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Real time Pressure-Volume loops in mice using complex admittance: measurement and implications

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Abstract—**Real time left ventricular (LV) pressure-volume (P-V) loops have provided a framework for understanding cardiac mechanics in experimental animals and humans [1]. Conductance measurements have been used for the past 25 years to generate an instantaneous left ventricular (LV) volume signal. The standard conductance method yields a combination of blood and ventricular muscle conductance; however, only the blood signal is used to estimate LV volume. The state of the art techniques like hypertonic saline injection and IVC occlusion, determine only a single steady-state value of the parallel conductance of the cardiac muscle. This is inaccurate, since the cardiac muscle component should vary instantaneously throughout the cardiac cycle as the LV contracts and fills, because the distance from the catheter to the muscle changes.**

The capacitive nature of cardiac muscle can be used to identify its contribution to the combined conductance signal. This method, in contrast to existing techniques, yields an instantaneous estimate of the parallel admittance of cardiac muscle that can be used to correct the measurement in real time. The corrected signal consists of blood conductance alone. We present the results of real time *in vivo* measurements of pressure – admittance and pressure – phase loops inside the murine left ventricle. We then use the magnitude and phase angle of the measured admittance to determine pressure volume loops inside the LV on a beat by beat basis. These results may be used to achieve a substantial improvement in the state of the art in this measurement method by eliminating the need for hypertonic saline injection.

I. Introduction

Determination of instantaneous volume in the murine LV is difficult due to the small heart size (5 mm length, 160 mg mass, and 40 µl LV volume) and its rapid heart rate (300-700 beats/min). Approaches such as ultrasonic crystals [2], magnetic resonance imaging (MRI) [3], [4], and echocardiography [5] have been used to measure instantaneous LV volume with varying degrees of success. These technologies all have severe limitations, particularly during dynamic maneuvers, such as transient occlusion of the inferior *vena cava* or aorta, that are required to generate load-independent indices of contractility.

A more reliable alternative to generate instantaneous LV P-V relations in the intact murine heart is to use conductance technology, as proposed by Baan *et al*. in 1984 [6]. Single frequency conductance has been used in mice to generate measures of ventricular function [5]–[8]. However, the traditional conductance method fails to correct for the parallel admittance of the myocardium and this results in an overestimation the LV blood volume [6]. The hypertonic saline injection technique developed for larger animals [9] cannot be used in such small animals since administration of even small volumes of hypertonic saline significantly alters both blood resistivity and hemodynamics (i.e., blood volume), violating the framework of the governing assumptions [6]. Simultaneous measurement at two frequencies combined with the hypertonic saline technique has been proposed by other investigators [10]-[12]. However, in all cases the methods used determine only a single value of steady state parallel conductance. Thus, conductance measurement in its present reduction to practice, in both small and large subjects, cannot calculate the instantaneous change in parallel conductance occurring throughout the cardiac cycle and as the LV cavity shrinks around the intra-cardiac electric field during occlusion of the inferior *vena cava*.

Pearce *et al.* [13] have shown that the effective electric permittivity of muscle *in vivo* is high enough that the admittance in the LV at a frequency of 30 kHz can be used to identify and separate the cardiac muscle component from the combined admittance measurement. Their fundamental observation is that blood is purely semiconducting at a frequency of 30 kHz, so all frequency-dependent admittance is entirely due to the muscle component only. That is, for a tetrapolar catheter in the LV, the admittance consists of two parallel components from blood and muscle:

$$Y_{meas} = Y_b + Y_m = G_b + G_m + j\omega C_m \quad (1)$$

where: Y is admittance (S), G is conductance (S), ω is the angular frequency (rad/s), C is capacitance (F), "meas" refers to the measured signal, "b" to the blood alone, and "m" to the muscle.

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For any electric field spatial distribution, **E**, in a homogeneous medium:

$$G = \frac{I}{V} = \sigma \frac{\mathbf{E} \bullet dA}{-\mathbf{E} \bullet dl} = \sigma F \quad \text{and} \quad C = \frac{Q}{V} = \varepsilon \frac{\mathbf{E} \bullet dA}{-\mathbf{E} \bullet dl} = \varepsilon F \quad (2)$$

where: I = current (A), V = potential (V), σ = electrical conductivity (S/m), Q = charge (C), ε = electric permittivity (F/m), and F is the electric field geometry factor common to both relations (m). The symmetry in the relationships of equations 2 leads to the familiar "conductance-capacitance" analogy. The symmetry is also the feature that allows identification and elimination of the cardiac muscle from the combined admittance signal: if C_m is measured, then $G_m = C_m \sigma_m/\varepsilon_m$. Pearce *et al.* [13] have determined the values for both σ_m and ε_m, enabling us to apply this new method for eliminating the parallel admittance of cardiac muscle.

II. Methods

A. In vivo LV catheter

The magnitude and phase of the electrical admittance as well as the LV pressure was measured using a miniaturized 1.4 Fr tetrapolar catheter manufactured by Millar Instruments, Houston, TX (part number SPR839) [Fig. 1]. The catheter contains four platinum ring electrodes aligned with an intra-electrode spacing of 0.5, 4.5, and 0.5 mm between electrodes 1 and 2, 2 and 3, and 3 and 4, respectively.

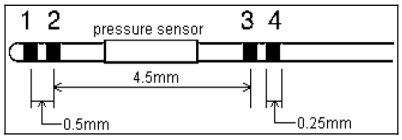

Fig. 1. In vivo LV conductance catheter used in the murine studies

In the tetrapolar technique electrodes 1 and 4 are driven with a current source and electrodes 2 and 3 are used for potential measurement at negligible current. This method is thus essentially insensitive to the series electrode-electrolyte interface impedance of the measurement electrodes. The 1.4 Fr pressure sensor is located between electrodes 2 and 3.

B. Instrumentation

We designed and built an instrument to measure the magnitude and phase angle of the measured admittance at a frequency of 30 kHz. The admittance magnitude measurement instrument is based on the system developed by Feldman *et al.* [14] redesigned to work at a single frequency and is illustrated in Fig. 2. The desired excitation frequency of 30 kHz was generated using a 4 MHz crystal and a digital counter chip (CD 4040, Texas Instruments Inc., Dallas TX) to divide the crystal frequency. The counter output was converted into a sinusoidal current signal that was applied to the two outer electrodes, #1 and #4 in Fig. 1. The instantaneous voltage signal between the inner electrodes, #2 and #3 in Fig. 1, was amplified with an instrumentation amplifier (AD 624, Analog Devices, Norwood MA), rectified and inverted with a divider chip (AD 734, Analog Devices) and finally scaled to $\pm$ 10V to represent the conductance signal over the range of expected values. The phase angle between the excitation current signal (across the two outer electrodes) and the measured voltage signal (across the two inner electrodes) was determined by converting the two sinusoidal signals into square waves using comparators (LM 339, National Semiconductor Corp., Santa Clara CA). The square waves were applied to the input of a NAND gate (CD 4011, Texas Instruments Inc., Dallas TX). The output of the NAND gate is a square wave whose duty cycle is proportional to the phase difference between the two input signals. This pulse width modulated output is converted to a DC voltage level using a true RMS to DC converter chip (AD 536, Analog Devices, Norwood MA). The phase circuit has a resolution of 100 mV/degree. The admittance magnitude output as well as the phase output were sampled and analyzed using Charts Acquisition Software (AD Instruments Pty Ltd., Castle Hill, NSW, Australia).

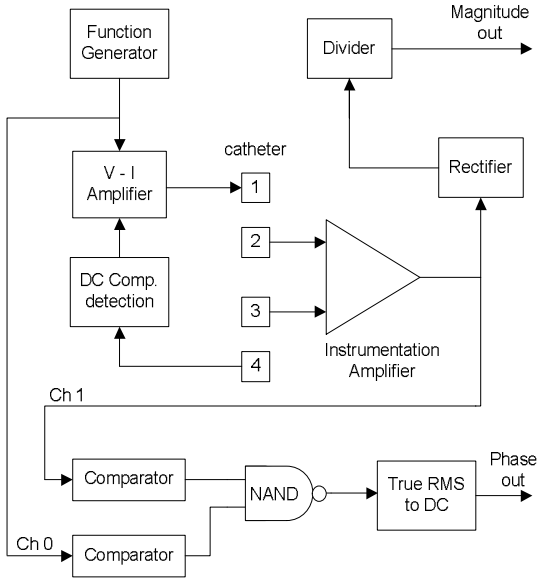

Fig. 2. Instrument used to measure complex admittance

The LV pressure was monitored using the pressure sensor on the catheter, connected to a pressure control unit (Model : TC-510, Millar Instruments, Houston TX).

C. Calibration

Calibration of the conductance measurement device was accomplished with 1% metal film resistors between 267 Ω (3,750 μS) and 5.33 kΩ (188 μS). The calibration resistors were tested on an Agilent Inc. Model 4194A Impedance / Gain-Phase Analyzer to ensure that no inductive or capacitive behavior was observable in them over the frequency range of

interest, 1 to 100 kHz. The miniaturized tetrapolar catheter cable has substantial inter-wire capacitance: there are six inter-electrode parallel capacitances among the four lead wires. The net effect of these capacitances was compensated by calibration of the catheter by immersing in a relatively large volume of saline of known electrical conductivity. The conductivity of the saline solutions was measured with a Hanna Model HI 8033 conductivity meter (Hanna Instruments, Woonsocket RI). A calibration curve was generated at the measurement frequency of 30 kHz to cover the range of expected effective conductivities for blood (8000 to 11000 μS/cm, or 0.8 to 1.1 S/m) at 37 C for both magnitude-only and magnitude-phase measurements.

D. Murine Studies

The Institutional Animal Care and Use Committee at the University of Texas Health Science at San Antonio approved all experiments. A total of 7 mice (+/+ MnSOD) were studied by complex admittance measurements. Mice were anesthetized by administration of urethane (1000 mg/kg ip) and etomidate (25 mg/kg ip), and mechanically ventilated with a rodent ventilator set at 150 breaths/min (100% O_2). Mice were placed on a heated, temperature-controlled operating table for small animals (Vestavia Scientific, Illinois). Experiments were performed at a murine body temperature of 37 C. The chest was entered via an anterior thoracotomy. The miniaturized conductance catheter was inserted into the LV through the apex of the heart and placed such that electrode #1 was positioned immediately above the aortic valve and electrode #4 was at the apex. The magnitude and phase angle of the admittance as well as the LV pressure were measured in the intact beating mouse heart. After the measurements were completed, the real time stroke volume was measured by placing an electromagnetic flow probe around the aorta. This stroke volume was used in the calculation of true LV volume from the measured admittance. The mouse blood was extracted from the aorta at the end of the experiment and the blood resistivity was measured.

III. RESULTS

A. Pressure - |Y| and Pressure – Phase loops

The real time pressure-|Y| and pressure-phase loops from two mice are shown in figure 3. The values of admittance and phase have been corrected for catheter and instrumentation effects. It is seen that the width of the pressure-phase loops is highly dependent on the positioning of the catheter within the LV since these loops are a result of the ventricular muscle moving towards and away from the catheter during each heart beat.

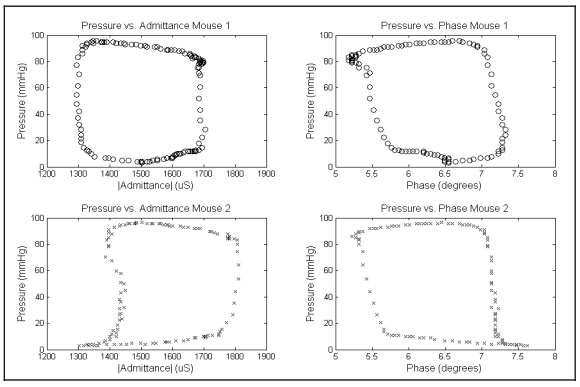

Fig. 3. Real Time *in vivo* Pressure - |Y| and Pressure – Phase loops obtained from two mice

B. Estimation of true volume from complex admittance measurements

The calculated admittance values are converted to volume using the non-linear conductance to volume equations developed by Wei *et al.* [15].

$$Vol = \frac{1}{1-\frac{g_b}{\gamma}}\rho L^2 g_b \tag{3}$$

where $\alpha(g_b) = 1 - \frac{g_b}{\gamma}$

and
$$\gamma = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \tag{4}$$

where
$$a = SV_m - \rho L^2 \left(g_{b-ED} - g_{b-ES}\right)$$
$$b = -SV_m \cdot \left(g_{b-ED} + g_{b-ES}\right)$$
$$c = SV_m \cdot g_{b-ED} \cdot g_{b-ES}$$

where ρ is the blood resistivity (S/m), g_b is the instantaneous LV blood conductance (S) measured using the conductance catheter, L is the distance between the voltage sensing electrodes (m), $g_{b\text{-}ED}$ and $g_{b\text{-}ES}$ are the blood conductance (S) at end diastole and end systole respectively, and SV_m is the stroke volume measured using the electromagnetic flow probe. The values of g_b, $g_{b\text{-}ED}$ and $g_{b\text{-}ES}$ have been corrected for admittance contributions from the instrumentation and the parallel admittance from the ventricular muscle.

The corresponding pressure volume loops from two mice are shown in figure 4.

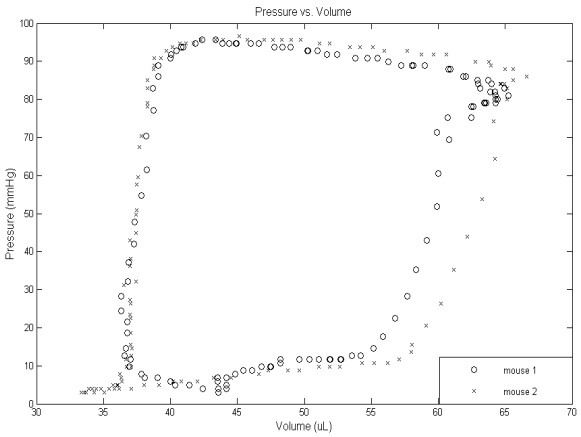

Fig. 4. Pressure – True Volume loops for the two mice

IV. Discussion

There are some features of the admittance system that make it hard to obtain an LV volume that is independent of catheter position inside the LV. This is because the phase angle measurement is very sensitive to the proximity of the muscle to the conductance catheter. This can be used as an advantage when trying to detect changes in the muscle properties, such as myocardial ischemia [16], [17]. The width of the pressure-phase loops is an indication of the blood volume in the LV. This width approaches zero if the electric field penetrated just the blood and not the ventricular muscle. This can be used as an indicator of an enlarged LV due to increased afterload from artery hardening or plaques.

The overall health of the cardiovascular system can be assessed using data from a set of murine or human PV loops. A miniaturization of the technology used to capture admittance signals from the heart can be used to determine a metric for patient's health after heart attack, and it could be incorporated onto existing pacemaker technology. The method for calibration outlined for mice is easily transferred to humans through the use of a larger sized catheter. Only the new stroke volume, resistivity, and length between the voltage contacts need to be changed in order to use any sized catheter on any animal.

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