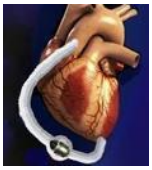

The VAD Journal: *The journal of mechanical assisted circulation and heart failure*

Peer-Reviewed Original Research

A Protocol for Collecting Human Cardiac Tissue for Research

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Abstract

This manuscript describes a protocol at the University of Kentucky that allows a translational research team to collect human myocardium that can be used for biological research. We have gained a great deal of practical experience since we started this protocol in 2008, and we hope that other groups might be able to learn from our endeavors. To date, we have procured ~4000 samples from ~230 patients. The tissue that we collect comes from organ donors and from patients who are receiving a heart transplant or a ventricular assist device because they have heart failure. We begin our manuscript by describing the importance of human samples in cardiac research. Subsequently, we describe the process for obtaining consent from patients, the cost of running the protocol, and some of the issues and practical difficulties that we have encountered. We conclude with some suggestions for other researchers who may be considering starting a similar protocol.

Keywords

Heart failure, Ventricular assist device, Human, Cardiac

Introduction

Heart failure is a clinical syndrome that manifests when there are structural and functional impairments to the heart that alters its ability to pump (1). The etiology of heart failure is complex but individuals who suffer from cardiovascular diseases including cardiomyopathy, myocarditis, hypertension, coronary atherosclerosis, and valvular and congenital heart malformations, are at increased risk (2). Currently, more than 23 million people worldwide suffer from the disease, which has a mortality rate of ~50% within the first five years of diagnosis (3). In the United States alone, heart failure affects ~6 million individuals with an estimated 650,000 new cases diagnosed annually. Taken together, heart failure costs the US healthcare system ~40 billion dollars a year (1, 4-6).

Despite intensive efforts, treatment options for patients who have advanced heart failure remain limited. Research that identifies mechanisms that contribute to impaired ventricular function can offer new hope to patients by supporting the development of better therapies. Many significant insights have been obtained to date by studying mice and rats. However, there are significant differences between the cardiac biology of rodents and humans, including (but not limited to) heart rate, oxygen consumption, and adrenergic receptor ratios (7, 8). Large animal models, such as dogs, pigs, and sheep, more closely approximate human cardiac function but also have limitations. For example, very few academic labs can afford to maintain dogs and pigs for research.

The aim of this manuscript is to describe a collection protocol that we have developed at the University of Kentucky to study cardiac samples from patients who are suffering from heart failure and research donors with non-failing hearts. It has taken us several years to develop and refine this protocol, and we would like to share what we have learned with the research community. We hope that reading about our experience will encourage other groups to start similar collection protocols. Human biospecimens cannot fully replace animal models in cardiovascular research. However, we think that many groups might be able to use human samples to accelerate their research and increase the translational significance of their work.

Informed Consent

Our program is approved by the University of Kentucky's Institutional Review Board (IRB) and is an opt-in consent program focusing on advanced heat failure. We aim to approach all patients who have been listed for heart transplant or will be implanted with a Ventricular Assist Device (VAD) and ask for their consent to use samples of their tissue for research. All of the samples that we procure are removed as part of normal clinical care and would be discarded if they were not saved for research. We do not currently perform pediatric heart transplants or

implant VADs into children at the University of Kentucky so all of our patients are 18 years or older.

Consents are normally obtained by a member of the patient's clinical team. We have found that it is simplest to obtain consent for potential heart transplant patients when they are being listed for transplant. Patients who will be receiving VADs are typically consented for research when they are being consented for the VAD implant. We explain that the samples we hope to procure will be discarded if they are not used for research and describe the potential risks of joining the study, which mainly relate to a potential loss of confidentiality if data are accidentally disclosed. We have sought consent from hundreds of patients since 2008 and virtually all of them have agreed to participate. Many of them are surprised that this sort of research is not more common. Patients often tell their team that they want to support the research because it might prevent somebody else from having to experience similar clinical problems.

Our protocol also allows us to collect myocardial samples from organ donors when the heart will not be used for transplant. For example, we might be able to acquire hearts that come from donors who have hepatitis or atherosclerosis. At the University of Kentucky, researchers and heart failure clinicians do not typically interact with the families of organ donors. Instead, the consent to donate samples is obtained by a member of the Organ Procurement Organization (OPO). Our OPO is Kentucky Organ Donor Affiliates (KODA) who typically ask the family of the donor for permission to (a) use organs for transplant, and (b) use organs for research if they cannot be used for transplant. If the family of the donor agrees to both of these requests, the OPO passes that information on to a member of our research team.

The protocol also allows us to extract data from the medical records of the patients who donated samples. We can use these data to link our research findings to specific medical conditions. We updated our protocol in 2013 so that we could share samples and the associated de-identified medical data (for example, age, diagnosis, body mass index, ejection fraction, diabetic status, etc.) with researchers at other institutions. This increases the impact of our work and further accelerates the research.

It is important to note that only researchers who are listed on the IRB protocol at the University of Kentucky are able to link specimens to specific people. Scientists at other institutions are never given access to the 18 types of data that are defined as Protected Health Information (PHI) by the Health Insurance Portability and Accountability Act (HIPAA). These data fields include names, addresses, and medical record numbers that are linked to a specific person.

At the moment, we have chosen not to return specific research results to the patients who donated samples. We explain this when we seek consent and we have adopted this approach because our current experiments won't impact the immediate treatment plan. Returning every experimental result to each patient might increase anxiety without having a positive effect. We recognize that this is

a complex area and might choose to revise the policy in the future as guidelines evolve.

Timeline for tissue procurement

Our procurement protocol is design to optimize the quality of the samples that we collect for research. The main goals are (a) to keep the heart or tissue samples cold as soon as they are removed from the patient or organ donor, and (b) to minimize the time before the samples are used for experiments with living cells or frozen in liquid nitrogen. Typically, samples that are frozen are placed in cryogenic vials and dropped into liquid nitrogen within ~30 minutes of being removed from the patient. Our experimental data show that this interval is short enough to preserve the integrity of sarcomeric proteins and mRNA (9).

As shown in Fig.1, the timeline for procurement starts when the circulating nurse who will be managing the operating room during the cardiac procedure calls a dedicated mobile phone. (Scientists in our lab take turns carrying this phone, which is answered 24 hours per day.) This first call typically occurs 2 to 3 hours before samples will become available so the scientist has time to travel to the university if they are not already there, and to prepare their equipment.

The circulating nurse calls the mobile phone a second time when the surgeon makes the first incision. This is the signal for the scientist to leave the lab and to walk to the clinical area. After changing into surgical scrubs, the scientist enters the operating room and notifies the circulating nurse that they are available to receive samples. If the operating room is very busy, the scientist may choose to wait outside. Typically though, the scientist waits in the back of the operating room for 5 to 60 minutes until the tissue is excised. The surgeon or scrub nurse hands the relevant samples to the circulating nurse who passes the tissue directly to the scientist. The scientist immediately places the tissue in a plastic container filled with cold saline slush, and then inserts that container into an insulated box. He/she then carries the insulated box back to the research lab (3 to 5 minute walk).

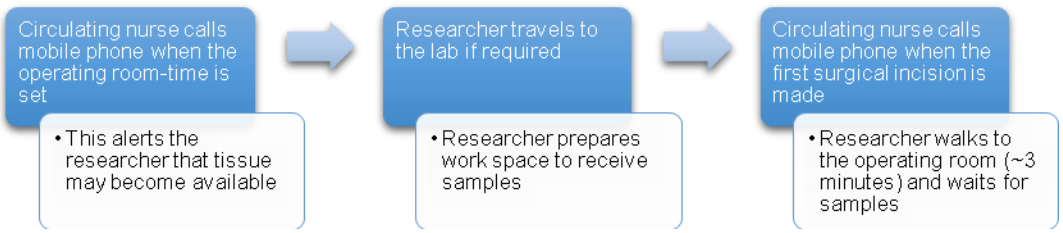

Fig. 1. Timeline for tissue procurement

Once back in the lab, the scientist removes the cardiac tissue from the insulated box and begins to dissect the samples. If they are going to attempt experiments with living cells, the scientist may isolate trabeculae or through-wall ventricular wedge sections. However, the main goal is to freeze samples for subsequent

analysis. As shown in Table 1, we aim to isolate sections from three transmural regions (sub-endocardial, mid-myocardial, and sub-epicardial) of tissue cores procured during VAD implants and from the left ventricular wall of explanted hearts. When possible, we also try to produce additional samples from the septum, the right ventricle, and both atria.

Tissue samples are cut into small blocks weighing 500 to 1000 mg and placed into pre-labeled 2 ml cryogenic tubes. These are then dropped into liquid nitrogen where the tissue freezes rapidly. After all of the samples have been frozen, they are placed in freezer boxes, and transferred to large tanks where they are maintained in the vapor phase of liquid nitrogen for long-term storage.

Table 1. Procurement procedure for heart transplant and LVAD samples

	Heart Transplant	**LVAD**
Location of samples collected	• Left ventricular free wall • Right ventricular free wall • Atria • Septum	• Through-wall core removed from the left ventricular apex before the VAD is implanted
Processing	• Cut tissue samples that are ~5 x 5 cm • Split left ventricular samples into sub-endocardial, mid-myocardial, and sub-epicardial regions (**Fig. 2a**) • Obtain 6 to 10 samples (each 500~1000 mg) for each region and place in cryogenic vials	• Split core into transmural regions if possible • Further dissect into a few (1 to 3) samples (each ~500 mg) and place in cryogenic vials
Storage of samples	• Flash freeze samples in liquid nitrogen • Place frozen samples in storage box (**Fig. 2b**) • Place box in cryogenic tank for storage in the vapor phase of liquid nitrogen (**Fig. 2c**)	

Data management

De-identification

Only scientists and clinicians who are listed on our IRB protocol are allowed to know the identities of the people who donated cardiac tissue. To preserve privacy, we therefore allocate a unique randomized identifier (which we term a hashcode) to each tissue procurement procedure. The vials containing the tissue samples are only labeled with this hashcode and the region of the heart that the sample was procured from. This allows us to share samples with other researchers without compromising the identity of the patient or donor.

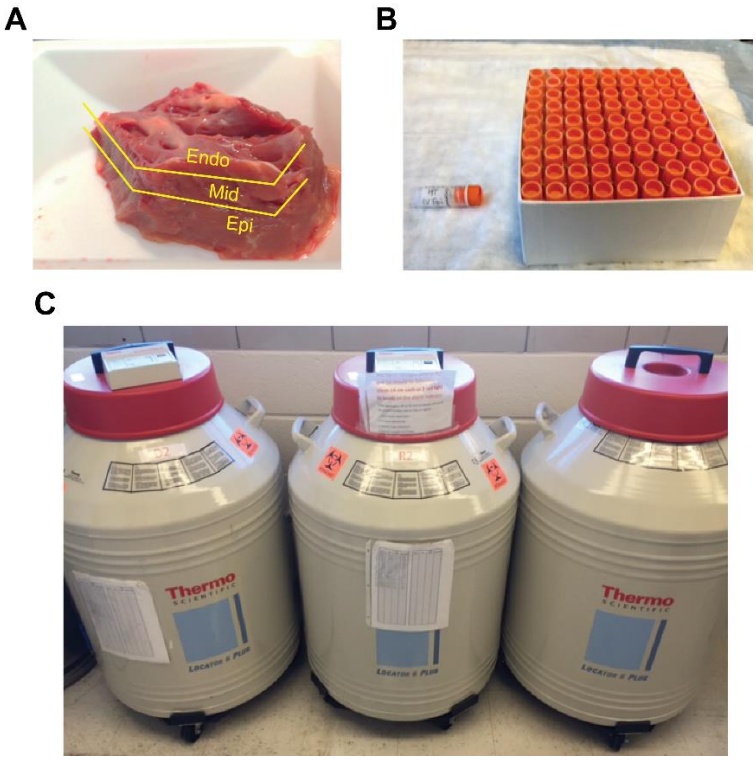

Fig. 2. Tissue dissection and storage.

Fig. 2a. ~5 cm x 5 cm piece of tissue from the LV annotated to show how the samples are separated into three transmural regions.

Fig. 2b. Storage vials with hashcode and storage box.

Fig. 2c. Cryogenic tanks that store samples in the vapor phase of liquid nitrogen

Each hashcode consists of 5 characters which are either digits (0 to 9) or letters in the range A to F (for example, 34F2A). We generated ~1,000,000 such codes at the beginning of our study by creating a random permutation of the integers from 1 to 2^{16} and then converting that list to hexadecimal. Hashcodes are

selected from the random list in turn so there is no practical way for an outside party to link the hashcode to a specific person, or even to an approximate procurement date.

Inventory

As described, sample vials are labeled only with the hashcode for the procurement and the region of the heart that the tissue was acquired from (for example, A231E, Right Ventricle). Vials are placed in storage boxes that have 81 positions (labeled from A1 to I9). These boxes are in turn placed in one of 144 slots in our 3 cryogenic storage tanks (Fig. 2c).

We record the hashcode, cardiac region, box position, slot number, and sample status (for example, available, or previously thawed) for each sample in Excel and use the resulting computer file to manage our inventory. We have several computer programs (written in MATLAB) that allow us to select samples and/or generate reports from the Excel file.

Clinical data

We use REDCap (Research Electronic Data Capture) software (10-13) to store and manage the clinical data that we collect for each patient and organ donor. This software is a secure, web-based database that is HIPAA compliant and approved by our IRB. One of the advantages of REDCap is that it allows us to restrict access to patient identifiers. Thus researchers who are not listed on our IRB can access the database to download clinical data but cannot obtain information about the identity of the patient or donor. This simplifies our logistics and makes it easier to manage our protocol.

Experimental techniques

We use the myocardial samples that we acquire to investigate cellular and molecular mechanisms that contribute to heart failure. We can perform experiments immediately after we procure the samples (fresh tissue) or using samples that have been previously frozen. Our laboratory specializes in experiments assessing contractile properties but we have also analyzed the samples using numerous histological and biochemical techniques.

The techniques that we have implemented using human samples include:

Fresh tissue:

- Mechanical studies
    - Single cells –ventricular myocytes are loaded with Fura-2 (a dye that indicates the intracellular Ca^{2+} concentration) and electrically stimulated. We use a photomultiplier and a high-speed camera to measure the Ca^{2+} transients and shortening profiles as the cells undergo unloaded twitch contractions.

- Trabeculae – intact trabeculae are attached between a force transducer and motor. We measure contractile force during electrically evoked twitch contractions and a range of parameters relating to myocardial relaxation.

Frozen tissue:

- Mechanical studies
  - Permeabilized single cells – single cells are chemically permeabilized using triton and attached between a force transducer and a motor. We typically measure contractile force, calcium sensitivity (pCa_{50}), and the rate of tension recovery (k_{tr}) at different levels of Ca^{2+} activation. These cells do not have large amounts of extracellular matrix so their passive stiffness is primarily due to titin molecules.
  - Permeabilized multicellular preparations – larger preparations (typically ~600 x 120 x 120 µm) are chemically permeabilized and attached between a force transducer and a motor and analyzed using similar techniques to those described for single permeabilized cells above. The passive stiffness of these preparations reflects titin molecules and an additional contribution from extracellular matrix proteins.
- Histology
- Microarrays to measure mRNA and microRNA levels
- Gel electrophoresis
- Collagen analysis
- PCR

Personnel and financial costs

Some of the clinical staff take on additional responsibilities to help us procure cardiac samples for research. We list some of these responsibilities along with an estimate of the time involved in Table 2. Table 3 shows the responsibilities and time commitment for research staff. Finally, Table 4 provides estimates of the financial costs. We are providing these data because we think that they may be useful to readers who are interested in starting their own procurement system.

Table 2 - Clinical staff

Title	Number of personnel involved	Responsibilities for this program	Time
Transplant surgeons	4	Agree to allow samples to be used for research	Minimal
Transplant and VAD coordinators	3	Obtain patient consents	20 minutes per patient
Operating room nursing staff	Many	Call research scientist, hand excised heart to scientist	10 minutes per procurement

Table 3 - Research staff

Title	Number of personnel involved	Responsibilities	Time
Scientists	3	Procure samples	Up to 4 hours waiting per heart plus ~1 hour to isolate samples, clean the procedure areas, and restock supplies.
IRB manager	1	Maintain IRB protocol, manage changes in personnel	20 hours per year
Clinical data manager	1	Maintain clinical database, extract data from medical records	40 hours per year plus 2 hours per patient
Sample management	1	Manage specimen inventory, maintain cryogenic tanks	6 hours per week

Item	Cost	Yearly estimate
Sample vials	$180 per case	$540 (~3 cases per year)
Liquid nitrogen	$38 per month	$456
Phone	$50 per month	$600
Cryogenic tanks	$5,000 per tank (holds ~1600 vials)	

Table 4 – Cost

Problems and weaknesses

One of the challenges that we have faced with this protocol is ensuring that scientists are available 24/7 to collect the samples from the operating room. This is a particular problem for heart transplants because these procedures can occur at any time of the day or night. (Most VAD placements take place during week-day mornings when the scientists are typically at work already.) Having a large team of scientists available to procure samples places less burden on each individual and makes it easier to provide 24/7 cover. However, having a team that is too large is also problematic. For example, if each scientist only performs a few procedures it can be difficult to ensure that samples are dissected consistently. A large team also makes it harder for each scientist to build their working relationship with the clinical staff in the operating room. This relationship is important because the clinical staff need to be confident that the scientist can collect the samples without compromising any aspect of the surgery. We have found that the system works best at our institution with a team of ~4 scientists.

Another challenge is obtaining non-failing hearts. Data provided by our local organ procurement agency (Kentucky Organ Donor Affiliates) show that thoracic organs are donated roughly once per week at the University of Kentucky. Since many hearts are not suitable for transplant, it might be possible to obtain non-failing hearts once every 2 or 3 weeks. In practice, we have collected an average of about two per year. The main problem has been that our clinical collaborators are not normally involved in organ procurements when the heart is not being used for transplant. Thus, we don't learn about organ procurements until after they have occurred. We are trying to improve our performance in this area and have recently developed stronger interactions with the organ donation team at our institution. We hope that this new development will help us to accelerate the rate at which we procure non-failing hearts.

One of the main interests of our lab is understanding how regional and transmural patterns of contraction influence ventricular function. To learn more about these effects, we measure the biochemical and contractile properties of cells from different parts of the heart. It is not yet clear how these properties are distributed, which makes it difficult to know the optimal way to dissect the samples. The practical approach that we have taken for left ventricular tissue is to separate the free wall into thirds (Fig. 2a). This is probably not a perfect solution but it has shown useful results in both rats and humans (9, 14).

We have also thought about how freezing the samples might influence the experimental results. To learn more about these potential effects, we would need to perform multiple experiments comparing fresh and previously frozen samples. This is difficult for our group because we don't normally have enough experienced scientists on hand to process the samples and to run the mechanical experiments at the same time. It is particularly hard to run mechanical experiments on tissue obtained from transplants since these procedures normally take place outside normal working hours.

Recommendations

We have gained a great deal of practical experience since we started this protocol in 2008. This section lists a few ideas and concepts that groups who are considering starting their own protocols may wish to consider**.**

Develop positive relationships with your IRB and biosafety teams

- We spoke to members of our IRB before we started writing our protocol so that we could gain from their experience and advice. We also initiated interactions with our biosafety advisors rather than waiting until our labs were inspected. This helped us to develop protocols that are good for our patients, our institution, and the researchers who perform the experiments.

Interact with patients and seek their opinions

- It is important that patients understand how their samples will be used and what our research is trying to achieve. We try to meet regularly with patients and make sure that they support our goals. The VAD and Transplant Support Group at our institution provides an ideal forum for these interactions.

Identify key personnel who are responsible for specific tasks

- Rather than taking turns on each aspect of the protocol, we ask key personnel to run specific components. For example, a transplant coordinator consents each patient who will be listed for a heart transplant, a scientist manages the inventory, and a faculty member collates clinical

data from the medical records. We have found that this helps to maintain consistency and allows team members to specialize in specific areas.

Simplify communication

- We provide a single telephone number that the operating room can call when cardiac tissue might become available. This number is associated with a single cell phone that the scientists take turns carrying. An alternative would be to use a forwarding system (such as Google Voice) that sends a call to multiple phones. On balance, our team prefers to use a dedicated phone rather than linking their personal cellphones to the tissue procurement process.

Do not hoard samples

- Patients do not donate their cardiac tissue so that it can be stored in a freezer. Instead, they hope that their samples might help other people from having to receive heart transplants or ventricular assist devices. We try to share any samples that we do not need for our own experiments with other research groups. Our goal is to work together to accelerate research focusing on advanced heart failure.

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