Scisense PV Technical Note

Ex-vivo Isolated Working Heart Model for Cardiac Assessment using PV Loops

The practise of isolating the heart from an animal's circulation simplifies the examination of inotropic (contractile) and chronotropic (heart rate) effects without confounding vascular responses. As isolated heart models lack fresh blood circulation, hormonal and autonomic nervous responses and otherwise very complex in-vivo factors are decoupled which helps to perform a variety of basic analyses of fundamental cardiac properties. This allows for testing of pharmacological compounds, unmasking potential direct action of studied compounds or studying basic cardiac muscle physiology. The preparation also allows direct inductions of ischemia or arrhythmias with precise mapping the conduction pathways, coronary blood flow regulation and cardiac metabolism.

There are two basic types of isolated heart models:

- Langendorff: retrograde perfusion via the cannulated aorta closing the aortic valve filling the Valsalva sinuses while entering into left and right coronary arteries through left and right coronary ostia. Perfusion buffer then passes through the coronary vascular bed before its drainage into coronary veins into coronary sinus in the RA. In this preparation the ventricle chamber(s) are not perfused. This set up is very important to discern mechanical behavior of smooth vascular muscle cells in the coronary vasculature expressed as changes in vessel radius. Can be either constant pressure via gravity-fed apparatus or using constant perfusion flow (3). See "Langendorff Model for Cardiac Assesment" for more informations.
- Working Heart: antegrade perfusion where perfusate enters through the mitral valve and is ejected through the aortic valve. This major modification in the isolated heart model was made by Neely and Morgan in 1967 (5). Isolated heart preparations that performed mechanical work are commonly referred to as the "working heart," but more appropriate term is the ejecting heart as the Langendorff heart is also "working" (6).

Flow and pressure measurements play important roles in maintaining these models.

EXCISION OF THE HEART AND ITS CANNULATION (MOUSE)

Note: All animal protocols have to follow local and institutional guidelines.

Heparinized blood is circulated through the heart before excision. Excision is done under general anesthesia using median sternotomy and quick cardiac dissection, or by ventilating the animal and slowly exposing the heart using a chest retractor and in-situ cannulation of the aorta before its full cardiac dissection. Note: cannulation time has to be kept to a minimum; the mouse heart is highly active metabolically and has oxygen and substrate for up to 60 sec. from the time of removal.

Removal of the heart is done by cutting across the arch of the aorta (leaving enough space for mounting on the cannula) along with all other vascular structures in the area. The heart is removed into ice-cold heparinized phosphate buffered saline (4°C) to arrest beating. The ascending aorta is then mounted on the aortic cannula. Gauge sizes of cannulas for mouse range from 22-16, for rats from 14-8(3).

During transfer of the mounted and cannulated heart the perfusate should be running to establish quick fluid perfusion. During cannula mounting pay special attention to preventing rupture of the aortic valves or associated structures. If valves are compromised, it limits the ability to maintain coronary perfusion pressure as the perfusate leaks inside the LV. Free drainage of coronary artery effluate/perfusate is enabled by an incision in the area of the pulmonary artery. At this time, an RV thermocouple wire can be inserted to monitor cardiac temperature (4).

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Working Heart Model for Cardiac Assessment

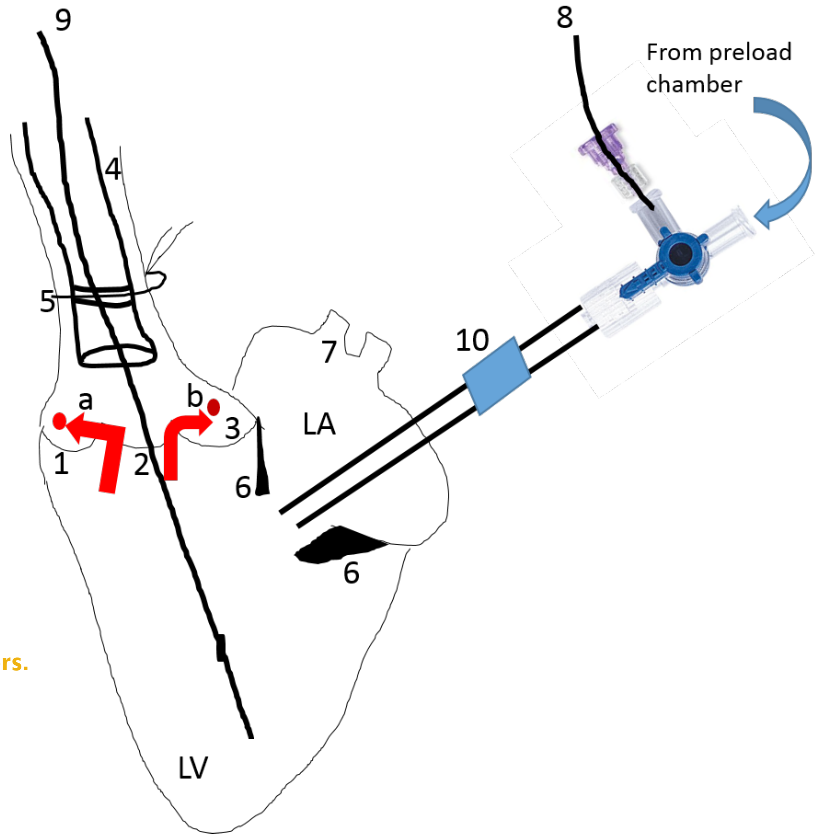
Please note: complete cardiac isolation will cause denervation and bradycardia partially due to limited blood supply to the SA pacemaker node (e.g. reducing heart rate in a mouse to 380-450 bpm from normal 600 bpm in vivo).

Perfusate is typically a nutrient rich (NR), oxygenated (O_2) solution-buffer (e.g. oxygenated Krebs-Henseleit bicarbonate-KHB or Thyrode's buffer at 37.5°C, pH 7.4, Conductivity of 16-20 ms/cm). This solution is supplied at a constant flow rate up to 15 ml/min/g or at a constant hydrostatic pressure (60-80 mmHg with commencement between 50-60 mmHg), allowing the heart to work for several hours after excision.

As the heart is mounted, the first minutes are critical in determining the success of the preparation. At this time it is also important to establish fluid to fluid infiltration to limit preconditioning due to delayed cardiac perfusion. Experiments can start 10-15 min after successfully establishing heart beats and last up to 4 hours. In most cases investigation will be time-limited as non-blood perfusion invites protein loss leading to tissue edema. Pacing can be used to increase heart rate to physiological levels allowing better direct comparisons with in-vivo cardiac contractility.

TIPS FOR SETTING UP THE SYSTEM

1. Ensure that the cannula position does not inhibit flow to the coronaries.
2. Correctly tighten the suture around the ascending aorta (best in the groove of the cannula).
3. Use the pressure sensor and flowsensor to control the preload.
4. Properly set up Pressure/PV Catheter in the LV using the CO from the flowsensor.
5. Temporary preload reduction is performed by clamping of the preload inflow line.



Ejecting heart set-up with pressure and flow sensors.

a. opening of the right coronary artery
b. opening of the left coronary artery

1. **Right semilunar cusp**
2. **Posterior semilunar cusp**
3. **Left semilunar cusp**
4. **Ascending Aorta**
5. **Suture location (in the groove)**
6. **Mitral valve**
7. **Left Pulmonary veins**
8. **Pressure catheter inserted into Tuohy Borst to monitor preload pressure**
9. **Pressure/PV catheter measuring cardiac load dependent and independent cardiac function (after temporary clamping of the preload line)**
10. **Tubing flowsensor placed on preload line. Tubing flowsensor placed on afterload line (not shown)**

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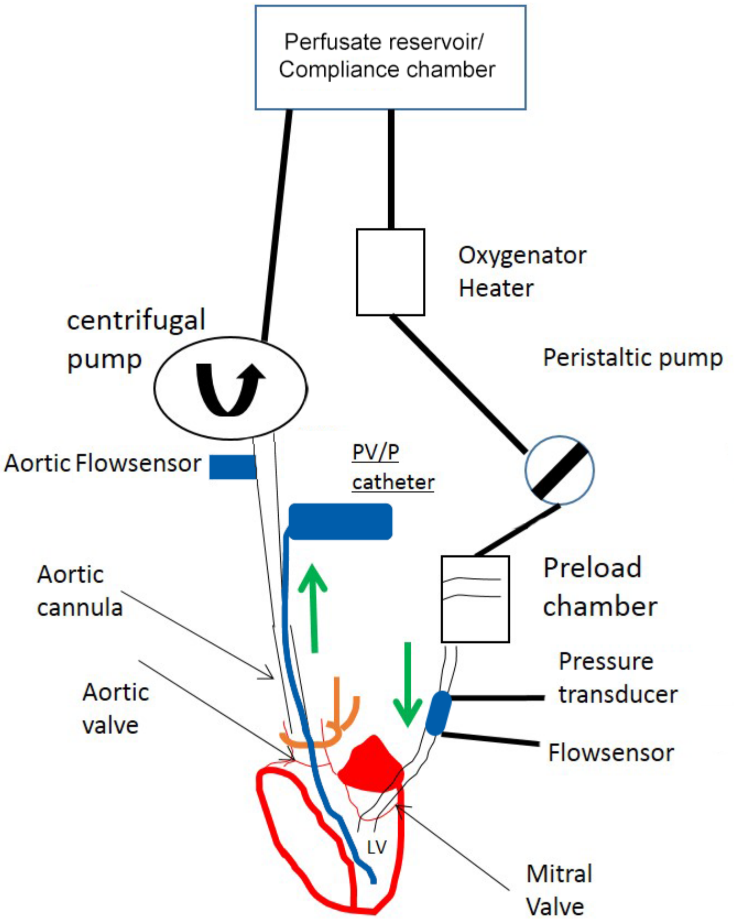
Working Heart Model for Cardiac Assessment

Isolated Ejecting Heart (IEH) Model

The difference of IEH, also know as fluid ejecting heart model or working heart, as compared to Langendorff is in the set-up and parameters measured. In the Langendorff set-up, the only perfused structures are the coronary arteries. In the ejecting heart model the ventricle is also perfused allowing control over both the preload and afterload with the ability to capture complete PV loops. Moreover, load independent parameters are able to be investigated including comparison of pressure-volume area (PVA) to myocardial oxygen consumption (mVO2), using temporary reductions in preload or afterload. Cardiac output in the ejecting heart is a combination of coronary flow with aortic flow. For this reason, compared to a Langendorff preparation, it is very important that ejecting heart aortic cannulas are as close to the inner aortic diameter as possible (1).

When mounting the working heart, the Left atrium (LA) is cannulated after the heart has been perfused through the aorta. The initial filling pressure (preload) is set up by introduction of a cannula into the left atrium with the pressure set to about 8 mmHg, whereas the afterload is created by the height of the compliance reservoir above the aortic cannula corresponding to about 55 -70 mmHg. The hearts are perfused with a nutrient rich (NR), oxygenated (O2) solution-buffer (e.g. oxygenated Krebs-Henseleit bicarbonate -KHB or Thyrode's buffer at 37.5°C, pH 7.4), usually with addition of pyruvate, fatty acids, platelets and plasma expanders etc (5).



Schematic representation of a set up of working heart model including possibilities for pressure, PV and flow measurements for ensuring control over the cardiac preparation. Please note: during the left ventricular ejection phase, a portion of the perfusate is forced into the coronary ostia enabling nutrient and oxygen rich perfusate to supply coronary vessels of the heart.

Instrumentation Sensor	Parameters measured
PV transducer in LV	LVP, LVV, load dependent and load-independent properties
Aortic pressure transducer	aortic pressure
Aortic flow sensor	aortic flow
Atrial pressure transducer	preload pressure
Preload flow sensor	atrial inflow

PXN in-line (above) and PXL clamp-on (not shown) tubing flowsensors can be implemented into the circuit giving information about preload or afterload of the isolated ejecting heart.

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Working Heart Model for Cardiac Assessment

Ejecting heart cardiac output (CO) is equal to the venous return from the lungs (represented by the oxygenator and heater in the circuit) to the left atrium (LA). The venous return is represented by the flow of perfusate from preload chamber via the atrial cannula. The LA perfusion line must be capable of delivering perfusate at a rate sufficient to support the maximum CO of a working heart at any particular preload. If the LA perfusion line is too small or there is an obstruction or debris decreasing flow and thus preload, it will falsely limit the CO. Using an inline flowsensor or in-line pressure sensor enables control of this parameter. Moreover, using a pressure or flow sensor ensures that LV filling is not limited by inadequate LA atrial inflow.

Filling of the LA can be determined by running the equipment without a heart attached and measuring the flow from the LA line. Flow rate of at least 150 ml/min is recommended for a 1g heart. The perfusion fluid enters via the mitral valve into the left ventricle and from there it is ejected through the aortic cannula against a hydrostatic pressure set via the compliance loop. The afterload is determined by the height of the compliance reservoir above the aortic cannula. The compliance bubble trap contains a 2 mm diameter air bubble and mimics normal vascular elasticity. It is an essential component of the perfusion circuit and greatly increasing the chances of successful working heart function. In the course of left ventricular ejection, a portion of the perfusion fluid is forced into the coronary ostia and thereby perfuses the coronary vessels of the heart. Cardiac output from the IEH is detected and measured by the flowsensor. See schematic picture for more circuit details.

APPLICATIONS OF WORKING HEART SET-UP USING PV MEASUREMENTS

Steady-state relationships can be obtained by varying the loading conditions of the heart over a wide range preload and afterload to obtain:

- Investigation of positive and negative inotropic effect (pharmacology) including load independent values
- Calcium antagonism
- Metabolic studies
- Arrhythmogenic, anti-arrhythmic, anti-fibrillatory effects (pharmacology)
- Electrophysiological evaluation (mapping) using cardiovascular agents
- Fluorescent/luminescent imaging

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