Published in final edited form as:

Drug Discov Today Dis Models. 2009 October 1; 5(3): 195–200. doi:10.1016/j.ddmod.2009.02.004.

Use of Larger Species such as Dog and Pig as Model Systems to Study Cardiac Disease

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1. Introduction

Small animals such as mice and rats are increasingly used to study cardiac disease, particularly in the context of drug discovery and development. This is primarily driven by the availability of transgenic mice, which can be used to identify proteins as pharmacological targets, and the low cost of rodents, which makes them useful in the testing phase. However, there are disadvantages to these small mammal models. Studies involving genetic manipulation are difficult to interpret; nearly all must be accompanied by some degree of compensation, even though it may be phenotypically irrelevant. Therefore the results of such experiments are often unsuitable for extrapolation to humans. In addition, many factors such as heart size, beating rate, and molecular composition are substantially different from human. This may have important implications for drug pharmacology. Larger species such as dogs and pigs overcome some of these limitations, and are better suited as models of complex human syndromes like heart failure. Though no model is perfect, several large animal models have been developed that mimic certain aspects of human cardiac disease.

In order to gain a more complete understanding of cardiac disease, which will facilitate the development of new and better treatments, cardiac function must be assessed at the whole organ level. This consists of measurements that may include mechanics, electrophysiology, and hemodynamics. This manuscript will review the methods currently used to study these quantities in large animals, with special emphasis on the differences from small animal models. In addition to functional measurements in the heart, large animal models are also important in cardiac device and surgical therapy fields where size is a severe limitation in the small animal models.

2. Intact Heart Mechanics

Pharmacologic agents are used in a wide variety of situations to alter global and regional function in the heart. In many cases, large functional gradients are present in the myocardium (for example with regional ischemia), and local (regional) measures of tissue function are needed. Many techniques have been used to study regional function in the ventricles of the heart, and each has advantages and disadvantages. In order to perform an in-depth, mathematically rigorous analysis of deformation, these techniques must employ some type of material markers. These are markers that are fixed to a particular point within the heart, and

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thus can be used to track its motion and deformation. While non-marker based methods have been used to describe myocardial deformation [1], these techniques are indirect estimations and will not be discussed here. Due to the larger size of dog and pig hearts, use of material markers is significantly easier and more widely used than with small animals.

a. Ultrasound

Ultrasound refers to any use of sound waves with frequency greater than 20 kHz, and has wide-ranging uses in medicine. Importantly, it can be used to visualize the heart inside of the chest with echocardiography [2,3]. However, because traditional ultrasound does not make use of material markers, it is unable to provide information about the deformation of the heart.

i. Intramyocardial crystals—In an alternative use of ultrasound, “crystal” pairs can be implanted into the wall of the ventricle and used to measure distance. The crystals are made from a piezo-electric ceramic material. One of the crystals emits sound in the 5-10 MHz range and the other receives it, providing a real-time measurement of the distance between them. In addition to the invasiveness of the open-chest preparation necessary for implantation, the crystals themselves are large (at least 2mm diameter) and have wires attached to them. This can create a significant amount of damage to the myocardium upon implantation, as well as limiting how closely the emitter/receiver pair can be placed. Therefore, intramyocardial crystals are best for measuring changes in segment length along a single dimension [4]. However, by using 3 or more emitter/receiver pairs, it is possible to measure 2D or 3D deformation [5]. Additionally, crystals have two major advantages: high temporal resolution and real-time viewing. Finally, arrays of crystals placed about the left ventricle can be used to estimate changes in cavity volume [6]. Due to their large size, intramyocardial crystals have limited applications in small animals, where they may be used only for large segment length changes, most notably diameter and wall thickness measurements.

ii. Speckle Tracking—Echocardiography is traditionally used to visualize the motion of the heart wall to determine the synchrony of contraction and quantities such as ejection fraction. However, it has been observed that the myocardium contains an acoustic signature, that is, a unique speckling pattern [7,8]. These speckles remain fixed within the myocardium over time, providing material markers. Therefore, post-processing of traditional echocardiographic images can be used to determine 2D or 3D cardiac deformation [9]. This technique is new, and still being validated against other more established methods. Like intramyocardial crystals, speckle tracking also has excellent temporal resolution. In addition, it has much better spatial resolution, with the ability to measure local 2D or 3D deformation in multiple regions and at different transmural depths. It also has the advantage of being non-invasive. With current technology, speckle tracking does not have adequate spatial resolution for use in small animals.

b. X-Ray

X-rays are typically used to image dense materials such as bone. Soft tissues, such as myocardium, provide limited resistance to x-ray penetration. By inserting small, metallic beads (<1 mm) into the heart, the x-rays can be blocked, thus providing a material marker. If these markers are imaged with biplane radiography or computed tomography, their three-dimensional coordinates can then be reconstructed. Records of this method date back to at least the 1960s, when lead beads were used to track changes in left ventricular dimensions [10]. Though invasive, this technique has also been applied to (transplanted) human hearts [11], to determine shortening and twisting of the left ventricle. The technique was modified by Fenton et al. to measure local three-dimensional strains [12], then improved by Waldman et al. [13], and is still in use today [14,15].

The major advantage of the x-ray technique is its ability to track remodeling in a chronic preparation [16,17], because the markers remain fixed within the tissue over time. However, like crystals, the x-ray technique also requires an open-chest preparation and implantation into the heart. Importantly, these markers are usually smaller ($<1\text{mm}$) and do not have attached wires, making chronic experiments possible. The temporal resolution is typically better than magnetic resonance imaging (MRI) based techniques, but equal to or worse than echocardiographic techniques. This technique is still considered the gold standard for myocardial deformation, particularly for describing three-dimensional deformation within a small volume of the heart ($\sim 25\text{mm}^2$). Beads can be used in small animals to a lesser degree, either by sewing them to the epicardial surface and, in cases of somewhat larger animals such as rabbits, implanting an endocardial-epicardial pair. Such an approach largely sacrifices information about transmural differences in mechanical function.

c. Magnetic Resonance Imaging (MRI)

Similar to echocardiographic images, MRI images do not contain material points. However, a technique has been developed known as myocardial tagging, in which “taglines” are created by saturation of tissue with radio frequency energy [18-20]. These taglines persist for a brief period, typically less than one cardiac cycle, before disappearing. The intersections of taglines provide material points which can be tracked through time. More recently, a harmonic phase (HARP) approach has been used to simplify post-processing [21]. HARP is analogous to echocardiographic speckle tracking, except that material points are tracked by the phase of the MR signal.

MRI is one of the best methods for overall assessment of myocardial strain. It can be used to determine deformation simultaneously over the entire heart non-invasively. High spatial and temporal resolution can be achieved using averaging, as part of a tradeoff with longer imaging times. Despite the obvious advantages, there are several limitations as well. Perhaps the most important is the inability to put ferrous metals in the scanner due to the magnetization effect. Other foreign objects, such as catheters, electrodes, etc. can introduce artifacts into the image. Another limitation is the inability to acquire data for the duration of the cardiac cycle simultaneously, but this can be avoided by triggering the taglines at different times within the cycle and piecing together the results [22]. Transmural resolution is limited even in large animal models [23,24], and is transmural resolution is extremely limited in mice and rats. Lastly, MRI cannot be used measure chronic remodeling because the material markers do not persist.

d. Optical Imaging

Optical imaging of the ventricular surface has also been used to study deformation. This technique utilizes markers affixed to the epicardial surface, which are then imaged by one or more cameras. The markers themselves can be of different compositions such as adhesive paper [25], suture [26] or reflective micro-particles [27]. The use of one camera prevents the tracking of fully three-dimensional material points; in this case the projection of these points onto the plane of the camera is used instead. This is equivalent to making the assumption that the radius of curvature at the point of measurement approaches infinity (and thus curvature is negligible). Using two cameras, three-dimensional points can be reconstructed. The major limitation with this technique is that the observed deformation is limited to the epicardium. In addition, the temporal resolution is limited by the camera's acquisition rate, while the spatial resolution is limited by the marker size and camera resolution, each of which make the use of this technique in small animals extremely limited.

3. Electrophysiology

Another important measurement in the study of cardiac disease is electrophysiology, in particular for antiarrhythmic drug testing. Changes in cardiac activation sequence may occur as a result of structural heart disease, and action potential duration can lengthen as seen in heart failure. Cardiac electrophysiology in its most basic form can be studied from the electrocardiogram (ECG), which can give important information about conduction sequence (QRS morphology), action potential duration (QT interval), and ischemia (ST segment changes). The ECG is, in effect, an integration of all of the electrical activity of the heart, measured along a particular axis (up to 12 different axes in a standard 5-lead system). As a result, the electrocardiogram cannot be used to directly examine regional differences in electrical function.

Cardiac electrophysiology is very important in drug development, for example due to the arrhythmogenic potential associated with prolonging the QT interval. Such risk is difficult to access in small animal testing, for a number of reasons. The action potential of the small mammal cardiomyocyte differs significantly in morphology from dog or human, and T waves are not as pronounced. Moreover, arrhythmia mechanisms such as reentry do not play a major role. Understanding of these types of complex phenomena requires a large animal model. Electrophysiology research in small animals is typically accomplished via optical mapping, which utilizes a voltage sensitive dye to visualize epicardial electrical activity, but cannot examine transmural activation or repolarization.

a. Plunge electrodes

Action potentials are a transmembrane phenomena, reflecting the change in voltage within a cell with respect to its extracellular environment. *In vitro*, it is possible to directly measure a transmembrane action potential by inserting one electrode inside the cell and referring the signal to a reference electrode in the solution. *In vivo*, it is not possible to insert an electrode directly into the cell. However, by placing a pair of electrodes into the myocardium, the local activity can be measured, analogous to the surface ECG. This signal is sometimes referred to as a local electrogram. Similar to the surface ECG, the local electrogram has a spike much like the QRS complex and a bump reminiscent of the T wave. Therefore, one can determine both the local activation time of this point in the myocardium, as well as the activation-recovery interval, which has been shown to correlate with the action potential duration [28].

The recording electrodes themselves can be constructed in different ways. In the simplest case, epicardial signals can be recorded by suturing wires to the surface of the heart, and endocardial recording can be made via a catheter. To make mid-myocardial recording, on the other hand, electrodes need to be plunged into the heart. This has been described by Scherlag et al. [29], in which two wires are delivered via a small needle. Anyukhovsky et al. modified the technique, using a multiple transmural needle electrode with electrodes at 1-mm depths through the ventricular wall [28]. A limitation of both techniques is the injury created by the needle insertion. In the case of the multiple transmural needle electrode, the post may also have some effect on the mechanics of the heart. Due to the smaller absolute wall dimensions in the small animal heart, intramural electrode recordings are rare.

b. Multi-electrode Sock

A less invasive alternative to plunge electrodes is the epicardial sock [30]. In this technique, a compliant material such as cloth is slipped over the apex of the heart. There are metal contacts sewn into the material, which can be connected to wires. These contacts pick up electrical signal from the epicardium and can be used to construct a map of epicardial activation, showing apex-base and septal-lateral gradients. The primary drawback to this method is that it is limited

to the epicardium. Also, if the sock material is not compliant enough, it may restrict movement of the heart wall.

c. Endocardial basket

Similar to the epicardial sock, an endocardial basket can be unfolded within the left ventricle [31]. The basket consists of multiple spines which are flexible and, when deployed, conform to the ventricular surface. Each spine has multiple electrodes for recording.

4. Hemodynamics

Various pharmacological agents have direct and indirect effects on cardiac hemodynamics: diuretics, inotropes, vasoconstrictors and chronotropes. Blood flow in the coronary circulation is regulated in similar ways to peripheral circulation, and measurement of coronary blood flow has obvious clinical applications. Within the cardiac chambers, blood pressure, flow and volumes are important parameters used to characterize the overall performance of the ventricles. Large animal models have been used for many years to quantify hemodynamics in the heart, in particular to study the effects and mechanisms of coronary artery disease and congestive heart failure. Several large animal heart failure models have been developed [32], some of which have small animal analogs. Examples include volume overload induced through an aortocaval shunt, and pressure overload hypertrophy induced by aortic banding. Other methods, however, are only possible in large animals, such as the coronary microembolism model of ischemic heart failure [33], because of constraints due to vessel size.

a. Angiography/Ventriculography

Angiography is the imaging of blood vessels, in the heart the most important are the large coronary arteries supplying the myocardium with oxygen. Angiograms of the heart chambers are important clinical measures for chamber size and shape, in particular for the ventricles (ventriculography). Coronary angiography has classically been performed with X-ray imaging after contrast injection, and clinical methods can be used in large animals. Coronary imaging may also be done with computed-tomography (CT) angiography [34] as well as magnetic resonance angiography [35]. This may also be possible in small mammals, using microCT [36] and small bore, high magnetic field MR scanners [37], but these methods are still being developed.

b. Microspheres for myocardial perfusion

Perfusion of the myocardium (regional myocardial blood flow) has been assessed with microsphere injections in large animal models [38]. These techniques provide for regional maps of tissue perfusion, essentially capillary perfusion, although they do not quantify blood flow in individual vessels. Radioactive microspheres have been used for several decades, and fluorescent or colored microspheres have been developed to reduce the use of radiation in the laboratory [38]. Multiple injections can be used for serial measurement of regional perfusion [39]. Besides microspheres, several indicator dilution techniques can also measure regional myocardial blood flow [40]. In a large animal such as the pig, there is enough tissue to analyze blood flow at multiple transmural depths, and this transmural resolution is limited in small animal models due to small tissue sample volumes.

c. Coronary vessel blood flow

Studies examining regional ischemia among others rely on local instantaneous measurement of coronary blood flow. Flow probes are commercially available using ultrasonic (i.e. Doppler) [41] as well as electromagnetic techniques [42], and are limited to use in larger coronary arteries. The most reliable results are obtained with the coronary vessel dissected and the probe

placed directly around the vessel. The same types of systems are used to measure aortic flow and cardiac output [43]. Other techniques such as local thermodilution [44], densitometry, and magnetic resonance angiography [45] have been developed for volumetric coronary blood flow measurement.

d. Pressure measurement in the ventricular chambers

Ventricular pressure is one of the most common and readily available parameters for characterizing ventricular function. Typically a catheter is inserted into one of the ventricles, usually through a peripheral artery or vein, or in some cases through the atria or directly through the myocardium, for example at the apex of the LV. Extravascular sensors consist of a fluid-filled catheter connected to the pressure sensor outside of the animal. Intravascular sensors (micro-manometers) use a catheter with a miniature pressure sensor near the tip of the catheter [46]. Thus intravascular sensors are directly coupled to the blood, which typically increases the frequency response of these systems compared to fluid-filled systems. The disadvantages of micro-tipped systems are cost, temperature drift and fragility. Ventricular contractility can be found from the derivative of a pressure signal. Several companies offer pressure-volume catheters, which measure ventricular volume with a conductance system in addition to the simultaneous pressure measurement. Unlike the other hemodynamic measurements described in this section, measurements of ventricular pressure and volume are possible in small mammals as well.

5. Summary

Advancements in therapeutics for cardiac disease in many cases involve animal testing, hence evaluation of hemodynamics, electrophysiology and cardiac mechanics in large animal models. Classical techniques and instrumentation continue to be used for pressure, flow and other important physiologic variables. Despite its limitations, X-ray imaging of implanted markers remains the gold standard for regional myocardial mechanics. However, MR tagging and echocardiographic speckle tracking will likely become increasingly popular in the coming years due to their suitability for use in patients. Combining of electrophysiologic measurements with regional mechanics has become an important technique for understanding of dyssynchronous contractions of the ventricles; other methods including angiography and electrical mapping have been developed in large animal models and are now helping to improve diagnosis and treatments for human heart disease.

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