

	Zebrafish	Chicken	Mouse
Development	External AV valve starts to form by 105 hpf	In ovo EMT in AV valve cushions initiates at HH Stage 13 and in OFT cushions at HH stage 16, initiating valve development	In utero AV Valve primordia starts to form around 9.5 dpc. OFT cushions form around 10 dpc
Chamber formation	2 chambers (single atrium and ventricle)	4 chambers	4 chambers
Valve formation	AV valve	Aortic valve, pulmonary valve, mitral valve, tricuspid valve	Aortic valve, pulmonary valve, mitral valve, tricuspid valve
Genetic manipulation	Excellent for forward genetics. Rapid improvement in reverse genetics	Rapid improvement in genetic editing with new CRISPR/Cas9 method	Strong in both forward and reverse genetics, including lineage tracing
In vivo imaging	Strong: high-speed confocal microscopy & light sheet microscopy	Strong: Optical coherence tomography, confocal microscopy & optical microscopy	Less advantageous due to access (methodologies in progress)
Surgical and pharmacological manipulation	Easy access for laser-targeted ablation and pharmacological interventions	Easy access for surgical, pharmacological manipulation and laser ablation	Less advantageous

outcome for a particular study. For example, ovine and swine models are both suitable for acute heart valve implant studies and to check whether the device provides acceptable hemodynamics (e.g., desired pressure drop, no regurgitation, acceptable opening, etc.). The chronic animal studies aim to check whether the valve is durable and can maintain its function for a relatively long period of time. During chronic studies, valve developers usually look for signs of calcification, body's adverse reactions to the implant, valve competency and hemodynamic performance in time. As a rule, if chronic study is the goal, sheep or minipigs are preferred since regular pigs grow faster, resulting in device-heart mismatch that will adversely affect the chronic study outcome. Furthermore, keeping larger animals is more difficult and requires larger vivarium space that will add to the cost of the study.

Overall, prosthetic heart valves are implanted to mitigate either a stenotic or a regurgitant native valve. Currently, there is no animal model that mimic either disease. Transcatheter aortic valves (TAVs) are implanted within a highly calcified native aortic valve. However, valvular calcification is a human disease and cannot be replicated in either of the ovine, canine or swine models. Calcified regions in the native valve help secure anchoring of the stented valve but technologies with staged deployment has overcome this limitation (Fig. 2) [41]. The anchoring limitation also pertains to transcatheter mitral valve (TMV) technologies. These valves are mainly aimed to alleviate native mitral valve incompetence. In most cases of mitral valve regurgitation, native valve annulus as well as the left atrium are significantly enlarged. Therefore, a very large prosthesis is needed to be implanted that requires a bulky delivery system. Imitation of enlarged native mitral annulus in animal models are close to impossible as such a disease does not naturally occur in large animal models. To summarize, the failure or success of both TAV and TMV implants in an animal model does not closely correlate with the human outcome. Having said that, many device features related to the transcatheter delivery and deployment can be safely studied and the results greatly mimic implantation in human.

In conclusion, one needs to carefully select an animal model to test a specific aspect of their prosthetic valve to achieve the best result at lowest cost with minimal number of animals used. All acute and chronic conditions should be considered to select the best animal model mimicking human conditions. Here, we briefly describe in more details the three common large animal models (swine, canine, ovine) used for heart valve studies.

Swine model

There are many similarities between the porcine and human heart [42,43]. The similarity among heart valves, major blood vessels, coronary arteries and cardiac conduction system of porcine heart to human's is remarkable and justifies their use for acute testing of the prosthetic heart valves and other cardiac devices. While the similarity can be justified, some studies have shown that the swine model's aortic valve can be different in size, geometry [44], fibrous continuity [45], and expression of metalloproteinase (MMP) I and proteoglycan [46]. As well, the wall thickness ratio between the left and right ventricle is much larger in the porcine heart compared to human's [45,47]. Moreover, human's left atrium receives blood from four or five pulmonary veins in contrast to porcine heart whose left atrium only receives flow from two pulmonary veins [48].

Alternatively, there are several limitations in the use of swine model to be considered; porcine heart is infamously sensitive to

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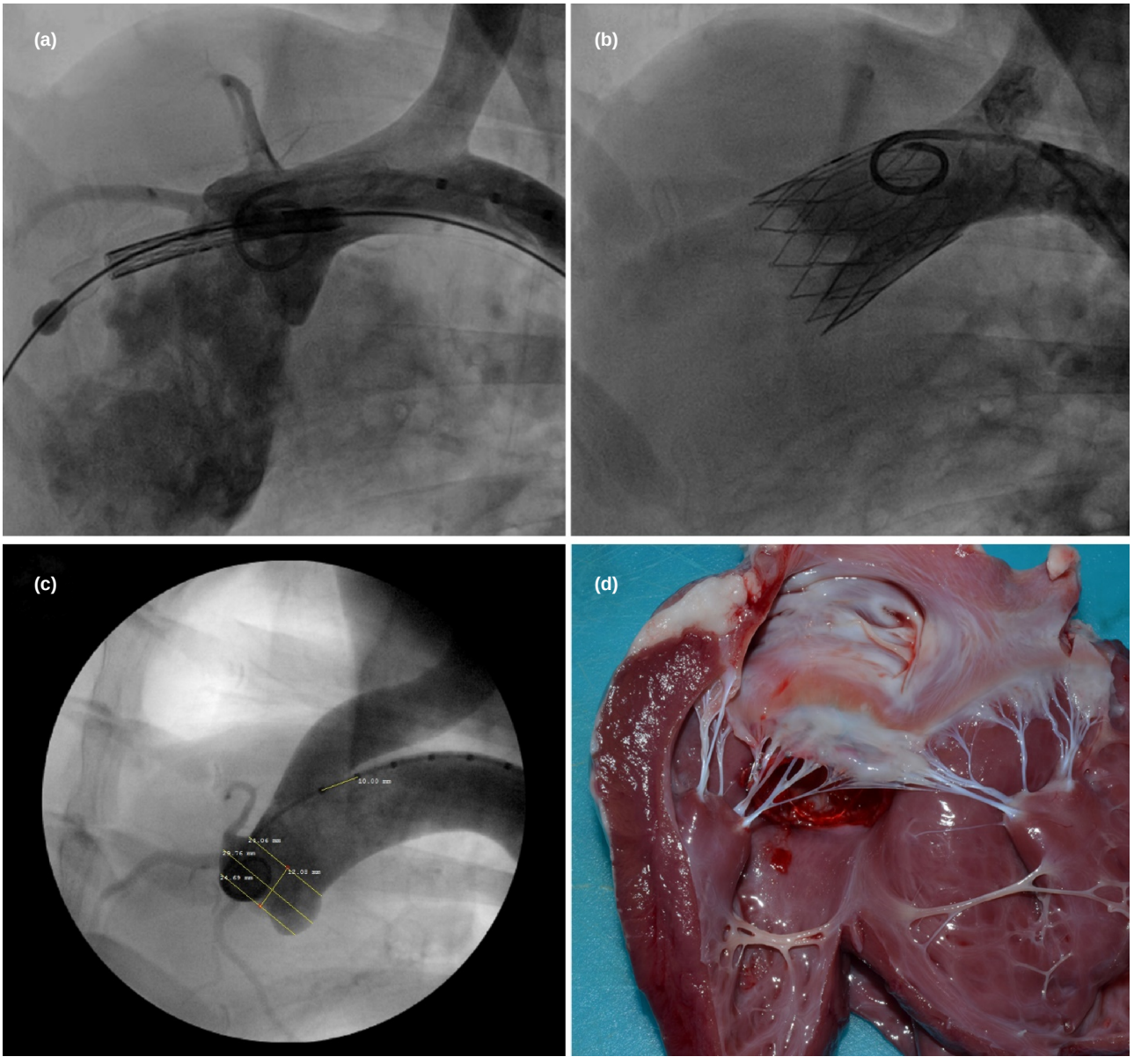

Fig. 2. Transcatheter implantation of FoldaValve in an ovine model's aortic valve. (a) fluoroscopic image of collapsed FoldaValve Advanced into the sheep's left ventricle through aortic valve, (b) FoldaValve deployed and expanded within the sheep's aortic valve. (c) Pre-procedural angiographic studies of sheep aortic annulus to measure the valve's and aortic root's dimensions, (d) post-procedural autopsy shows FoldaValve properly positioned within the sheep's native aortic valve. The images are reproduced from Kheradvar et al. [41].

anesthesia and surgical manipulation that frequently leads to intra-surgical and post-surgical complications including arrhythmia and even death [49]. More specifically, there are several factors that may lead to fatal arrhythmia. For example, human's and swine's cardiac electrophysiology is quite similar with the exception of Ca^{2+}-independent transient outward K^+ current (I_{to1}) whose expression is absent in pigs, so that only I_{to2} is present in porcine ventricular myocytes [50,51]. As well, it has been shown that 'hibernating' the myocardium, commonly practiced during swine surgery, may lead to chronic arterial occlusion leading to cardiac death [52]. The difference in cardiac electrophysiology has led to higher heart rate in pigs compared to human heart [53].

In addition, pigs are prone to post-surgical infection and overall need more regular follow up and attention. To avoid infection, special sterile techniques need to be used. Grehan et al. [49] reported difficulty in maintaining safe levels of anticoagulation with warfarin, when used in a study involving swine model for mechanical heart valve testing. Additionally, they reported marked fibrous formation and thrombosis around the valve as well as perivalvular defects, due to normal somatic growth occurring in young swine [49].

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Canine model

There are some similarities between canine and human heart, but in comparison to human's and ovine hearts, the canine heart has a more connected network of coronary collateralization between right and left coronary circulation that protects their heart from ischemia. Accordingly, canine heart was considered as a large animal model for myocardial ischemia studies. Generally, using catheters in canine model is easier than ovine and swine models during surgery. They have thinner skin, which makes it easier for interventionalist to advance catheters into the vasculatures. On top of that, imaging and cardiac monitoring are more accessible in canine compared to ovine and swine models. Although, canine tricuspid valve normally has two leaflets [54], the lower risk of post-surgical infection after valve implantation was a main advantage to select canine for the valve replacement procedures in the past [55]. However, at the present time, canine models are not commonly used for heart valve implantations mainly due to restrictions in obtaining the necessary approval for performing experiments in this species, which is unique to canines [56,57].

Ovine model

The ovine model is currently considered the best animal model for valve replacement survival studies that satisfy Food and Drug Administration (FDA) and CE mark requirements [58]. The anatomy of the sheep heart as well as physiological parameters such as heart rate and blood pressure are similar to human's [59]. More importantly, valves' sizes of an adult sheep are comparable to human's, which makes it an ideal model for heart valve replacement (Fig. 2) [60]. Alternatively, ovine aortic valves leaflets are thinner and more fragile in comparison with human's [61], and the fibrous continuity that is present in human's mitral and aortic valve leaflets is absent in ovine heart valves [62]. As well, the coronary collateral network in ovine heart is low in comparison to swine and human heart, which makes it an ideal animal model for myocardial ischemia research [63,64]. Finally, ovine growth rate is generally lower than swine, which makes it an ideal model for overall heart valve prosthetics including regular and hybrid tissue-engineered heart valves [65–69].

Since acquired human heart valve disease does not naturally occur in animals, many have tried developing their desired animal model through different methods. For example, Simmons and colleagues identified 584 genes as differentially expressed by the endothelium on the aortic side versus ventricular side of normal porcine aortic valves and used this model to study the correlation between phenotypic heterogeneity and regions of susceptibility in normal valvular endothelium [70]. Some studies show evidence that hypercholesterolemic diets in swine models would lead to human-type disease with small early calcific nodules observed in 6–7 months [71]. Others have used surgical banding to produce graded aortic and mitral valve stenosis [72]. As well, use of synthetic calcified polymeric valves has been suggested to imitate valve-in-valve transcatheter procedures [73].

Conclusion

This paper aimed to briefly review the current state-of-the-art information on animal models used for heart valve research and development, and justification to use an animal model for a particular project. In summary, studies that aim to test novel devices or procedures for replacement and repair of diseased valves may use large animals whose heart size and valve anatomy closely mimic human's. As well, studies with a focus on developmental malformation, genetic and/or disease epigenetics usually employ small animal models that are easily accessible for *in vivo* imaging and can be genetically or physically modified as needed.

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Conflict of intent

Prof. Kheradvar is a co-founder of Folda LLC that commercializes FoldaValve™. He and has an equity interest in Folda LLC, a company that may potentially benefit from the research results. The terms of this arrangement have been reviewed and approved by the University of California, Irvine in accordance with its conflict of interest policies. The other authors have no conflicts of interest to declare.

References

[1] Unger P, et al. Management of multiple valve disease. Heart 2011;97(4):272–7.
[2] Iung B, Vahanian A. Epidemiology of valvular heart disease in the adult. Nat Rev Cardiol 2011;8:162.
[3] Kheradvar A, Groves EM, Dasi LP, Alavi SH, Tranquillo RT, Grande-Allen KJ, et al. Emerging trends in heart valve engineering: part I. Solutions for future. Ann Biomed Eng 2015;43(4):833–43.
[4] Kheradvar A, Groves EM, Goergen CJ, Alavi SH, Tranquillo RT, Simmons CA, et al. Emerging trends in heart valve engineering: part II. Novel and standard technologies for aortic valve replacement. Ann Biomed Eng 2015;43(4):844–57.
[5] Kheradvar A, Groves EM, Simmons CA, Griffith BE, Alavi SH, Tranquillo RT, et al. Emerging trends in heart valve engineering: part III. Novel technologies for mitral valve repair and replacement. Ann Biomed Eng 2015;43(4):858–70.
[6] Wittig J, Münsterberg A. The early stages of heart development: insights from chicken embryos. J Cardiovasc Dev Dis 2016;3(2):12.
[7] Manner J. Cardiac looping in the chick embryo: a morphological review with special reference to terminological and biomechanical aspects of the looping process. Anat Rec 2000;259(3):248–62.
[8] Garita B, Jenkins MW, Han M, Zhou C, VanAuker M, Rollins AM, et al. Blood flow dynamics of one cardiac cycle and relationship to mechanotransduction and trabeculation during heart looping. Am J Physiol Heart Circ Physiol 2011;300:H879–91.
[9] Martinsen BJ. Reference guide to the stages of chick heart embryology. Dev Dyn 2005;233:1217–37.

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[10] Schleiffarth JR, Person AD, Martinsen BJ, Sukovich DJ, Neumann A, Baker CVH, et al. Wnt5a is required for cardiac outflow tract septation in mice. Pediatr Res 2007;61(4):386–91.
[11] Srivastava D, Olson EN. A genetic blueprint for cardiac development. Nature 2000;407(6801):221–6.
[12] Markwald RR, Fitzharris TP, Manasek FJ. Structural development of endocardial cushions. Am J Anat 1976;148:85–120.
[13] Nakajima Y, Yamagishi T, Hokari S, Nakamura H. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)-beta and bone morphogenetic protein (BMP). Anat Rec 2000;258:119–27.
[14] Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. Int Rev Cytol 2005;243:287–335.
[15] Combs MD, Yutzey KE. Heart valve development: regulatory networks in development and disease. Circ Res 2009;105(5):408–21.
[16] Rugonyi S. Genetic and flow anomalies in congenital heart disease. AIMS Genet 2016;3(3):157–66.
[17] Moon A. Chapter 4 mouse models of congenital cardiovascular disease. In: Current topics in developmental biology. Academic Press; 2008. p. 171–248.
[18] Meilhac SM, Lescroart F, Blanpain C, Buckingham ME. Cardiac cell lineages that form the heart. Cold Spring Harb Perspect Med 2014;4(9) a013888.
[19] Bakkers J. Zebrafish as a model to study cardiac development and human cardiac disease. Cardiovasc Res 2011;91(2):279–88.
[20] Lee J, Fei P, Packard RRS, Kang H, Xu H, Baek KI, et al. 4-Dimensional light-sheet microscopy to elucidate shear stress modulation of cardiac trabeculation. J Clin Invest 2016;126(5):1679–90.
[21] Fujimoto JG. Optical coherence tomography for ultrahigh resolution in vivo imaging. Nat Biotechnol 2003;21:1361–7.
[22] Jenkins MW, Adler DC, Gargesha M, Huber R, Rothenberg F, Belding J, et al. Ultrahigh-speed optical coherence tomography imaging and visualization of the embryonic avian heart using a buffered fourier domain mode locked laser. Opt Express 2007;15:6251–67.
[23] Rugonyi S, Shaut C, Liu A, Thornburg K, Wang RK. Changes in wall motion and blood flow in the outflow tract of chick embryonic hearts observed with optical coherence tomography after outflow tract banding and vitelline-vein ligation. Phys Med Biol 2008;53:5077–91.
[24] Li P, Yin X, Shi L, Rugonyi S, Wang RK. In vivo imaging of blood flow and wall strain rate in outflow tract of embryonic chick heart using ultrafast spectral domain optical coherence tomography. J Biomed Opt 2012;17:096006.
[25] Jenkins M, Watanabe M, Rollins A. Longitudinal imaging of heart development with optical coherence tomography. IEEE J Sel Top Quantum Electron 2012;18:1166–75.
[26] Gregg CL, Butcher JT. Quantitative in vivo imaging of embryonic development: opportunities and challenges. Differentiation 2012;84 (1):149–62.
[27] Manner J, Thrane L, Norozi K, Yelbuz TM. High-resolution in vivo imaging of the cross-sectional deformations of contracting embryonic heart loops using optical coherence tomography. Dev Dyn 2008;237(4):953–61.
[28] Tavares ALP, Mercado-Pimentel ME, Runyan RB, Kitten GT. TGFβ-mediated RhoA expression is necessary for epithelial-mesenchymal transition in the embryonic chick heart. Dev Dyn 2006;235(6):1589–98.
[29] Midgett M, López CS, David L, Maloyan A, Rugonyi S. Increased hemodynamic load in early embryonic stages alters endocardial to mesenchymal transition. Front Physiol 2017;8(56).
[30] Steed E, Faggianelli N, Roth S, Ramspacher C, Concordet JP, Vermot J. klf2a couples mechanotransduction and zebrafish valve morphogenesis through fibronectin synthesis. Nat Commun 2016;7:11646.
[31] Tavares ALP, Brown JA, Ulrich EC, Dvorak K, Runyan RB. RUNX2-I is an early regulator of epithelial-mesenchymal cell transition in the chick embryo. Dev Dyn 2018;247:542–54.
[32] Lee YM, et al. Vascular endothelial growth factor receptor signaling is required for cardiac valve formation in zebrafish. Dev Dyn 2006;235: 29–37.
[33] Midgett M, Rugonyi S. Congenital heart malformations induced by hemodynamic altering surgical interventions. Front Physiol 2014;5.
[34] Tan H, Biechler S, Junor L, Yost MJ, Dean D, Li J, et al. Fluid flow forces and rhoA regulate fibrous development of the atrioventricular valves. Dev Biol 2013;374(2):345–56.
[35] Goodwin RL, Biechler SV, Junor L, Evans AN, Eberth JF, Price RL, Potts JD, et al. The impact of flow-induced forces on the morphogenesis of the outflow tract. Front Physiol 2014;5.
[36] Menon V, Eberth JF, Goodwin RL, Potts JD, et al. Altered hemodynamics in the embryonic heart affects outflow valve development. J Cardiovasc Dev Dis 2015;2(2):108.
[37] Cheung DY, Duan B, Butcher JT. Current progress in tissue engineering of heart valves: multiscale problems, multiscale solutions. Expert Opin Biol Ther 2015;15(8):1155–72.
[38] Pérez RA, et al. Naturally and synthetic smart composite biomaterials for tissue regeneration. Adv Drug Deliv Rev 2013;65(4):471–96.
[39] Sodian R, et al. Tissue engineering of heart valves: in vitro experiences. Ann Thorac Surg 2000;70(1):140–4.
[40] Sodian R, et al. Early in vivo experience with tissue-Engineered trileaflet heart valves. Circulation 2000;102(Suppl. 3). p. Iii–22.
[41] Kheradvar A, Groves EL, Tseng EE. Proof of concept of FOLDAVALVE a novel 14Fr totally repositionable and retrievable transcatheter aortic valve. Euro Intervent 2015;10(11):591–6.
[42] Naimark WA, et al. Correlation of structure and viscoelastic properties in the pericardia of four mammalian species. Am J Physiol 1992;263(4 Pt. 2): H1095–106.
[43] Kozlowski T, et al. Porcine kidney and heart transplantation in baboons undergoing a tolerance induction regimen and antibody adsorption. Transplantation 1999;67(1):18–30.
[44] Sim EKW, et al. Comparison of human and porcine aortic valves. Clin Anat 2003;16(3):193–6.
[45] Crick SJ, et al. Anatomy of the pig heart: comparisons with normal human cardiac structure. J Anat 1998;193(Pt. 1):105–19.
[46] Stephens EH, Kearney DL, Grande-Allen KJ. Insight into pathologic abnormalities in congenital semilunar valve disease based on advances in understanding normal valve microstructure and extracellular matrix. Cardiovasc Pathol 2012;21(1):46–58.
[47] Lelovas PP, Kostomitsopoulos NG, Xanthos TT. A comparative anatomic and physiologic overview of the porcine heart. J Am Assoc Lab Anim Sci 2014;53(5):432–8.
[48] König HE, Ruberte J, Liebich J. Organs of the cardiovascular system (systema cardiovasculare). In: König HE, Liebich J, editors. Veterinary anatomy of domestic mammals. New York, NY: Schattauer; 2004.
[49] Grehan JF, et al. Development and evaluation of a swine model to assess the preclinical safety of mechanical heart valves. J Heart Valve Dis 2000;9 (5):710–9. discussion 719–20.
[50] Li G-R, et al. Calcium-activated transient outward chloride current and phase 1 repolarization of swine ventricular action potential. Cardiovasc Res 2003;58(1):89–98.
[51] Schultz J-H, et al. Molecular and functional characterization of Kv4.2 and KChIP2 expressed in the porcine left ventricle. Pflügers Arch 2007;454 (2):195–207.
[52] Canty JM, et al. Hibernating myocardium. Circ Res 2004;94(8):1142.
[53] Bianco RW, Callegos RP, Rivald A, Voigt J, Dalmasso AP. Animal models for cardiac research. In: Laizzo PA, editor. Handbook of cardiac anatomy, physiology, and devices. Minneapolis, MN: Springer; 2009.
[54] Evans HE. The heart and arteries. In: Evans HE, editor. Miller's anatomy of the dog. Philadelphia, PA: Saunders; 1993. p. 586–681.
[55] Bianco RW, et al. Canine model for long-term evaluation of prosthetic mitral valves. J Surg Res 1986;41(2):134–40.
[56] Milani-Nejad N, Janssen PML. Small and large animal models in cardiac contraction research: advantages and disadvantages. Pharmacol Ther 2014;141(3):235–49.
[57] Camacho P, et al. Large mammalian animal models of heart disease. J Cardiovasc Dev Dis 2016;3(4):30.
[58] Iaizzo PA, et al. Heart valves: from design to clinical implantation. Springer; 2013.
[59] Barnhart GR, et al. Bioprosthetic valvular failure. Clinical and pathological observations in an experimental animal model. J Thorac Cardiovasc Surg 1982;83(4):618–31.

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[60] Sands MP, et al. An anatomical comparison of human pig: calf, and sheep aortic valves. Ann Thorac Surg 1969;8(5):407–14.
[61] Sands MP, Rittenhouse EA, Mohri H, Merendino KA. An anatomical comparison of human pig, calf, and sheep aortic valves. Ann Thorac Surg 1969;8(5):407–14.
[62] Hill AJ, Iaizzo PA. Comparative cardiac anatomy. In: Iaizzo PA, editor. Handbook of cardiac anatomy, physiology, and devices. New York, NY: Springer; 2009.
[63] Schaper W, Flameng W, DeBrabander M. Comparative aspects of coronary collateral circulation. Adv Exp Med Biol 1972;22:267–76.
[64] DiVincenti L, Westcott R, Lee C. Sheep (*Ovis aries*) as a model for cardiovascular surgery and management before, during, and after cardiopulmonary bypass. J Am Assoc Lab Anim Sci 2014;53(5):439–448.
[65] Alavi SH, Kheradvar A. A hybrid tissue-engineered heart valve. Ann Thorac Surg 2015;99(6):2183–7.
[66] Syedain Z, et al. Tubular heart valves from decellularized engineered tissue. Ann Biomed Eng 2013;41(12):2645–54.
[67] Syedain ZH, et al. Implantation of a tissue-engineered heart valve from human fibroblasts exhibiting short term function in the sheep pulmonary artery. Cardiovasc Eng Technol 2011;2(2):101–12.
[68] Driessen-Mol A, et al. Transcatheter implantation of homologous off-the-shelf tissue-engineered heart valves with self-repair capacity: long-term functionality and rapid in vivo remodeling in sheep. J Am Coll Cardiol 2014;63(13):1320–9.
[69] Gottlieb D, et al. In vivo monitoring of function of autologous engineered pulmonary valve. J Thorac Cardiovasc Surg 2010;139(3):723–31.
[70] Simmons CA, et al. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res 2005;96(7):792–9.
[71] Guerraty MA, et al. Hypercholesterolemia induces side-specific phenotypic changes and peroxisome proliferator—activated receptor-γ pathway activation in swine aortic valve endothelium. Arterioscler Thromb Vasc Biol 2010;30(2):225–31.
[72] Taylor DE, Whamond JS. A method of producing graded stenosis of the aortic and mitral valves in sheep for fluid dynamic studies. J Physiol 1975;244(1):16–7.
[73] Falahatpisheh A, Morisawa D, Toosky TT, Kheradvar A. A calcified polymeric valve for valve-in-valve applications. J Biomech 2017;50:77–82.
[74] Midgett M, et al. Blood flow through the embryonic heart outflow tract during cardiac looping in HH13–HH18 chicken embryos. J R Soc Interface 2015;12(111).

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