Production of living myocardial slices from circulatory death hearts after ex vivo heart perfusion

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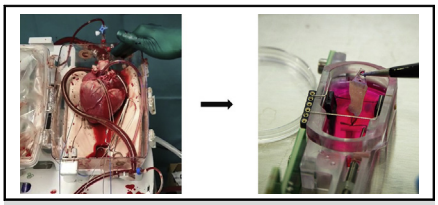


Ex-vivo heart perfusion followed by in-vitro cardiac slice culture.

CENTRAL MESSAGE

Living myocardial slice production from ex vivo perfused porcine slaughterhouse hearts provides a unique combination of 2 research platforms for translational cardiothoracic research purposes.

Video clip is available online.

Living myocardial slices (LMS) are ultrathin sections of beating cardiac tissue that maintain 3-dimensional microarchitecture and multicellularity and are cultured under conditions of near-physiological preload and continuous electrical excitation.[1] LMS are mostly produced from human surgical biopsies obtained from heart transplantations or mechanical support implantations, limiting the type and availability of provided tissue. Here, we present a recently validated novel technique for LMS[1] that we implemented for donation after circulatory death (DCD) porcine slaughterhouse hearts on ex vivo heart perfusion (EVHP). Integration of these 2 research modalities introduces an innovative platform for translational DCD studies.

TECHNIQUE

Hearts were excised and blood collected as per standard procedure of the local abattoir. Cardioplegic solution (2 L of 4 °C St Thomas Hospital with 2500 U/L heparin) was rapidly administered resulting in a total warm ischemic time of less than 10 minutes. The heart was placed on ice and transported to the laboratory for EVHP, similar to the human EVHP transplantation protocol from Transmedics (Transmedics Inc).[2] In short, cannulas were fixed in the ascending aorta and pulmonary artery, caval veins were ligated, and a vent was positioned in the left ventricle (Figure 1). The heart was reperfused in Langendorff mode with a mixture of priming solution and porcine blood, subsequently leading to active coronary perfusion and spontaneous contractions. Blood perfusate exiting the pulmonary artery cannula and left ventricle vent was collected and recirculated. Aortic pressure (75-85 mm Hg), coronary flow (700-800 mL/min), blood gas (pH: 7.35-7.45, partial pressure of oxygen: 80-105 mm Hg, partial pressure of carbon dioxide: 35-45 mm Hg), metabolic (glucose: 5-7 mmol/L, lactate: downward trend), and electrolyte values (Na^+: 135-145 mmol/L, K^+: 3.5-5.0 mmol/L, Ca^{2+}: 1.1-1.3 mmol/L) were continuously monitored and optimized.

After 2 hours of Langendorff perfusion, the heart was cooled and arrested using cardioplegia. Biopsies were taken from the left and right ventricular free wall and immediately submerged in 4 °C Tyrode solution for LMS production. Specimens were dissected in tissue blocks of 1 cm^3, embedded in 4% low-melting agarose, and cut to 300-μm thin slices with a precision-cutting vibratome. Slices were fixated between small plastic triangles with surgical Histoacryl glue (B. Braun) in alignment with the myocardial fiber orientation. LMS were mounted in the culture chambers and cultured at 38 °C in standard cell culture medium. A

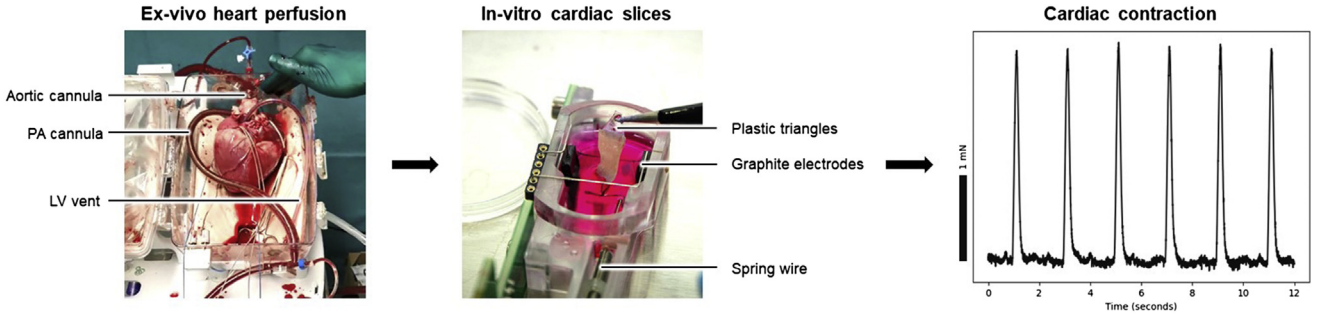


FIGURE 1. Hearts were mounted on an ex vivo heart perfusion system and subsequently cultured as living myocardial slices (*LMS*) in biomimetic cultivation chambers. When electrically stimulated and when preload is applied, LMS are contracting with real-time contractile feedback. *PA*, Pulmonary artery; *LV*, left ventricle.

physiological preload of ∼1 mN was set via a spring wire to mimic volume loading of the heart (Figure 1). LMS showed contractions at each electrical impulse sent via graphite field electrodes (frequency: 0.5 Hz, pulse duration: 3 milliseconds, stimulation current: 50 mA).[1] LMS culture was stopped after 1 week of contractions. A summary of the technique is presented in Video 1.

COMMENTS

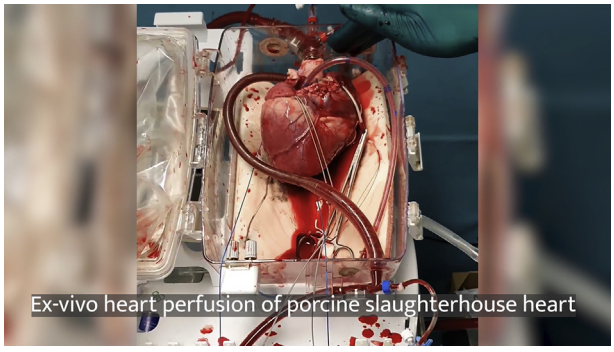
The presented technique was applied to 3 DCD hearts and ∼10 slices per heart were made, resulting in 32 slices of which 22 LMS showed contractions. Ex vivo perfusion was needed to revive these LMS, since viable production of LMS from slaughterhouse hearts without normothermic perfusion cannot be accomplished. Clinical transplantation procedures using DCD hearts corroborate this finding and also need revival on EVHP, due to the detrimental effects of prolonged warm ischemia. Similarly, slaughterhouse hearts are obtained after circulatory death and it appears that these hearts also need normothermic reperfusion before viable LMS can be produced. Hence, slaughterhouse hearts present a clinically relevant model for DCD transplantations while decreasing unnecessary animal experimentation[3] and providing an unlimited supply of tissue for biomimetic examination using LMS.

The combination of LMS with EVHP requires extensive practice,[4] thereby making quality of LMS dependent on the practical and technical skills of the researchers. For this reason, we recommend a multidisciplinary research team with experts from surgery, perfusion, and laboratory science, which only aids to the translational approach and learning curve of the presented technique.

The combination provides the best of both worlds, as EVHP may be limited to acute experimentation of several hours, LMS provide prolonged experimentation up to several weeks.[1] As such, the model can be used for a wide variety of innovative DCD experiments, including conditioning of the donor heart to protect against ischemia–reperfusion injury. Cardiac conditioning with pharmacologic compounds and mesenchymal stem cell therapies are exciting routes to further explore.[5] EVHP provides the perfect platform to deliver such substances to the heart, where the effect of those compounds can be evaluated on cellular level with beating LMS for prolonged periods of time.

In the future, the technique can be used for personalized medicine studies by first testing different medications on the donor heart's LMS and then translating those outcomes to the heart recipient's treatment plan.

In conclusion, we present a novel platform for translational cardiac research, combining LMS with EVHP. This setup allows for prolonged contractility studies in light of the current expansion of human DCD heart transplantations, without the use of animal experimentation.



VIDEO 1. Summary of the technique to produce living myocardial slices after ex vivo heart perfusion. Video available at: https://www.jtcvs.org/article/S2666-2507(22)00207-3/fulltext.

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