Animal Models for Cardiac Research

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Abstract

The modern era of cardiac surgery is largely considered to have begun in the animal research laboratories. Today, animal models continue to be used for the study of cardiovascular diseases and are required for the preclinical assessment of pharmaceuticals, mechanical devices, therapeutic procedures, and/or continuation therapies. This chapter was written to provide readers and potential investigators with important background information necessary for the process of matching an experimental hypothesis to an animal species that will serve as an appropriate model for studying a specific cardiovascular disease or for testing a given medical device. A review of the current animal models used in cardiac research is provided and arranged by disease state. Critical factors to consider when choosing an appropriate animal model including costs, reproducibility, and degree of similarity of the model to human disease are discussed. Thus, this chapter can be utilized as a practical guide for planning of research protocols.

Keywords

Animal model • Isolated cardiomyocytes • Isolated perfused heart • Valve disease • Atrial fibrillation • Myocardial ischemia • Heart failure • Heart transplantation • Mechanical device testing • Cardiomyoplasty • Stem cell research

27.1 Protocol Development

Several scientific governing bodies have developed guidelines and periodic review processes to ensure that research animals are used in an ethical and scientifically appropriate manner. Investigators who plan to utilize animal subjects in their research should first familiarize themselves with the document entitled "Guide for the Care and Use of Laboratory Animals" prepared by the US National Academy of Sciences [1]. In addition, investigators should use these guidelines in conjunction with accepted scientific methods in order to develop a standardized protocol for each research project. It is a requirement that prior to commencing research, a detailed protocol undergoes review and is approved by the local governing body responsible for the safe and ethical use of animals in research. In most organizations in the USA where research using animals is performed, the standard governing body is known as the Institutional Animal Care and Use Committee, or IACUC (www.iacuc.umn.edu).

Both large and small animals have been extensively used in cardiovascular research. The choice of animal model should be primarily based on: (1) the scientific hypotheses; (2) the laboratory's capability to safely employ the model in the species chosen (i.e., expertise in the selected procedure, appropriate animal housing and care, equipment, laboratory resources); and (3) the degree of the species similarity to the human anatomy relative to the device or procedure to be tested. It is important to note that many of the best animal models can be expensive to establish and maintain and, as

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such, funding must be appropriate to complete the required number of animal experiments to satisfy a pre-calculated statistical power. Several obvious technical limitations for the use of small animals exist, for example in the case of implanting mechanical devices such as heart valves. The sheer size of the animal is not large enough to implant a valve fit for a human; the animal species must be chosen to fit the device to be studied. Great strides have been made in both imaging (ultrasound and MRI) and miniaturizing electronic equipment that can be used for monitoring physiological parameters, allowing for more intensive cardiac monitoring within small animal models. In choosing the animal species, the researcher should attempt to match physiological parameters (of the animal) as closely as possible to those of humans to obtain results that are the most clinically relevant. Note that many tables of physiological values are available for commonly used research animal species, and these should be used to assist in choosing the appropriate model [2–6]. For more details see Chap. 6.

27.2 Spontaneously Occurring Animal Models of Congenital Cardiac Disease

Naturally occurring animal models of cardiac disease arise infrequently; however when they do occur, there is marked variability in the observed phenotype and they also frequently occur with other congenital abnormalities that hinder breeding efforts. Furthermore, genetic manipulation of breeding stock for specific mutations has economical, ethical, and moral issues that preclude the development of such breeding lines. As a result, the commercial availability of animals for such specific research purposes continues to be quite limited, necessitating the development of contrived models in most cases. Yet, great strides have been made in the application of classical breeding techniques and, more recently, molecular engineering to develop breeding stocks of mice and, in some cases, rats. Specifically, the use of transgenic mice and mice with gene deletions or other induced genetic changes has been considered essential for investigating pathophysiologic mechanisms of disease, including those of the cardiovascular system. Moreover, these genetically modified animals, in some instances, are useful for the initial in vivo testing of pharmaceuticals and/or certain devices [7].

27.3 Alternatives to Whole Animal Models

In some cases, isolated cardiac cell lines in culture, segments of myocardium, or isolated heart models may provide an effective alternative to whole animal research. Isolated preparations have been particularly useful for the study of metabolic pathways, as the perfusate can be modified while the effluent can be easily collected for analysis. Additionally, functional measurements can be easily completed in this in vitro environment. Given the need to reduce cost and limit the number of animals used in research, the attraction of using alternatives to whole animal models is strong. However, regulations typically do not permit the direct extrapolation of the experimental findings from isolated in vitro models to subsequent clinical trials. For example, in vitro studies often demonstrate whether a pharmaceutical agent is potentially useful; however, the concentrations used may be either toxic or the agent may lack efficacy when pharmacokinetics and pharmacodynamics are investigated in the live animal. Nevertheless, these in vitro alternatives are essential for initial studies pertaining to myocardial ischemia, transplantation, and/or pharmaceutical development.

27.3.1 Isolated Cardiomyocytes

The use of isolated cardiomyocytes has allowed researchers to eliminate confounding interactions with surrounding tissue elements. It has also allowed for the measurement of intracellular changes at the single cell level. Yet, care must be taken to match the culture conditions to those of the intact organ to ensure both the viability of the cells used and the quality of the data collected [8, 9]. This simple model provides an important approach before the use of whole animals in early phase testing of experimental protocols, and is of particular interest for use in the testing of new pharmacological agents and/or gene therapies. Cardiac myocyte cultures can be obtained from freshly isolated tissue, differentiated embryonic stem cells or multipotent adult progenitor cells, or immortalized tumor cell lines such as HL-1 from the At-1 mouse [10]. Some functions of cardiac myocytes that can be examined include: (1) contractility, using optical or mechanical detectors; (2) RNA expression (e.g., using Q-PCR); and/or (3) membrane integrity, and measuring release of lactate dehydrogenase, creatine phosphokinase, or troponin [9, 11–15]. Nevertheless, one has to consider that cultured cells may respond differently to a given therapy than those within a living organism.

27.3.2 Isolated Perfused Hearts

An isolated heart perfusion system replicates the physiological conditions outside of the body, allowing for easy access for measurement of the perfused effluent. Isolated in vitro perfusion studies have been performed using the entire heart or a portion of the heart (e.g., intraventricular septum, papillary muscle). Commercially available setups for such in vitro studies are available for the mouse, rat, and guinea pig hearts (ADInstruments, Colorado Springs, CO, USA; Fig. 27.1).

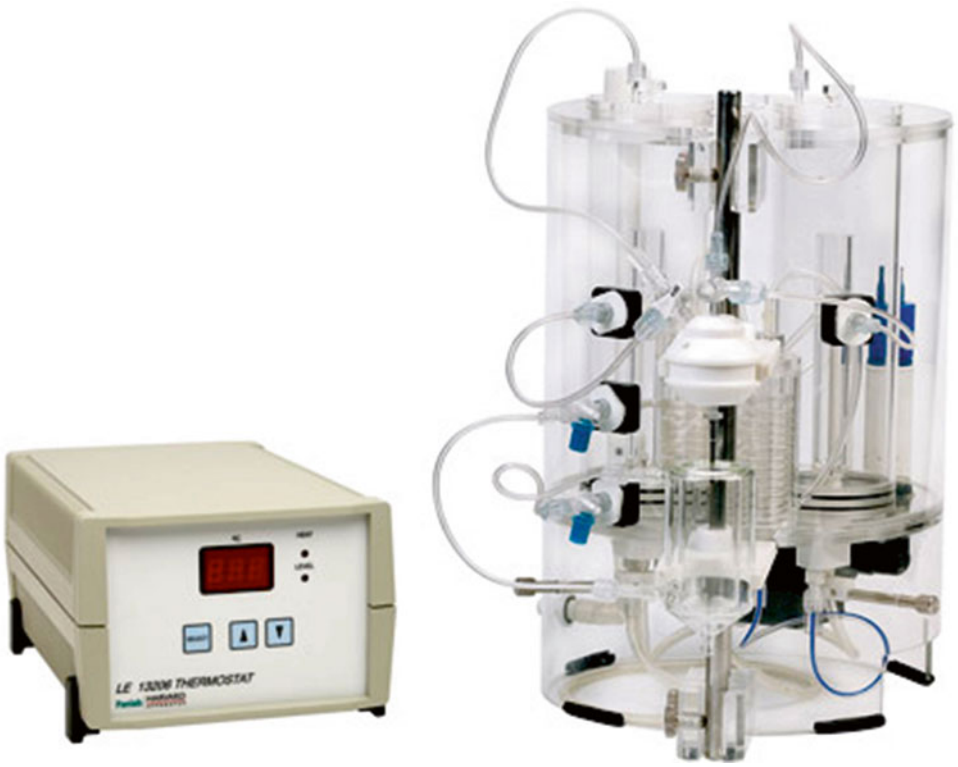

Fig. 27.1 ADInstruments Langendorff perfusion setup. Courtesy of ADInstruments

Larger setups have also been described to accommodate canine, porcine, ovine, and human hearts. An excellent example of an application of the isolated heart model can be seen on the Atlas of Human Cardiac Anatomy website (www.vhlab.umn.edu/atlas) and on The Visible Heart website (www.vhlab.umn.edu).

Several different methods for studying the isolated heart are possible, two of these methods are the Langendorff perfusion approach and the isolated working heart model [16, 17]. In the Langendorff system, constant pressure flow through an aortic cannula forces the aortic valve closed, and the perfusate passes through the coronary arteries without retrograde flow entering the left ventricle. This perfusion provides the myocardium with a physiologic solution, allowing the heart to beat without blood flowing through the four chambers. This method was named after Oscar Langendorff who, in 1895, was the first to describe an experimental model of an isolated mammalian heart as a technique to assess its required contractile activity. The advantage of the Langendorff perfusion method is that the measurement of EKG changes can be easily assessed, as well as measurement of metabolites that drain from the coronary sinus. Yet, the lack of flow in the left ventricle may limit its usefulness, i.e., minimal blood entering into the ventricle may promote clot formation, in turn, affecting the viability of the preparation. Additionally, the lack of flow in the ventricle may result in abnormal three-dimensional conformational changes in the heart that may cause coronary vascular compression. However, placement of a fluid balloon connected to a pressure transducer may allow for partial control of this problem, and may also be useful experimentally to assess changes in left ventricular function. Recent studies utilizing variations of the Langendorff working heart have adapted the setup for a range of different heart sizes. The classic hydrostatic afterload column is replaced with a centrifugal pump, allowing for easy adjustment and tight regulation of perfusion pressures, meaning the same setup can be used for various species or heart sizes [18].

The major disadvantage of the Langendorff preparation is that it does not eject the perfusate from the left ventricle and is therefore a non-work-producing model. This problem was initially overcome by Neely who used an isolated working heart which simulates physiological flow through the heart's four chambers [19]. In this model, the perfusate is supplied by a cannula inserted into the left atrium; outflow through the left ventricle is monitored, while left atrial pressure or aortic pressure is controlled. This setup is considered ideal for the study of pressure and flow in the aorta as well as the left and right ventricles.

27.3.3 Additional Problems with Isolated Perfused Heart Models

Both types of isolated heart preparations have problems in common that should be considered when attempting to

extrapolate results to the in vivo condition. First, the isolation process used for these models requires global myocardial ischemia (a period of no perfusion). Typically, once the organ is reperfused, baseline data (heart rate, left ventricular pressure, coronary blood flow) must be collected after a stabilization period to ensure relative viability of the preparation. Clearly, both the ischemic time and stabilization time may influence research outcomes. Therefore, any results obtained must be carefully analyzed with reference to the preparation's baseline state as well as to the normal in vivo values, to avoid falsely attributing changes in cardiac function to the experimental protocol.

The composition of the perfusate can greatly impact the function and viability of the preparation in both of the aforementioned models. Early studies utilizing isolated heart models have employed whole blood as a perfusate [20]. However, significant problems with clotting and hemolysis may limit the time that the preparation remains viable. Saline compounds, which lack the potential for clotting and hemolysis, are considered useful alternatives to whole blood. However, such buffers have a lower colloid osmotic pressure and, coupled with the lower coronary vascular resistance, will typically result in progressive and severe edema; this results in interstitial edema formation and nonuniform perfusion. To extend the usefulness of the preparation, one can add osmotically active substances to the medium used for bathing and perfusing the preparation in an attempt to limit edema [21, 22]. Nevertheless, despite the technical difficulties associated with these models, isolated hearts have been used in research ranging from ischemia to transplant studies. For more details on isolated heart experimentation, the reader is also referred to Chap. 41.

27.4 Animal Models Used to Test Devices for Treatment of Valvular Disease

The significant morbidity and mortality associated with heart valve disease has produced a highly competitive market for manufactured prosthetic valves. Efforts to develop the ideal replacement heart valve have focused on producing a device that functions like the native valve (Table 27.1). To this end, certain basic principles of physics are fundamental in the design of mechanical valves, as evidenced by the evolution of various designs. The dynamics of blood flow through a tube with its specific viscosity is such that the flow is greatest in the center of the tube. Thus, any structure in the center of the valve (i.e., mechanical valve leaflets) will reduce the velocity through that valve (Fig. 27.2).

Table 27.1 Qualities of the ideal device for heart valve replacement

• Durable
• Does not leak
• Biologically inert
• Nonthrombogenic
• Facilitates laminar flow
• Easily implanted by the surgeon
• Quiet

Guidelines for the design and testing of bio-artificial and/or mechanical heart valves have been established by the Center for Devices and Radiological Health of the Food and Drug Administration (FDA) and the International Organization for Standardization (ISO). The FDA has provided industry assistance in the form of guidance documents, advice, reporting, premarket approval, development of standards, and third-party reviews. Typically, prosthetic valve replacements are classified as either tissue (Fig. 27.3) or mechanical (Figs. 27.2 and 27.4), yet despite their common purpose, specific valve composition and function vary widely. Nevertheless, all valves must undergo performance-based testing to examine hydrodynamic performance (Table 27.2). For example, accelerated cyclic testing provides wear information, allowing for estimates of structural performance by providing data on fatigue, endurance limits, and damage tolerances of the valve.

Importantly, the FDA requires the demonstration of both efficacy and safety of prototype heart valve replacements prior to final approval for human implantation. This is based on the principle that additional technical and biological information can be gained by observing the valve in actual use. As a result, animal studies remain a crucial component in the overall evaluation of replacement heart valves [23]. To date, all investigational valves undergo a preclinical animal study with valve implantation in the orthotopic or anatomically normal position (with a required 20-week minimum period of evaluation). Specifically, the FDA looks for separate data from mechanical and biological valve studies. For example, mechanical valves generally place extreme shearing forces on the red blood cells and platelets, causing hemolysis and thrombosis that necessitate chronic anticoagulation after valve implantation. On the other hand, biologic valves place very low shear forces on the red blood cells and platelets, and thus there is no need for anticoagulation; however, they are sensitive to formation of calcium deposition, requiring the incorporation of some measures in their manufacturing that will attempt to prevent calcification after implantation. The lack of naturally occurring models of valve disease and the need for standardized models for FDA/ISO approval has led to the use of iatrogenic models of valve disease. To date, the ovine model has been used for producing a graded stenosis in the aortic and mitral valves by banding the aorta in young animals [24]. In contrast, aortic supravalvular stenosis, as well as aortic valvular stenosis, has been commonly induced in the canine model [25, 26]. Additionally, induction of mitral valve regurgitation in the

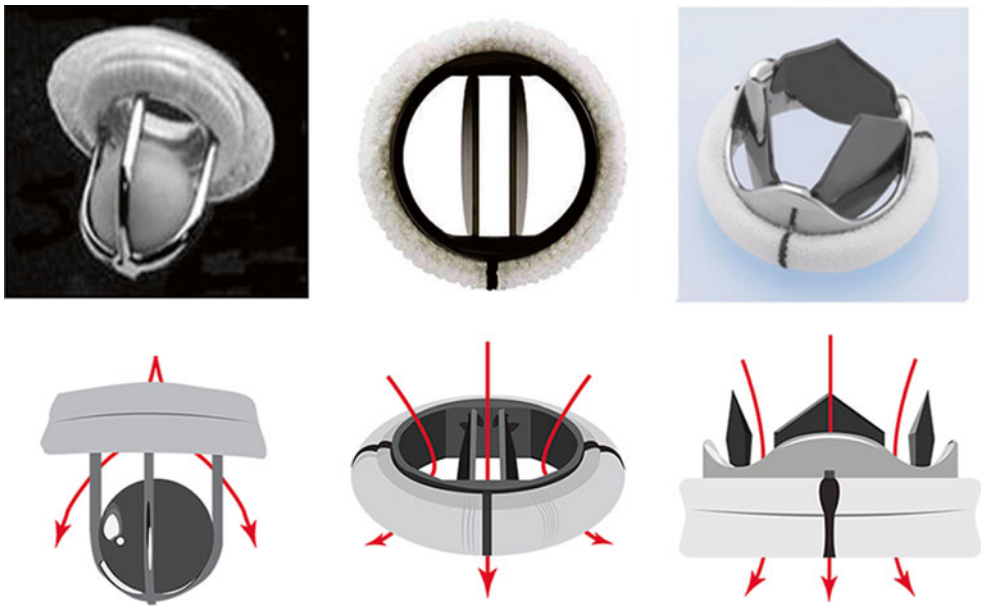

Fig. 27.2 Comparison of different mechanical valves with their flow characteristics. The evolution of the valve from the Starr-Edwards ball (*left*), the current standard bi-leaflet (*center*; St. Jude Medical, St. Paul, MN, USA), and a novel trileaflet design (*right*, Triflow Medical Inc.) currently in development. Below each valve is a stylized representation of the flow patterns reflecting the improvement in valve design

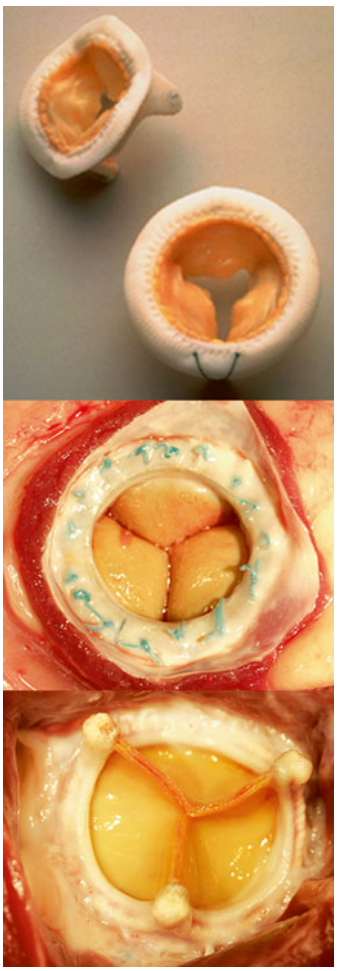

Fig. 27.3 (*Top*) The Medtronic Mosaic® stented tissue valve (Medtronic, Inc., Minneapolis, MN, USA). (*Middle*) Carpentier-Edwards Perimount Plus 6900P stented tissue valve, inflow aspect in the Mitral position in a sheep. (*Bottom*) Carpentier-Edwards Perimount Plus 6900P stented tissue valve, outflow aspect in the Mitral position in a sheep

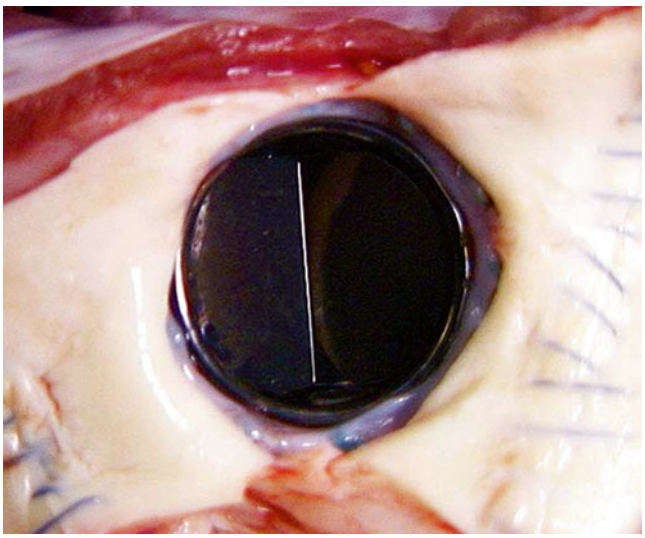

Fig. 27.4 Ovine model of a normal bileaflet mechanical valve implantation

canine is possible by placement of a shunt [27] or by transection of the chordae tendineae. Interestingly, experiments have also been performed to induce stenosis or regurgitation in the tricuspid and pulmonic valves [28]. However, most valve implantation studies approved for human use are completed in normal animals and their primary goals are to strictly examine valve performance (Fig. 27.4). A thorough understanding of the background and natural history of heart valve disease in standard laboratory animal species is needed to ensure that spontaneous valve lesions are not misinterpreted as treatment related [29].

A principal advantage of employing the canine model is the large amount of background information available in the cardiovascular surgical literature. Historically, the dog was

Table 27.2 Mechanical valve fluid dynamic testing

• Forward flow testing
• Backflow leakage testing
• Pulsatile flow pressure drop
• Pulsatile flow regurgitation
• Flow visualization
• Cavitation potential
• Verification of the Bernoulli relationship

Source: "Replacement Heart Valve Guidelines," formulated by the US Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health

considered to be the gold standard for both acute and chronic models of valve replacement that was accepted by the FDA. Early success with the canine model in valve replacement identified the need for minimizing the risk of surgical infection at the time of prosthesis implantation. Specifically, the use of preoperative parenteral and postoperative topical antibiotics, strict sterile techniques, minimum numbers of operative arterial and venous lines, and short cardiopulmonary bypass times were all noted parameters to minimize the risk of bacterial valve implant seeding [30].

As described in Chap. 6, the anatomy of the porcine heart is most similar to that of the human heart in regards to the conduction system, coronary arteries, blood supply to the conduction system, and great vessels. In addition, the coagulation cascade of the swine is quite similar to that of humans. Despite these advantages, several problems have been identified in using this model for valvular research. First, the porcine heart is extremely sensitive to anesthesia, and surgical manipulation often results in postsurgical complications, arrhythmias, and/or death. Second, the growth of young swine is rapid, resulting in heart size and physiological flow that is not constant over required follow-up periods of several months; yet, if you want to investigate the effects of heart growth on a device, using the swine may be beneficial. Specifically, these alterations often result in fibrous sheathing and obstruction of the valve orifice, thrombus formation, or dehiscence (separation) of the sewing cuff from the native annulus. Finally, significant bleeding complications due to application of anticoagulation therapy and poor survival have limited the use of the pig in studying valve-related thrombosis [31]. A recent porcine model for aortic valve sclerosis was able to mimic early human aortic valve disease by feeding swine a high-fat/high-cholesterol diet. This study showed the efficacy of modifying certain factors in a study, allowing for animal models to mimic changes seen in humans [32].

The ovine model is currently accepted as the gold standard for valve replacement using defined survival surgeries that meet FDA requirements. Normal cardiovascular physiological parameters of sheep approximate those of humans in blood pressure, heart rate, cardiac output, and intracardiac pressure [33]. In addition, the anatomy of the adult heart provides valve orifice diameters that are similar to humans [34]. The use of animals of similar age and weight (8–12 months, 30–40 kg) allows for the testing of replacement valves using a single orifice size for comparison of valve performance to an appropriate standard. Although the heart and vessels are small in animals within this weight range, the sheep's relatively large left and right atria allow for straightforward surgical approaches to either the mitral or tricuspid valves.

In general, sheep as experimental animals allow for easy handing and long-term husbandry. Furthermore, juvenile sheep grow at a rate that does not cause excessive mitral or aortic stenosis during the postimplantation test periods, as compared to the porcine model [31]. However, specific attention to gastric decompression, perioperative antibiotics, sterile techniques, and minimally invasive interventions in the postoperative period will all increase the success of valve implantation studies in the ovine model [35].

27.4.1 Animal Models of Atrial Fibrillation for Preclinical Valve Testing

Given the increasing number of patients afflicted with atrial fibrillation worldwide, an animal model of the disorder is needed to predict valvular function and its effects on the natural course of the disease. For example, in one study, atrial fibrillation was associated with morbidity secondary to stroke (13 %) and congestive heart failure (24 %) despite anticoagulant treatment and independent of New York Heart Association (NYHA) functional classification, type of surgery, coronary artery disease, history of coronary artery bypass graft surgery, or other cardiac risk factors [10]. Previous research has uncovered a number of cardiovascular structural and electrophysiological alterations associated with atrial fibrillation [23–26]. More specifically, the fibrillating heart will have a shorter refractory period at the right atrial appendage, shorter action potential duration, electrophysiological remodeling, and changes in gene expression [24, 26]. Myocardial remodeling leading to atrial enlargement appears to be a direct result of atrial fibrillation. From a structural standpoint, the fibrillating left atrium is larger, has relative stasis of blood particularly in the atrial appendage, and fails to give the "atrial kick" which comprises approximately 20 % of ventricular filling. These characteristics also explain the increased thromboembolic risk and decreased cardiac output associated with atrial fibrillation.

27.4.2 Pacing-Induced Atrial Fibrillation

Control of the heart beat using electrical stimulation is usually achieved using an intracardiac or transesophageal

approach. Intracardiac pacing, subdivided into burst pacing and continuous pacing, is the most commonly used procedure for the induction of atrial fibrillation in the sheep model and in animal models overall. Rapid atrial pacing is the most common method of inducing atrial fibrillation for in vivo investigation. The transesophageal approach to pacing represents another possibility; however, it is used in humans and animal models primarily in the detection and assessment of irregular cardiac rhythms and coronary artery disease [36]. It should also be noted that implantable systems offer another alternative for the delivery of right atrial rapid pacing in order to induce atrial fibrillation in conscious animals [37].

27.4.3 Pharmacologic-Induced Atrial Fibrillation

Administration of catecholamines and acetylcholine perfused through the sinoatrial nodal artery can induce atrial fibrillation. Isoproterenol (nonselective beta-adrenergic agonist) and adrenaline (alpha-and beta-adrenergic agonist) induce atrial fibrillation in dogs [38]. Atropine treatment prevented catecholamine-mediated atrial fibrillation, indicating a critical role of cholinergic tone in these atrial fibrillation episodes. Acetylcholine-mediated atrial fibrillation is facilitated by isoproterenol, which decreases the threshold of acetylcholine concentration required for atrial fibrillation induction and increases the atrial fibrillation duration. The focal delivery of these agents into atrial tissues can also cause episodic fibrillation.

27.4.4 Other Potential Atrial Fibrillation Models

Given that many genes are associated with cardiac contractility, it is reasonable to postulate that genetic engineering may have a potential role in the development of an atrial fibrillation model. The first important advance in this direction has been the identification of a genetic locus for familial atrial fibrillation on chromosome 10q22-q24 [39]. The discovery that stem cell-derived cardiomyocytes have an intrinsic arrhythmic potential further leads to the question whether stem cell therapy could be the basis for a model of atrial fibrillation [40].

27.5 Animal Models in Myocardial Ischemia

Despite great advances in treatment options, atherosclerotic coronary vascular disease remains one of the leading causes of death worldwide. As a result, this disease continues to be an active area of cardiovascular research. Originally defined by the Greeks as a lack of blood flow, the modern definition of ischemia emphasizes both the imbalance between oxygen supply and demand as well as the inadequate removal of waste products. Impaired oxygen delivery causes a reduction in oxidative phosphorylation, resulting in myocardial dependence on anaerobic glycolysis for the production of high-energy phosphates. This shift in metabolism produces excess lactate which then accumulates in the myocardium. As impaired ATP production and local tissue acidosis prevails, there is a resultant decline in cardiac contractility. Ultimately, if ischemia is not reversed, myocardial infarction occurs with permanent cellular loss and impaired cardiac function. Multiple experimental techniques have been developed for the study of cardiac ischemia. Currently, scientists consistently use isolated myocytes to examine single cell responses, while isolated perfused hearts and whole animal models allow for a better understanding of the whole organ responses. Regardless of the model type, experimental animals remain a crucial tool in the area of research.

27.5.1 Experimental Methods for Creating Ischemia

The ideal model for investigations of ischemic myocardium would theoretically be the intact chronically instrumented awake animal, as acute surgical trauma and anesthetic agents both depress cardiac function [2]. The conscious animal model also has the advantage that it can be used in studies requiring physiological stress, e.g., stress produced by exercise. However, the high cost of the implanted transducers and probes as well as difficulties with measurement techniques often preclude the use of such an approach. To date, the majority of studies use anesthetized animal models for the study of ischemia in either closed or open chest models. Closed chest models have the advantage that tissue trauma is minimized, but in such models, direct access to the heart for metabolite measurement is a major limitation. In contrast, the open chest preparation has the advantage that regional function and metabolism can be studied in detail. The open chest models suffer from drawbacks that include a greater susceptibility to temperature variations and a potential for surgical trauma that may considerably alter cardiac function (Fig. 27.5).

Multiple techniques have been used to create models of myocardial ischemia for research purposes, depending on whether the desired occlusion is to be permanent, temporary, or progressive. Methods to produce complete permanent occlusions include surgical coronary artery ligation or radiological embolization with microparticles. Furthermore, permanent or temporary partial coronary occlusions are commonly induced by ligation, balloon occlusion, or clamping.

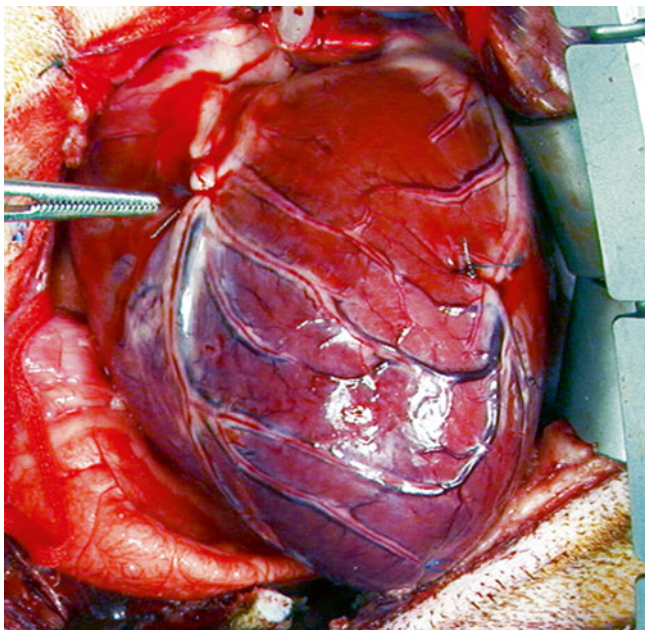

Fig. 27.5 Ligated left anterior descending coronary artery (adjacent to forceps) in an open chest canine model

Typically, models of progressive coronary artery occlusions use either balloon/catheter occlusion or ameroid constrictors (Fig. 27.6). Regardless of the method chosen, the researcher must be aware that the concentric experimental lesions that are created will differ from those of naturally occurring atherosclerotic coronary vascular disease which are typically eccentric. Normally, such eccentric stenoses remain vasoactive and are capable of altering coronary blood flow by changing their lumen diameter. It should be noted that no such vasoactivity remains in experimentally created concentric lesions which will prohibit humoral agents from altering regional coronary flow (Fig. 27.7).

Experience has shown that an induced occlusion of the left anterior descending coronary artery is favored over that in the left circumflex coronary artery for the production of regional myocardial ischemia. It is generally accepted that the occlusion of the left anterior descending coronary artery results in a larger area of myocardial ischemia, and therefore greater impairment of global left ventricular function. However, estimates of infarction size alone have not correlated well with ventricular function [41]. Thus, it has been demonstrated that for the same amount of ischemic myocardium, the compensatory increase by the nonischemic myocardium is different for the left anterior descending coronary artery and the left circumflex coronary arteries [42]. Therefore, in an ideal model, both infarct size and its location must be similar in order to achieve the same degree of impairment in left ventricular global function. If possible, one should also estimate the ischemic area at risk due to an imposed occlusion, e.g., with imaging or the use of dyes.

27.5.2 Localizing and Quantifying Myocardial Ischemia

Blood samples collected from the coronary sinus or from a regional coronary vein are commonly obtained and used for metabolic studies. Yet, such results must be interpreted with the knowledge that these samples include blood from adjacent noninjured myocardium. The use of coronary venous samples for studying metabolism is decreasing because of new approaches using microdialysis, MRI, nuclear magnetic resonance spectroscopy, and positron emission tomography [43–45].

The size and location of myocardial infarction can be determined by Triphenyltetrazolium chloride (TTC) staining, which has been the gold standard for quantifying the extent of myocardial infarction in pathological specimens [46] (Fig. 27.8). In addition, the assessment of localized tissue blood flow using microspheres (radioactive or colored) remains another important standard. However, newer noninvasive methods of determining blood flow in the live animal that allow for repeated follow-up determinations are being developed and improved upon, including spectroscopy and MRI.

27.5.3 Specific Animal Models for Ischemia Investigations

Both large and small animal models have been developed for the study of myocardial ischemia. Advantages of large animal models are their similarity in physiology to humans and ease of instrumentation, and disadvantages include significantly greater care and cost issues that may make small animal models more attractive, particularly when large numbers of animals are required to achieve significant statistical power [47].

The dog has been the most frequently utilized species for in vivo studies of chronic ischemia because dogs have a well-developed coronary collateral circulation, similar to humans with chronic ischemia (progressive heart failure). Furthermore, dogs are easy to handle and lack significant growth as adults, which allows long-term follow-up. However, the significant variability in coronary collateral circulation may hamper efforts to create consistent sizes of ischemic regions between animals, or may result in a minimized ischemic zone.

The pig heart is closer to the relatively healthy human heart with limited collateral blood flow; this makes the swine heart ideal for acute ischemia studies. However, long-term follow-up using the swine model, in general, is considered problematic; if juvenile animals are utilized, significant changes in animal weight will result in both increased difficulties with handling as well as alterations in basic cardiac

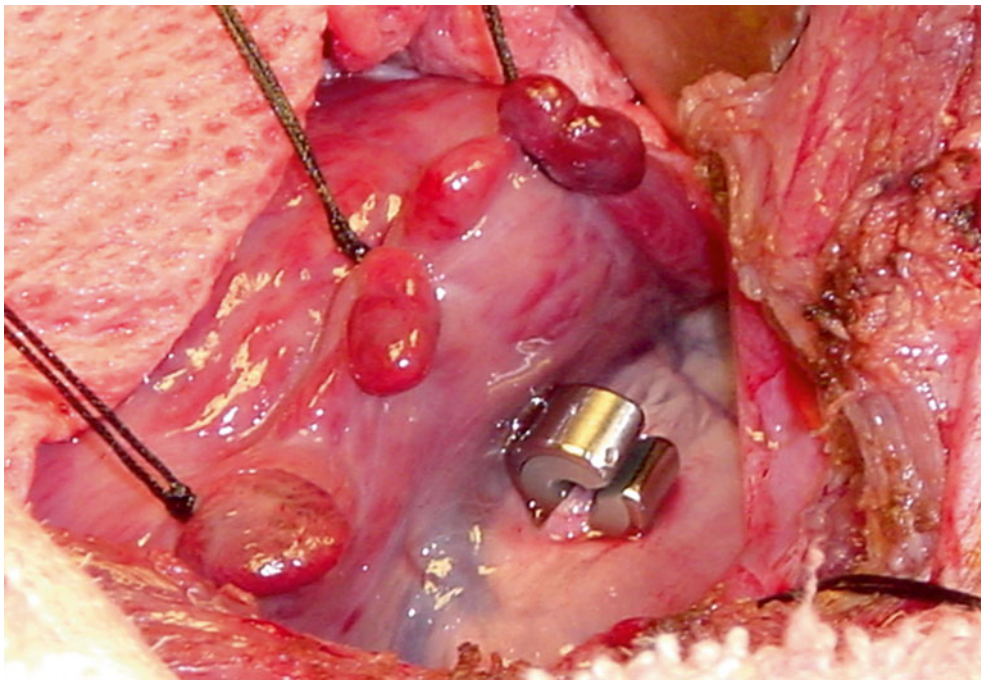

Fig. 27.6 Ameroid occluder in the canine model. Photo courtesy of Michael Jerosch-Herold and Cory Swingen

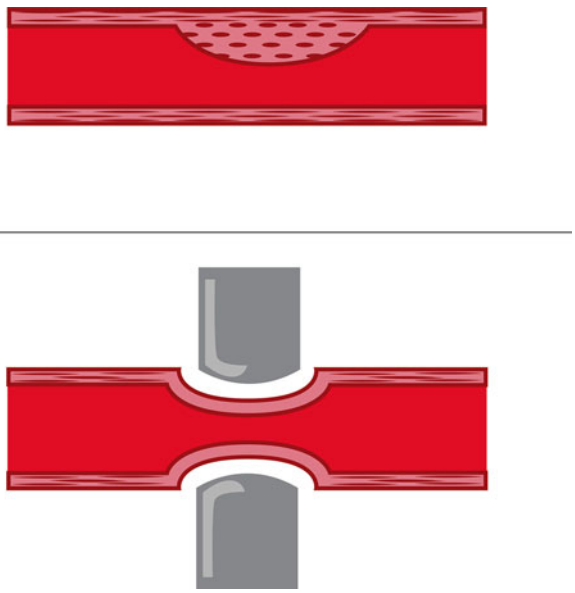

Fig. 27.7 (*Top*) Example of an eccentric vascular constriction as with coronary artery disease. (*Bottom*) Concentric lesion as created by experimental ligation or ameroid occlusion

physiology. More specifically, in consideration of heart to body weight ratios, in a healthy person the ratio is about 5 g/kg, for pigs weighing between 25 and 30 kg, the ratio is similar to that in humans, but for animals exceeding 100 kg, it is only half that value [42]. Importantly, such ratio changes must be considered when interpreting experimental results.

Small animals have also been used as models for investigations of regional myocardial ischemia. However, it has been established that the collateral circulation of the rat is sparse and that of the rabbit may show intraspecies differences [48]. In turn, the guinea pig has such an extensive collateral network that normal perfusion is maintained after a coronary artery occlusion and often infarction does not develop. Another problem with using these animals is that the small vessel diameters may delay or prevent instantaneous reperfusion following transient vessel occlusion, which is further complicated by the inability to make quantitative assessments of coronary blood flow in these small vessels to verify reperfusion. Nevertheless, the use of small animal models for studying myocardial ischemia remains important, including recent studies using stem cells for treatment. See also Chap. 6 for additional details on the comparative coronary circulations.

27.6 Animal Models in Heart Failure and Transplantation

Alexis Carrel reported the first heterotopic transplantation (Table 27.3) of a canine heart connected to the neck vessels of another dog in 1905, but the transplant succumbed to massive clotting and the animal survived for only 2 h. Many years later, Richard Lower and Norman Shumway perfected an orthotopic transplantation technique in the canine and achieved heart graft survivals of up to 21 days. Translation of this research to clinical practice was first performed by Christiaan Barnard in 1967, but acceptable graft survival required further studies in animal models to overcome rejection by the host immune system.

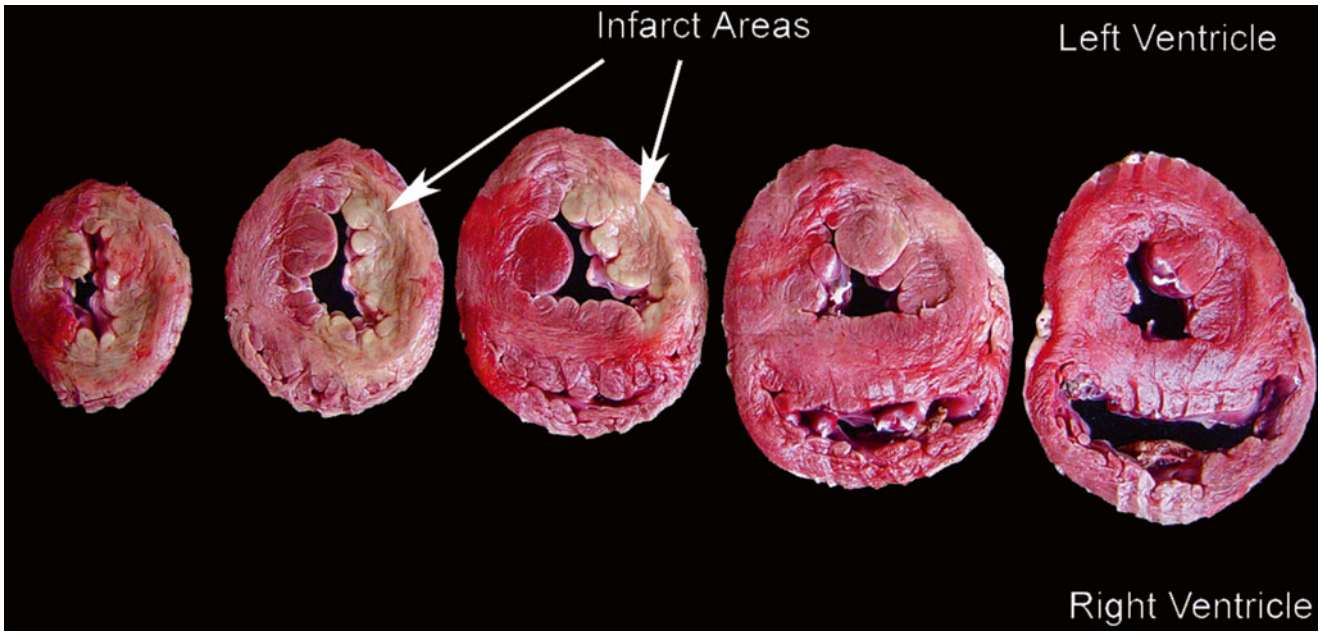

Fig. 27.8 Triphenyltetrazolium chloride (TTC) staining in canine infarct model showing pallor of myocardium (infarcts, *arrows*) in left anterior coronary artery distribution

Table 27.3 Definition of graft types

Graft Type	Definition
Autograft	Transplant from one site to another in the same individual
Isograft	Transplant from a donor to a genetically identical individual (monozygotic twin)
Syngraft	Transplant from a donor to a recipient with no detectable genetic difference (inbred strain)
Allograft (homograft)	Transplant from a donor to a genetically different individual of the same species
Xenograft (heterograft)	Transplant from a donor to a recipient of another species

Today, the successful treatment of end-stage cardiac failure is possible with organ transplantation. In addition, mechanical assist devices are employed for patients who may not initially qualify for transplantation. However, it is clear that too few suitable donor organs are available to meet the current needs (Fig. 27.9). This lack of a reliable and stable source of donor hearts serves as the main impetus for further research into: (1) stem cell therapy used before heart failure; (2) the means to expand cardiac donor pools (e.g., the use of Organ Care System, TransMedics, Boston, MA, USA); and (3) the use of mechanical assist devices and xenotransplantation.

27.6.1 Methods in Transplantation Research

Extensive research has been conducted in the field of cardiac transplantation (Fig. 27.10). *Orthotopic* heart transplantation (the placement of the donor heart in the anatomically correct position) was made possible only after the pioneering efforts of C. Walton Lillehei (refer to Heart Transplantation by Kirklen et al. [49] for a complete discussion of the surgical technique). Orthotopic transplantation is technically feasible using available cardiopulmonary bypass circuits in both the canine and porcine animal models and has often been chosen for the study of organ preservation, graft rejection immunology, immunosuppressive regimens, and/or ischemia/ reperfusion injury [50–53].

Heterotopic cardiac transplantation places the heart in an anatomical location other than the mediastinum. Clinically, a heart transplanted into the heterotopic position ("working" model) is performed by connecting the donor aorta to the recipient aorta, and the donor pulmonary artery to the recipient pulmonary artery while the donor and recipient right atria are anastomosed. Experimentally, a "non-working" model of heterotopic heart transplantation is achieved by connecting the donor aorta to the recipient aorta, and the donor pulmonary artery to the recipient vena cava [54]. As a result, blood

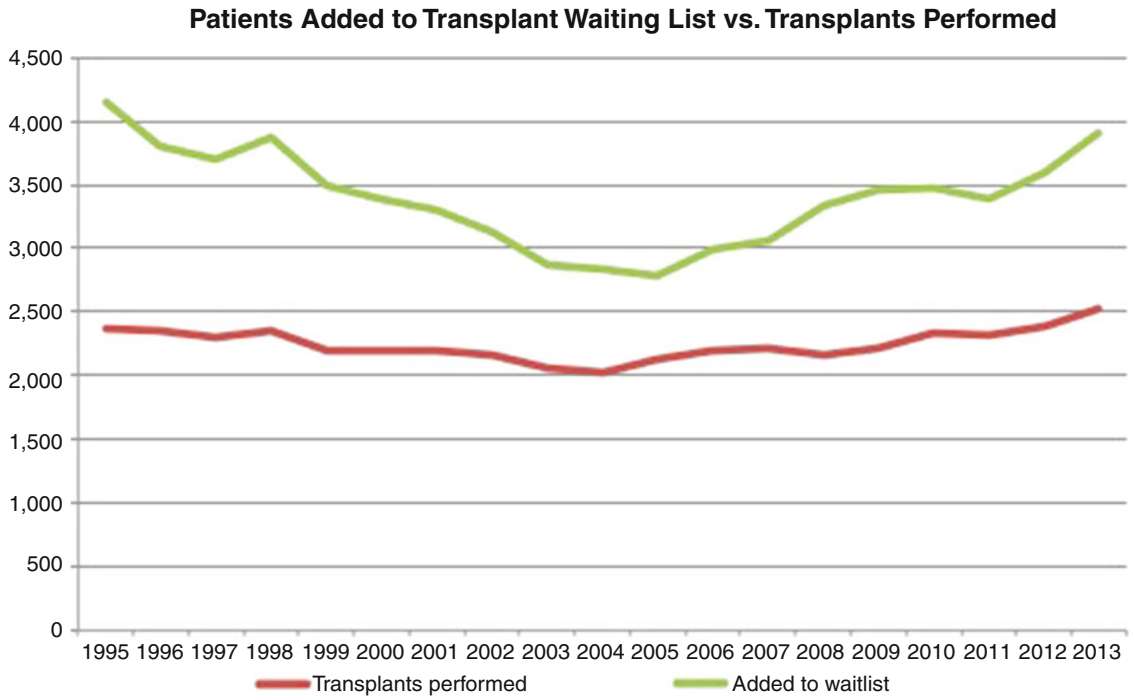

Fig. 27.9 Total number of United Network for Organ Sharing (UNOS) cardiac transplant waiting list registrants and donor hearts per year. Adapted from the UNOS database

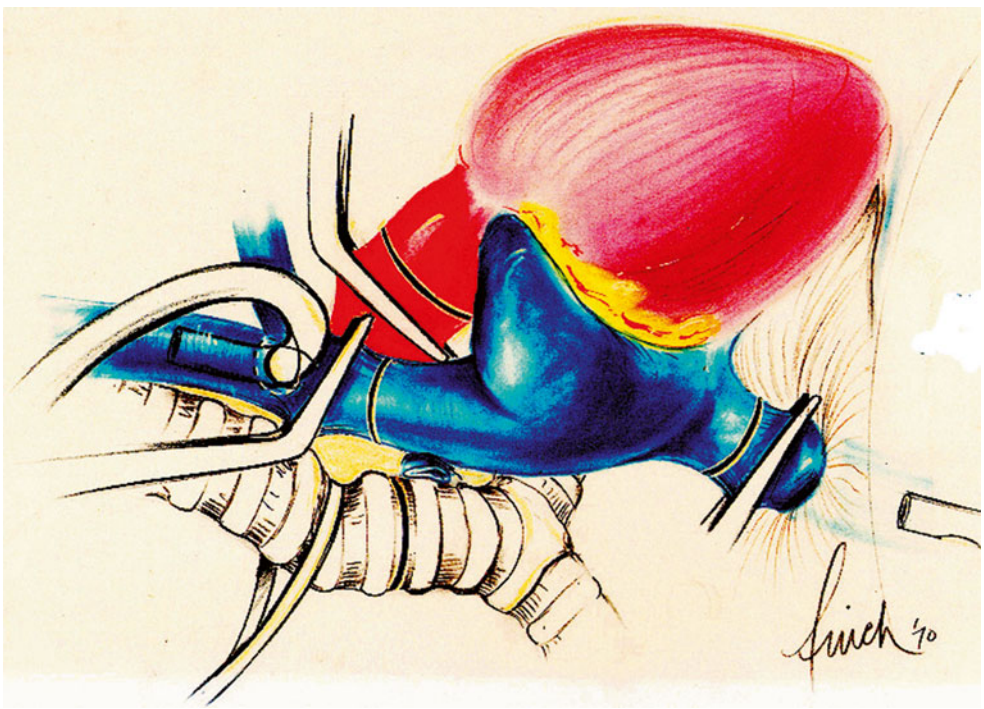

Fig. 27.10 Orthotopic heart transplant (original art by Martin E. Finch)

flow is typically non-physiologic; normal patterns are limited to the coronary arterial and venous system. Absence of significant flow in the ventricles, except for drainage of blood from the coronary sinus into its right ventricle, may promote clot formation and graft failure. Heterotopic transplantation is typically used for studies on ischemia/reperfusion injury [55], prevention of rejection and immunosuppression [56], xenotransplantation, and/or coronary vascular pathology [57]. Heterotopic heart transplantations are most commonly performed using small mammal models such as the mouse, rat, hamster, guinea pig, or rabbit; yet additional skills with microsurgical techniques are then

required. An advantage of this approach is that recipients may retain complete function of their native hearts whether or not the heterotopic donor hearts survive.

27.6.2 Specific Animal Models for Transplantation Research

The choice of an animal model for cardiac transplantation depends greatly upon the area of pathophysiological research. The following is a brief introduction to models that are currently used in this field.

27.6.2.1 The Rodent Transplantation Model

The development of microsurgical techniques has allowed the performance of heart transplantation in rodents. Importantly, the use of rodents in transplantation research can dramatically reduce the costs associated with larger animal models. Typically, Lewis rats have been used for transplantation experiments related to ischemia and reperfusion, prevention of rejection, immunosuppression, and coronary vascular pathology. Because of the small size of rodents, the technique of heterotopic heart transplantation to the abdominal aorta and inferior vena cava, as described by Ono and Lindsey, has been used extensively [58].

Anatomically, the coronary artery blood flow in rodents differs somewhat from higher order mammals in that the left and right coronary arteries traverse the lateral wall of the right ventricle rather than the atrioventricular sulcus. In addition, an extra vessel branching from the cardiomediastinal trunk supplies the sinoatrial node. Some further disadvantages of the rodent model are that hemodynamic measurement of the transplanted hearts can be difficult and transplantation requires microvascular surgical techniques and a surgical microscope. Yet an advantage of this model is its use for xenotransplantation experiments with grafts from mouse to rat, hamster to rat, guinea pig to rat, or hamster to guinea pig. In addition, preservation solutions can be fairly easily evaluated for the end points of survival, histology, and/or for high-energy phosphate analyses. Furthermore, the heterotopic rat transplant model has been extensively used in the pharmaceutical industry to evaluate the effectiveness of anti-rejection medications. More recently, the availability of transgenic or "knock-out" rodents continues to have a large impact in this area of research, i.e. animals with a gene deleted to induce a desired phenotype.

27.6.2.2 The Canine Transplantation Model

The anatomy of the canine heart is similar to that of the human heart (for more details see Chap. 6). As mentioned above, the dog heart has an extensive collateral circulation connecting the left and right coronary circulation. In contrast, nonathletic humans elicit few bridging collaterals. This collateral circulation in the dog is considered to be theoretically advantageous in heart transplantation experiments, as it may protect marginal areas of the heart from ischemia. From the perspective of an easy-to-employ model, dogs have a minimal amount of adipose tissue and their skin is loose, allowing tunneling of catheters if vascular access is needed postoperatively. Furthermore, the dog's relatively large thorax and mediastinum allow for clear visualization of the heart and great vessels. Thus, the canine model for heart transplantation is generally considered most easy to employ for animal studies on organ preservation, reperfusion injury, rejection studies, and/or post-transplant organ monitoring.

27.6.2.3 The Swine Transplantation Model

The porcine heart is often considered the most anatomically similar to the human heart. Specifically, as noted above, the porcine heart has few collateral vessels and an end artery coronary anatomy predominates. Yet, cannulation for cardiopulmonary bypass may be difficult, and the right atrial tissue has typically been described as fragile or friable [2]. In addition, a surgical cut-down for venous and arterial access may be required, secondary to the thick subcutaneous layer of adipose tissue. The pig transplantation model is also prone to postoperative wound infections, necessitating strict sterile techniques during cardiac surgery. Furthermore, juvenile pigs have a rapid rate of somatic growth, which can challenge long-term foreign body implantations. Physiologically, the porcine heart is considered to be prone to arrhythmias and is sensitive to physical manipulation. Bretylium tosylate can be given to limit such arrhythmias; however, ventricular fibrillation can be a recurrent problem following cardiopulmonary bypass [59]. The swine model is considered appropriate for heart transplantation; however, it is often described to be more suited to acute or short-term graft survival studies [60]. Ongoing projects to create a porcine heart with compatible tissue antigens to be used as a substitute for the human donor heart are exciting areas of research that will make the increased use of swine heart donors more likely. Thus, transgenic pigs that express human membrane-associated complement inhibitors in the vasculature have been used for studies of xenotransplantation in nonhuman primate recipients. Moreover, pigs with genetic deletions to prevent the expression of certain antigens that are involved in rejection are currently used as donors for nonhuman primates, making it possible to achieve significant prolongations of graft survival.

27.6.2.4 The Nonhuman Primate Transplantation Model

Researchers in the field of cardiac transplantation have used the nonhuman primate model extensively in developing both

the technique of transplantation and the scientific background necessary for the survival of the donor heart [61, 62]. Numerous programs have successfully used the nonhuman primate in small and large cardiac transplant studies [63–65]. Yet, particular problems with the use of primates have been associated with their veterinary care requirements during preoperative and postoperative times, and thus appropriate facilities are needed. Furthermore, nonhuman primates are extremely susceptible to mycobacterium tuberculosis and appropriate precautions must be taken to minimize the risk of infection. It should be noted that baboons are sensitive to stress and are prone to develop gastroenteritis and bacteremia after surgery, and handling of the baboon typically requires sedation. Nevertheless, the use of baboons and other nonhuman primates has many advantages as an experimental model in transplant research. First, the anatomy of baboons is similar to that of humans, except that the baboon heart has only two aortic arch vessels compared to the three found in humans. Second, the growth of the baboon can be controlled, and adult weights in the 20–30 kg range are maintained for 20–30 years. Additionally, physiologic characteristics of the baboon heart are similar to those of humans, allowing for the use of standard operative instrumentation. From a technical standpoint, the cardiac tissue of the nonhuman primate is not considered as friable or prone to serious arrhythmias as that of swine.

Adverse immunological responses in the primate are a main concern with xenotransplantation and also with antirejection treatments. Interestingly, the human ABO blood type system is applicable with simian tissue and saliva, but not with simian blood [66]. Tissue typing with the major histocompatibility system using primate tissue is also possible. Hyperacute rejection of a pig heart is inherent to xenotransplantation in this model because of the preexisting antibodies in the recipient. Specifically, the donor antigens on the surface of the endothelium of the donor's heart react with antibodies of the recipient, resulting in rejection upon subsequent activation of complement [67]. Inhibition of complement activation or depletion of complement factors was shown to abrogate cardiac xenotransplant rejection [68]. Heterotopic (nonanatomic and nonfunctional) heart transplantation in the nonhuman primate is an established surgical procedure appropriate for investigation of immunosuppressive drug therapies and study of immune reactions between the donor heart and recipient. Typical locations for heterotopic implantation of the donor heart include the neck or abdomen of the primate [69].

27.7 Animal Models for the Testing of Mechanical Devices

Maximizing surgical therapies in end-stage heart failure is a field of great interest. Recently, devices [i.e., ventricular assist devices (VADs)] have become increasingly important because of the large numbers of patients presenting with end-stage heart failure. Interestingly, mechanical VADs are filling a niche where they are both a "bridge to transplant" and a "destination therapy" at centers such as the University of Minnesota. Before such a device can be implanted into a human, the procedure requires years of high-level preclinical and animal testing.

27.7.1 Animal Model Selection for Device Testing

Animal models are necessary for research and development of mechanical devices such as the VAD and for training of the medical personnel involved in their use. The justification for use of a particular animal model is primarily based on: (1) the investigator's past and current success using a particular animal; (2) device size; and/or (3) the relative comparative anatomy. Careful selection of an appropriate model will decrease the difficulty of implanting such devices; devices designed for human use will require a comparably sized research animal for testing. For example, the size of in-line axial flow pumps (Fig. 27.11) has become relatively compact, and they have been implanted into the dog, sheep, and calf models [70, 71]. Larger pumps still require a larger animal [72] (Figs. 27.12 and 27.13). See Chap. 39 for more details.

27.7.2 Federal Guidelines for Device Testing

Although VAD technology is a rapidly advancing field, the FDA has not yet published official guidance documents for such devices. However, the FDA does have specific evalu-

Fig. 27.11 Example of an axial flow pump impeller

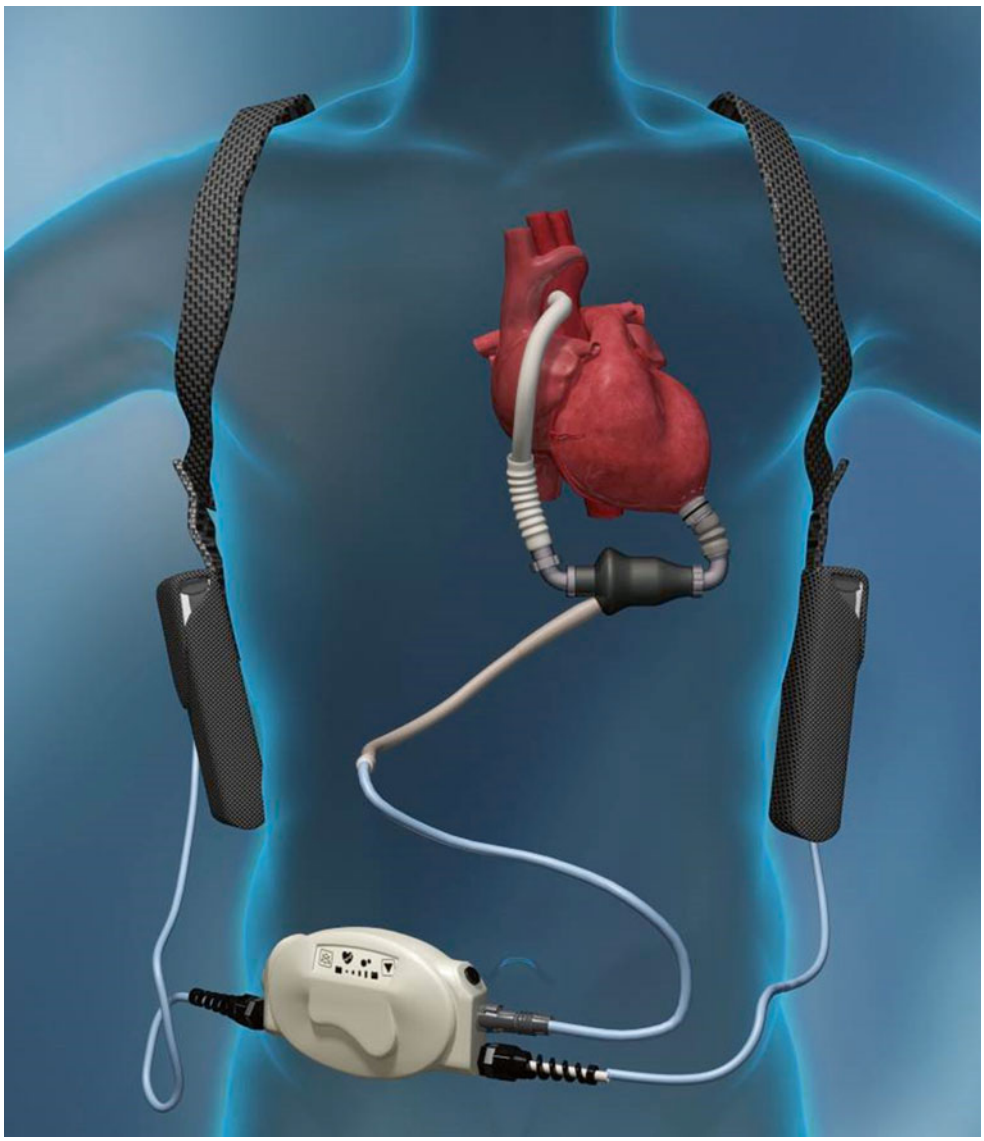

Fig. 27.12 Schematic of a left ventricular assist device (Heartmate II, Thoratec) in human use. Courtesy of Thoratec Corporation

ation criteria for VADs that are designed to identify possible hazards prior to clinical usage. Long-term reliability is a current issue of concern with such devices, as some patients waiting for a transplant survive longer, and destination therapy has become a reality for several device recipients. Consequently, long-term biocompatibility, thrombogenesis (long-term surface coating antithrombogenic integrity), bacterial infection, battery life, and hardware and software reliability have become important parameters for FDA evaluation of such devices. Ultimately, the ease of patient use related to VADs is also of central importance.

27.7.3 Explant Analysis

27.7.3.1 Background

The major types of prosthetic valves currently used in clinical practice are the mechanical valve and the bioprosthetic (or tissue) valve. Percutaneous delivery technology is just beginning to be applied to bioprosthetic valves within the last few years; there is a wave of clinical application in Europe which is gaining momentum in North America in certain select patients.

The role of pathology in the evaluation of cardiac devices is integral for the end results, which is to have data that is

Fig. 27.13 Left ventricular assist device (Heartmate II, Thoratec) in human use. Courtesy of Thoratec Corporation

acceptable for regulatory submission. This is not an exhaustive review of cardiovascular pathology in the context of cardiac devices, but an overview of how regulatory agencies use industry-derived standards to deliver guidance on pathology evaluation.

The use of pathology as the gold standard for explanted device analysis requires more than classical pathology analysis used in diagnostic settings. The integration of regulatory standards is critical to achieve an outcome from each study that will be acceptable for submission to the desired regulatory agency. There are numerous guidance documents from different regulatory bodies and organizations that contribute to the regulatory framework that exist for complete preclinical analysis of medical devices. The ISO 5840 series of documents are not prescriptive in the approach to certain areas of the device or surrounding anatomy, but do require that broad areas be investigated (e.g., calcification). The ISO 5840 document relies on approximately 25 other supporting ISO documents that include risk assessment, pathology evaluation, and marketing approaches. The most heavily involved companion document is ISO 10993, which currently is comprised of 18 different documents, the most important for heart valves being the one pertaining to biocompatibility. In addition to the ISO documents there are FDA guidance documents and, of course, relevant published literature. Interpreting these guidance documents often relies upon experience; researchers should keep themselves abreast of updates to these documents.

27.7.3.2 Pathology in Context of the Study

The two largest divisions of a study for a pathologist are the in-life and explant (i.e., postmortem) stages. These two stages are separated by timing; an animal that goes to the full study term is euthanized and is evaluated based on the study protocol (Fig. 27.14). The departure from this occurs when there is an early "unexpected" death. The unexpected death introduces a number of additional factors to the postmortem analysis, primarily the investigation of whether or not the death was device related. In a well-maintained animal colony, there should be few cases in which the death is not device related in some way (whether this is device failure or surgical error). Careful investigation of the device is often difficult, as an unexpected death precludes administration of heparin to the animal allowing large postmortem clots to surround the device.

The in-life stage involves the analyses of clinical pathology samples which can range from complete blood counts (CBC) and blood chemistries to blood cultures. Clinical pathology tests used can be separated into three categories: (1) hematology; (2) clinical chemistry; and (3) coagulation profiles. There are many natural disease entities that clinical pathology can screen for, but the device-induced changes are the areas of interest, particularly device-induced hemolysis and blood calcium levels. Hemolysis is analyzed by weighing multiple factors such as hematocrit, red cell morphology, haptoglobin, and free hemoglobin. Most often, the serum is macroscopically unremarkable but, in severe cases, there can

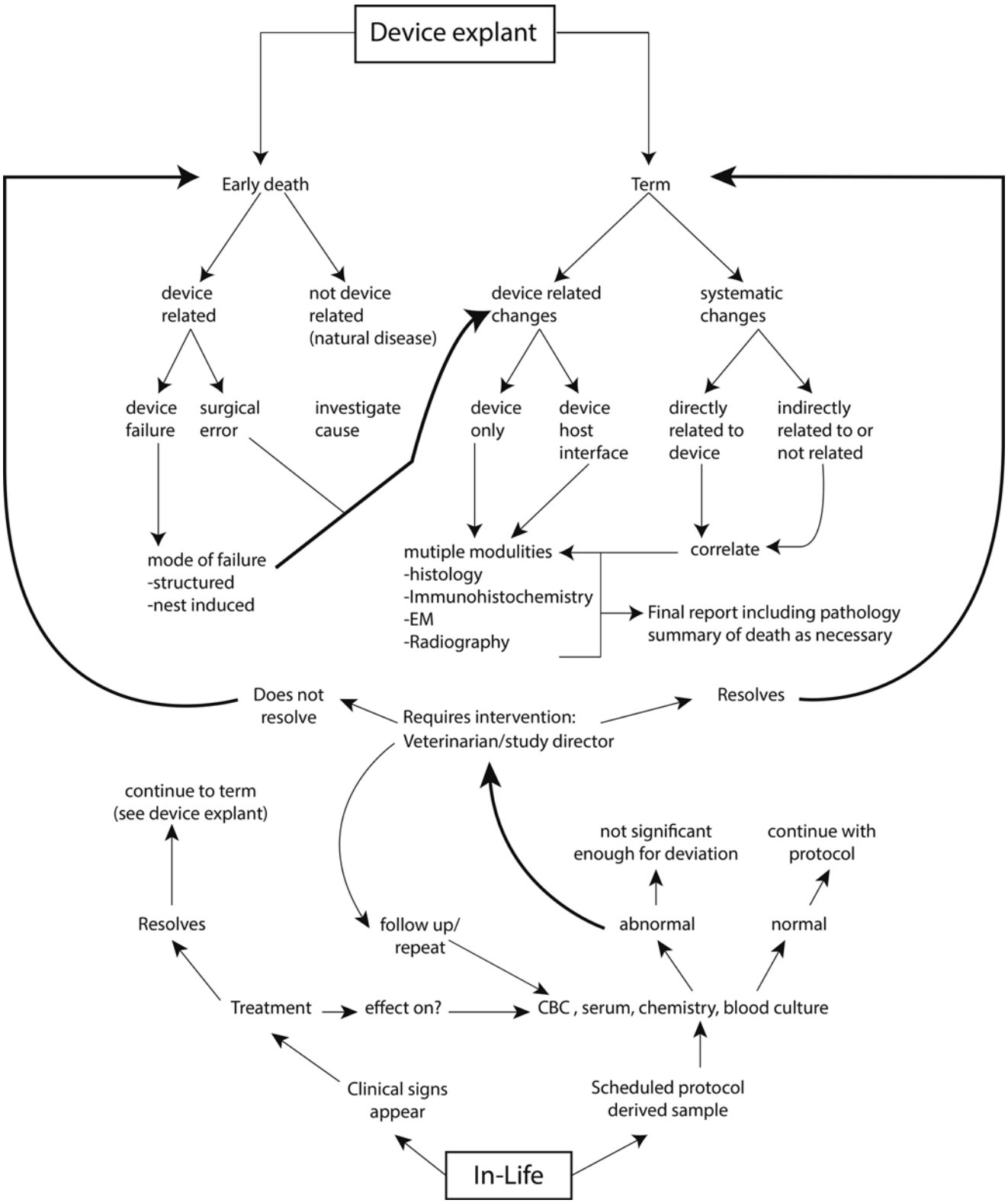

Fig. 27.14 The intricate relationship that pathology has to the in-life phase and the explant phase of a preclinical study

be overt reddening of the serum indicating intravascular hemolysis. Calcification is a particular problem in bioprosthetic (tissue) valves; while dystrophic calcification is found to be the cause in the overwhelming majority of cases, serum calcium must still be assessed to rule out metastatic calcification. This is particularly true if sheep are housed outside in regions of the world where calcinogenic plants are known to exist; such plants contain glycosides of the active metabolite of vitamin D.

27.7.3.3 Process of Using Pathology

The overall analyses of the post-implant device have gross, microscopic, and radiographic components. Yet, typically there are small differences in the way that 4-chambered mammalian hearts are analyzed when they contain devices. Depending on the objectives of the study, the analyses performed under non-Good Laboratory Practice (GLP) conditions can differ substantially from those performed under GLP conditions. On the other hand, while the depth of analyses can differ widely between GLP and non-GLP studies, most laboratories will employ similar techniques or approaches.

In general, there is a core set of methods that needs to be included. First, the radiographic analyses of devices allow the structural integrity (i.e., presence of metal fractures), presence of radio-opaque material, and orientation within the ventricular outflow tract (particularly with TAVI devices) to be assessed. Second, the gross necropsy represents the first time the pathologist is able to lay hands on the test system. This procedure can be conducted in a number of different ways, and standard approaches to necropsy have been covered in other texts [73]. Prior to the necropsy of each animal, terminal procedures are often performed such as echocardiography and angiography, after which heparin is delivered and the animal is euthanized. Necropsy procedures can vary widely depending on the location of the device and whether sterile collection is to be attempted. For cardiac devices that are not collected in a sterile manner, the animal is presented in either left lateral or dorsal recumbency and the abdomen is opened; all organs are evaluated in situ and removed for further evaluation. Following the abdominal cavity evisceration, half the thoracic cage is removed to expose the thoracic viscera. This allows close evaluation of the thoracotomy site and the relationship that the heart and lungs have to the mediastinum and parietal pleura. The tongue, esophagus, and trachea are removed "en bloc" with the heart and lungs (often called the "pluck"). The lungs can then be carefully dissected from the heart to allow detailed external evaluation. At this time, the heart can either be further dissected to reveal the internal device or it can be perfusion fixed (in formalin). More often, dissection of the heart and device is performed at the time of necropsy, and photographs are taken of the inflow and outflow aspects. It is important to note that some of the photographs should be taken directly over the sewing ring to evenly include all features of the device. Third, as part of the regulatory guidance documents, a gross evaluation is performed. It is imperative that a thorough gross examination of each organ is performed, as this is the only opportunity for adverse events to be identified. The most important distant (or end) organs to be examined depends on which side of the heart the device is located. For devices on the left side (aortic and mitral valve devices), the aorta (full length to the iliac bifurcation) should be examined. Kidneys, brain, and eyes should be investigated for embolic debris of various types, and the lungs should be investigated for evidence of left-sided heart failure (pulmonary edema). For the right side (right AV valve, pulmonic valve), the lungs and liver are the major organs of interest. Due to the systemic nature of right-sided heart failure, body cavities and other organs must be thoroughly investigated. It should be noted that bioprosthetic valves delivered to the aortic position via a transcatheter approach (transcatheter aortic valve implantation, or TAVI) have an additional set of analytical criteria that need attention such as device migration. In addition, the approach to device analyses must include considerations relative to the delivery of the device into the vascular site; these include biofilms, host reparative response to surgery, and materials used to implant the device compared with the device itself (i.e., suture material).

Histologic analyses of the device are accomplished primarily by the use of formalin-fixed paraffin-embedded sections of the device stained with hematoxylin and eosin (HE). Additional stains that can be used for paraffin sections can include: Von Kossa, alizarin red (mineralization/ calcification), Mason's trichrome, and Movat's pentachrome (fibrosis/smooth muscle hypertrophy/hyperplasia). For plastic sections (embedded in resin), HE, toluidine basic fuschin, or elastin trichrome can be used as a starting point. For immunohistochemistry, smooth muscle actin (Table 27.4) and a wide range of CD markers can be used for inflammation classification.

For assessing biocompatibility, in particular, it is highly desirable for the device to be visible while it is in contact with the host tissue. This often necessitates the use of other embedding modalities such as plastic embedding. Plastic embedding allows hard materials such as hardened plastics and metals to be analyzed in situ (in close contact with the host tissue), rather than the hard structures removed for paraffin processing. Similar stains can be used between plastic and paraffin (Table 27.4); however, there are some minor issues with tinctorial properties in tissues embedded in plastic. The major drawback with plastic embedding is cost and the length of time to complete the preparation.

The written pathology report is the final document that, once signed, represents the pathology data that has been col-

Table 27.4 Histology stains[a]

Technique	Stain/method	Use
Histochemistry (paraffin)	Hematoxylin and Eosin (HE)	General stain used for initial survey of the tissue. Many changes can be identified with this stain
	Movat's pentachrome (MP)/Masson's trichrome	Identifies (smooth) muscle, collagen fibers and with MP elastic fibers, mucin and fibrin
	Alizarin red (AR)/ Von Kossa (VK)	Highlights calcification (AR) and mineralization (VK)
	Toluidine blue-Basic Fuschin or elastin	Demonstrates mucopolysaccharides and other metachromatic substances in tissue sections
	Periodic Acid Schiff (PAS)	Highlights glycoproteins present extracellularly as well as in chondroid matrix
Immunohistochemistry (paraffin)	Factor VIII, CD31	Identifying endothelial cells
	Alpha smooth muscle actin	Identifies smooth muscle presence within
Plastic embedding	HE, Toluidine blue-Basic Fuchsin or elastin, PAS	See above for use

[a]This list is not exhaustive, but highlights some of the major methods and stains used in histologic valve analysis

lected throughout the study and is now used as a major piece for the regulatory authorities to decide whether the device can move to human clinical trials. There are many different versions of the pathology report that can be created; the authors prefer to have a complete pathology report with all findings included in chronological order of the study. In general, these parts include: (1) clinical pathology; (2) gross pathology including photographs (animal and device); (3) digital radiographs of the device; and (4) microscopic analyses of the device and organs of interest. This allows the report reader to have the complete analyses of each animal/device interaction in one document. The second document one should typically prepare is the pathology summary which should combine all findings from each animal in the study and provide conclusions based on the study protocol and any other regulatory document related items.

27.8 Cellular Cardiomyoplasty

Cellular cardiomyoplasty, the process by which the injured myocardium is repaired by cell transplantation, has significant clinical potential [74, 75]. Much of the excitement about cell-based therapy lies on the premise that repairing the injured heart will overcome inherent limitations for the broad application of organ transplantation and mechanical assist devices. Validation of such therapies needs to be performed in appropriate animal models. A thorough review of the literature for stem cell-mediated cardiac therapy has been provided in Chap. 40, and thus will only be reviewed briefly in this section.

27.8.1 The Ideal Cell Population for Cardiomyoplasty

While advances in the field of cardiomyoplasty have recently been achieved with the advent of stem cell technology, the "ideal" population of cells that is able to effectively engraft damaged myocardium and restore cardiac function without improper differentiation to other contaminating cell types is still an issue of debate. Many cell types with the potential to repair the injured heart have been considered, including differentiated cells (fetal myocytes or satellite muscle cells) and undifferentiated cells (embryonic or adult stem cells). While pluripotent embryonic stem cells offer the promise of functional plasticity and the ability to differentiate into any cell type in vitro, extensive experimentation in vivo is still necessary to properly direct the formation of integrated, functional cardiac tissue at the site of injury without improper differentiation to form teratomas (tumors) or other noncardiac cell types. Multipotent tissue-specific cells that have already committed to a distinct lineage, such as hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells, have also produced encouraging results [76]. However, to date, the use of these cells often results in incomplete engraftment and a failure to restore cardiac function over time [77]. Regardless of the cell type used in cardiomyoplasty, it is clear that animal models will again play a crucial role in the translational research that will be necessary to advance this theory into clinical practice. Hence, such cell preparations need to be appropriately prepared for each selected species of animal to be included within a given trial.

27.8.2 Animal Models for Stem Cell Research

Although multiple animal species have been used for the study of cellular cardiomyoplasty, most investigators have chosen acute ischemia as their experimental model of choice. However, while effective treatment options for acute ischemia do exist, only limited options are available for chronic myocardial ischemia. This observation strongly suggests that further development of the chronic ischemia models using cardiomyoplasty is warranted. As with most research, the experimental hypothesis will remain fundamental in choosing the correct animal model. However, the lack of appropriate stem cell lines in the desired species will add more limitations in selection. Fully characterizing cell lines are important and advantageous so that functional changes of the therapy are correctly attributed to the appropriate precursor cell.

The multiple types of stem cells available for the rodent, specifically the rat and mouse, have made small animal models effective for investigations of stem cell engraftments. However, the differences in myocardial perfusion and ventricular thickness may confer differences in nutrient supplies that would support engraftment in small animal models, but the results obtained may not be translatable to either large mammalian models or humans.

Ultimately, large animal models that better approximate the diseased human heart will be required to fully assess stem cell engraftment, differentiation, and/or functional improvement. Furthermore, large animal models, in general, are considered better suited for assessment of myocardial function via angiography, echocardiography, or MRI; however, to date, the limited availability of appropriate stem cell lines for use in these models has prevented the widespread use of large animal models. Nevertheless, stem cell lines are currently being developed for the pig, dog, and monkey at a number of institutions. More specifically, one lab has developed a canine model of cardiomyoplasty for chronic ischemia in which bone marrow-derived stem cells are used (Fig. 27.15). Two of this chapter's authors, (RPG, RWB), have demonstrated successful engraftment and statistically significant, sustained long-term improvement in regional myocardial function by MRI follow-up. Though successful, this first effort has resulted in additional questions that need resolution: (1) How and when should we deliver the cells? (2) How many cells should be implanted? (3) How often do cells need to be delivered? Much work has yet to be completed before one can be certain that stem cell therapies can be considered a viable treatment for various forms of myocardial disease.

27.8.3 Stem Cell Delivery Methods

Multiple methods of stem cell delivery have been investigated including: (1) direct myocardial injection; (2) peripheral transfusion; and/or (3) stem cell mobilization [78]. Direct epicardial-myocardial injection can be fairly consistently completed intraoperatively during procedures such as coronary arterial bypass or valve operations. Endocardial injection will likely be completed by using commercially available, radiographically guided stem cell injection catheters (Fig. 27.16). Both transfusion and mobilization of resident stem cells offer the least invasive means of stem cell delivery, but require the availability of effective cellular homing signals to direct the correct location for engraftment. This latter hurdle could possibly be overcome by using guided direct myocardial injections, either surgically or via interventional catheter techniques. Available information suggests that multiple stem cell injections may be required to achieve full myocardial regeneration for therapeutic repair. As a result, the use of stem cell injection catheters may become the standard of practice. In addition, advanced imaging techniques such as MRI could be used to localize the injured myocardium and direct, in real-time, stem cell injection catheters to the damaged area.

27.8.4 Stem Cell Engraftment Issues

The ability to track the implanted cell is critical not only to assess the potential of engraftment but also for later determination of differentiation and incorporation into the native tissue. Multiple techniques of cell labeling are currently under investigation including the use of viral gene transduction (e.g., DAPI, Green Fluorescent Gene, Lac Z), incorporation of dyes, and the use of metallic microparticles [79, 80]. For example, gene insertion can be fairly easily accomplished, i.e., allowing for fluorescence microscopy or Q-PCR identification of the stem cell. However, the exact insertion site into the DNA of the cell cannot currently be well controlled, introducing the possibility for nonexpression of the gene or potential disruption of normal cellular transcription and translation processes. The use of dyes incorporated into the cells by pinocytosis has been reported. The primary disadvantage of this technique has been the potential for dye incorporation into native cells in vivo. The use of metallic microparticles has received recent attention, since such particles may allow for real-time identification of cells by MRI imaging and later pathologically by staining. However, information about the potential disruption of cellular function and possible uptake in vivo by native cells has yet to be fully elucidated.

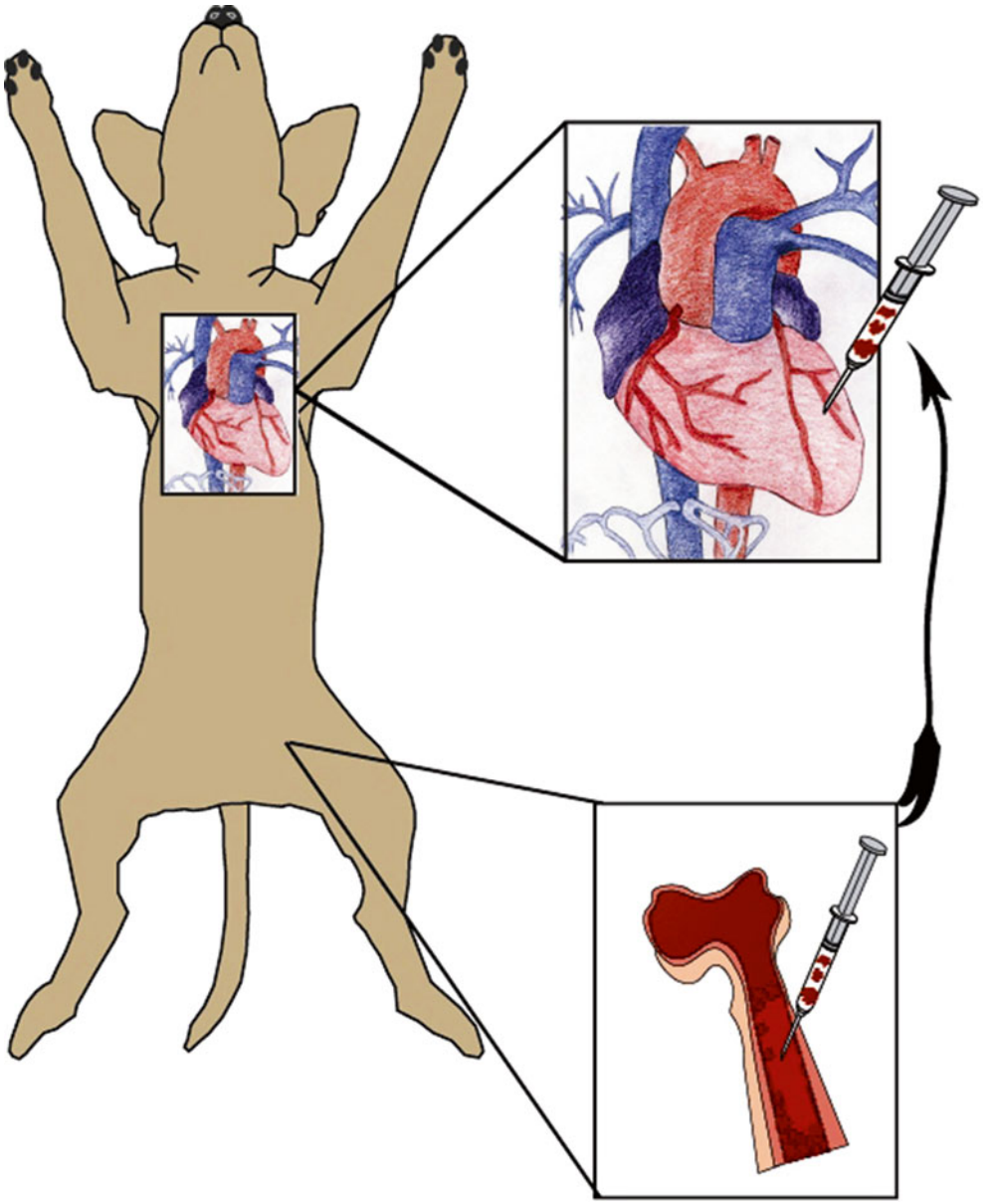

Fig. 27.15 Canine model for bone marrow-derived multipotent stem cell cardiomyoplasty (Illustration courtesy of Kathy B. Nichols)

27.8.5 Functional Assessment of Stem Cell Therapies

Many efforts to demonstrate improvement in cardiac function following cellular cardiomyoplasty have been undertaken. Methods include pressure measurements, ultrasonic microcrystal placement, echocardiography, and/or MRI. Regardless of the specific method used by the investigators, to date, there are only a few significant long-term follow-up studies that exist in the literature. Thus, we conclude that much more research is required before this approach can be applied to humans.

27.9 Summary

In preparing to embark on a preclinical study of a new cardiovascular device, procedure, drug, and/or therapy, it is important to carefully select the animal model. Once the model has been selected, the study design to be followed carries immense importance, particularly with regard to the rigor of the scientific method, regulatory intricacies and, last but not least, the ethical considerations involved with animal use. Attention to all these details is essential and will allow a successful preclinical study outcome. New cardiovascular technologies will continue to be introduced into an ever-

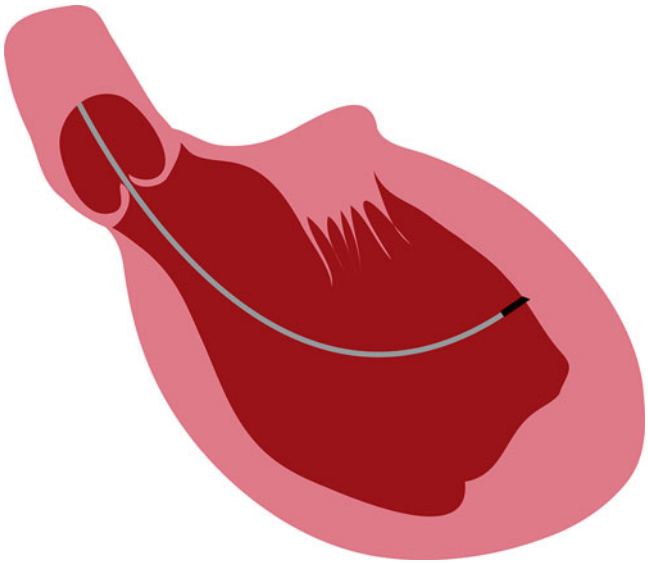

Fig. 27.16 Example of catheter-guided stem cell therapy for myocardial infarction

increasing environment of tighter regulations for animal use and human safety. At some stage of a device's evolution, consultation with experienced centers of experimental research is recommended, as they can provide additional assistance for preparing research protocols and completing the research necessary for well-performed preclinical studies. The progression of a device from design to clinical use is the end goal of these studies and, as such, preclinical research will remain a necessary step for the foreseeable future.

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