Development of an isolated MR-compatible working pig heart setup for structural and functional analysis of cardiac diseases

Fanny Vaillant[1,2], Julie Magat[1,2], Jérôme Naulin[1,2], Virginie Loyer[1,2], Delphine Vieillot[3], Véronique Arsac[1,2], Philippe Diolez[1,2], and Bruno Quesson[1,2]
[1]*IHU LIRYC, Pessac, France,* [2]*Centre de Recherche Cardio-Thoracique de Bordeaux INSERM U1045, Université Bordeaux Segalen, France,* [3]*Université Bordeaux Segalen, France*

Target audience: MR Scientists, clinicians and biologists specialized in imaging of cardiac structure, function and metabolism.

Purpose:
Magnetic Resonance (MR) imaging and spectroscopy allow non invasive characterization of cardiac structure, function and metabolism. However, the vast majority of *ex vivo* studies on isolated perfused hearts are performed using Langendorff setup (perfusion is maintained but without filling the heart cavities) (2) and are designed for rodents. However, cardiac structure and metabolism vary between species and make conclusions of the observed cardiac characteristics in small animal models difficult to extrapolate in humans. In order to provide a model closer to the human heart, we developed a MR-compatible set-up of isolated working heart for pig, which ensures complete perfusion of the left atria and ventricle and where the working load can be adjusted to mimic *in vivo* conditions, allowing assessment of the intrinsic myocardial properties in the absence of extra cardiac influences (e.g. neurohumoral influences, respiratory motion…).

Methods: *Animal preparation and ex vivo set-up*: after anaesthesia, pigs (≈60kg, N=3) received an injection of heparin, the thorax was opened and the heart was rapidly excised (after induction of ventricular fibrillation to avoid presence of air bubbles in the coronary arteries). The aorta and pulmonary veins were canulated to perfuse the heart with autologous blood diluted with a modified Krebs buffer (v/v: 1/3, gassed with O_2/CO_2 95/5%) containing hormones (8 nM insulin, 5 nM epinephrine) and metabolic substrates (16 mM glucose, 0.5 mM pyruvate, 1 mM lactate). A schematic view of the perfusion device is shown in Figure 1. Perfusion of the heart was maintained at physiological pressure (preload at 15cm H_2O). During systole, the heart pumped in the aortic cannula against a physiological afterload (at 85cm H_2O). MR-compatible electrodes (lead needle electrode, SA instruments, New York USA) were positioned into the myocardium (right atrium, ventricular apex and fat for the reference) and connected to the ECG device of the MR system, in order to record ECG signals and synchronise the MR acquisition sequences. Two additional carbon electrodes were implanted in the right atria and apex and connected to a function generator (model 33120A, Agilent Technologies) to allow pacing of the heart. Blood temperature was continuously monitored (and maintained at 37°C) with an optic fibre probe (Luxtron) inserted into the pulmonary artery. In order to validate parameters for *ex vivo* cardiac function recorded via MR measurements, additional hearts were perfused outside of the MR environment and cardiac function was recorded with a pressure-volume catheter (Millar Instrument, TX USA) inserted into the left ventricle via the apex.

MR experiments: all experiments were performed at 1.5T (Avanto; Siemens, Erlangen Germany). A 16 elements cardiac array coil was positioned below the thermo-regulated reservoir surrounding the perfused heart, and two 8 elements coils were positioned laterally. After scout images were recorded, ECG-gated cine short axis (SA) images were acquired using a Balanced-SSFP sequence with TE/TR/slices/cardiac phases/flip angle/Res=1.43ms/3.7ms/12/20/66°/0.9x0.9x2mm^3. The ejection fraction (EF) of the Left Ventricule (LV) was evaluated from these images by measuring end-diastolic (EDV) and end-systolic volumes (ESV). EF was calculated from EF = (EDV-ESV)/EDVx100. After injection of 0.5 mM of Gadolinium in the perfusion medium, T1-weigthed images were acquired using the inversion-recovery MOLLI (1) sequence with TE/TR/T1start/Flip Angle/Resolution=1.06ms/595ms/116ms/35°/1.82x1.82mm and partial Fourier (6/8) acquisition. Five inversions were applied to recover 17 inversion delays points of measures between 116ms and 4692ms. Image series were processed for each pixel with a three parameters non-linear fitting routine written in Matlab to compute T1 parametric maps (in ms). These parametric maps were used to assess potential alteration of the myocardium.

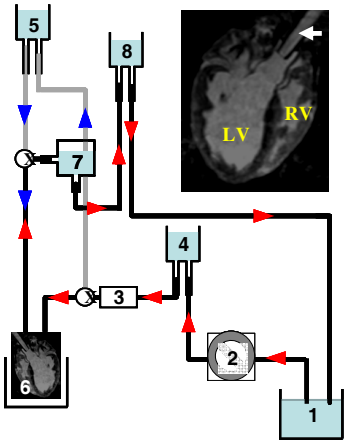

Figure 1: MR-compatible set-up for the isolated working heart.
1.Main reservoir, 2.Pump, 3.Oxygenator, 4.Preload, 5.Langendorff reservoir, 6.Heart chamber, 7.Compliance chamber, 8.Postload.
Image on the top right is a MR slice of a 3D IR sequence showing the cannula in the aorta (white arrow).

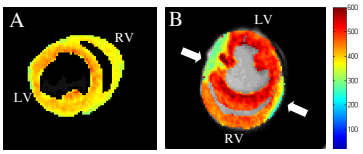

Figure 2: T_1 mapping of myocardium. T_1 values (in ms) are indicated on the scale. White arrows indicate anterior and posterior faces of the heart.

Results: After cannulas were positioned, hearts were reperfused in the Langendorff mode for 20 minutes to wash out cardioplegic solution. Then, perfusion was switched into the working mode. After stabilization of the *ex vivo* cardiac function in the working mode, MR recordings were started.

Typical EF values were 20±5%, in agreement with values obtained using Millar catheter. Figure 2A shows a typical example of a normal heart with homogeneous T_1 values (~400 ms) over the complete myocardium. Figure 2B displays spatial variations of the distribution of T_1 values (see arrows) on anterior and posterior faces of the heart. The macroscopic analysis of the samples at the end of the experiment (Fig. 3) confirmed the presence of lesions at these locations. The maximal perfusion duration in the working mode was 200 min, with nearly stable cardiac function, although a slow progressive reduction of aortic flow was observed (750 mL/min at t=0 min, 550 mL/min at t=150 min and 450mL/min at t=200 min). The heart rate increased from 94 bpm to 110 bpm during that period. Other MR measurements (data not shown) were performed (tagging, coronary artery imaging …) to supplement assessment of cardiac function and structure. Physiological pacing of the heart (with frequency ranging from 100 to 180 bpm) was successfully tested during MR acquisition, without noticeable image degradation or alteration of cardiac function. Transient fibrillation was also successfully induced using burst stimulation at 30 Hz during 1 s.

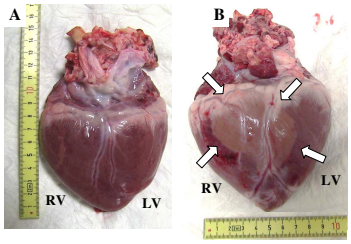

Fig.3. picture of the posterior face of the two hearts shown in Fig. 2, at the end of the experiment. White arrows indicate the presence of a large myocardial lesion.

Discussion:
The isolated working heart setup presented in this study is a physiologically relevant model to evaluate the cardiac function and a powerful approach to assess intrinsic mechanical and metabolic functions under physiological conditions or during induced arrhythmogenic periods. We demonstrate here for the first time the possibility (i) to perform blood-perfusion of hearts from large animals with a working left atrium and ventricle inside a MR environment, and (ii) to record parameters currently assessed in clinic as cardiac structure and dynamics under well controlled conditions. This *ex vivo* model approach gives the opportunity to strictly control the cardiac loads and metabolic supply and to study their modifications during a wide range of experimental conditions (ischemia, heart failure, arrhythmia…).

Conclusions:
Our *ex vivo* working heart model provides a new powerful tool to investigate cardiac mechanical and electrophysiological functions, structure and metabolism without any impact of extra cardiac environment. This experimental model will be useful for a better understanding of cardiac function regulation and to assess efficiency of new diagnostic methods and therapies.

References: (1) Messroghli DR, et al. Magn Reson Medd 2007, 58, 34-40. (2) Schuster et al. Journal of Cardiovascular Magnetic Resonance 2010 12:53.