Evidence of Time-Varying Myocardial Contribution by *In Vivo* Magnitude and Phase Measurement in Mice

Chia-Ling Wei[1], Jonathan W. Valvano[1], Marc D. Feldman[2], Anil Kottam[1], David Altman[1], Karthik Raghavan[1], Daniel J. Fernandez[1], Maricela Reyes[2], Daniel Escobedo[2], John A. Pearce[1]
[1]Department of Electrical and Computer Engineering, The University of Texas at Austin, TX, USA
[2]Department of Medicine, The University of Texas Health Science Center at San Antonio, TX, USA

Abstract—**Cardiac volume can be estimated by a conductance catheter system. Both blood and myocardium are conductive, but only the blood conductance is desired. Therefore, the parallel myocardium contribution should be removed from the total measured conductance. Several methods have been developed to estimate the contribution from myocardium, and they only determine a single steady state value for the parallel contribution. Besides, myocardium was treated as purely resistive or mainly capacitive when estimating the myocardial contribution. We question these assumptions and propose that the myocardium is both resistive and capacitive, and its contribution changes during a single cardiac cycle. *In vivo* magnitude and phase experiments were performed in mice to confirm this hypothesis.**

Keywords—**Capacitive myocardium, catheter, constant parallel admittance, phase measurement**.

I. Introduction

Pressure-volume analysis is the gold standard for assessing myocardial function. The left ventricular (LV) pressure-volume relationship generated on a beat-by-beat basis during transient occlusion of the inferior vena cava allows precise hemodynamic characterization of LV systolic and diastolic function independent of loading conditions [1]-[3]. There is some interest in applying LV pressure-volume relationships to characterize the cardiac performance of gene-altered mice. The measurement of instantaneous volume has been problematic due to the small size of the mouse heart and its rapid rate (up to 600 bpm). Conductance technology has been miniaturized to generate an instantaneous conductance signal, which is proportional to volume, to solve this problem [1].

A LV volume signal can be estimated with a conductance catheter system. Experimentally a four-electrode catheter (see Fig. 1) is inserted into the mouse LV to generate an electric field and continuously measure the instantaneous conductance as the LV fills and ejects blood. Unfortunately, the measured conductance is a combination of blood and left ventricular myocardium, but only the blood conductance is desired. Hence, the parallel myocardial contribution needs to be removed from the total measured conductance. Previous studies only determine a single steady state value for the parallel myocardial contribution [3]-[7]. Numerous investigators have confirmed this finding and it has, in part, been the basis for the acceptance of the technique to generate a reliable instantaneous LV volume signal during steady state conditions. However, electromagnetically the electric field decreases dramatically with increasing distance from sources [8]-[10]. When a heart beats, the distance between the catheter and myocardial wall changes, which should make the myocardial contribution to the total measured conductance change as well. Hence, we question the assumption of constant myocardial contribution and propose that the parallel myocardial contribution changes during a single cardiac cycle.

Furthermore, myocardium has been proved to have both resistive and capacitive properties, but all the myocardial contribution estimation method are based on the assumption that myocardium is either purely resistive or mainly capacitive [3]-[7], [11]-[14]. Actually the myocardial resistive property has been found to be substantial [12]. Hence, the controversy is whether or not the capacitance is negligible compared to the conductance. We propose that both of them are significant, which makes it more appropriate to label the measured result admittance, instead of conductance.

If this hypothesis is sustained, the phase of total measured admittance should increase measurably with frequency, since the imaginary part of myocardium admittance would increase with frequency. Further, if the myocardial contribution becomes less significant with increasing LV volumes as we hypothesized above, the phase measured at larger LV volumes should be smaller. Therefore, if we can demonstrate experimentally that the phase does increase with increasing frequency and decreasing volume, the hypotheses of time-varying myocardial admittance and non-negligible capacitance are confirmed.

In this study, measurements of phase and magnitude of the admittance between the two inner sensing electrodes (see Fig. 1) were performed to examine the proposed hypotheses.

II. Methodology

A 1.4-Fr pressure-volume catheter (SPR-839, made by Millar Instruments, Houston, Texas) was used in these studies. The catheter has four 0.25-mm-length platinum electrodes with interelectrode spacing of 0.5, 4.5, and 0.5 mm respectively. A combined 10 and 100 kHz sinusoidal signal generated from a signal function generator board was

fed into an instrument designed by us and then was converted into a current signal. A constant 30 μA peak-to-peak excitation ac current, composed of 10 and 100 kHz, was applied to the two outermost electrodes to generate an intraventricular electric field. The voltage differences between the two inner electrodes were measured continuously. The magnitude of the voltage between the two inner electrodes is inversely proportional to the admittance between the inner electrode pair. This dual-frequency signal was separated by two band-pass filters (BPF) and then amplified. These filtered signals together with the input signal were sampled by a digital oscilloscope (TDS 350, Tektronix). The scope communicates with PC through a GPIB interface, as shown in Fig. 1. LabVIEW™ 6.1 (National Instruments, Austin, TX) was used to record data and MATLAB® (Mathworks, Inc., Natick, MA) was used to analyze data. This phase measurement is not accomplished in real-time. The oscilloscope is capable of recording 1000 samples for two channels at up to 20 MHz. The data recording process is triggered manually. The recorded data from both input and output signals are converted into the frequency domain by Fast Fourier Transform (FFT). The measured phase is the phase difference between the input and output signals at either 10 or 100 kHz. The magnitude of the measured admittance is inversely proportional to the amplitude of the output signal.

Calibration is required for both magnitude and phase. The magnitude calibration was performed to convert the voltage output into conductance. Known resistors were used to calibrate the instrumentation. The phase calibration is to eliminate the phase shift due to the electronic system, the stray capacitance of the catheter, and the electrode-electrolyte interfaces [15]. The phase calibration was performed by inserting the catheter into saline of known conductivity close to the conductivity of murine blood. The phase shift from our instrumentation was subtracted from the phase measured in *in vivo* mouse experiments. The calibration offsets are 14.7° at 10 kHz and 96.5° at 100 kHz.

The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and conformed with "Guidelines for the Care and Use of Laboratory Animals" (NIH publication No. 86-23, revised in 1985) and "Principles of Laboratory Animal Care" (published by the National Society for Medical Research). Six wild type C57 black mice were used for these experiments. Mice were anesthetized by urethane (1000 mg/kg IP) and etomidate (25 mg/kg IP). Respiration was controlled through a tracheotomy cannula, and the mice were mechanically ventilated with a rodent ventilator at 100 breaths/min supplemented with 100% oxygen. The chest was entered by anterior thoracotomy. An apical stab was made in the heart with a 30-gauge needle, and the miniaturized mouse conductance catheter was advanced retrograde into the LV along the long axis with the proximal electrodes just within the myocardial wall of the apex.

III. Results

Fig. 2 shows the waveforms of pressure, measured magnitude at 10 and 100 kHz, and the magnitude difference between the two frequencies. At end-diastole, the measured magnitudes at both frequencies are the largest in a cardiac cycle, while the magnitude difference is the smallest. On the other hand, at end-systole, the magnitudes are the smallest within a cycle, while the difference reaches its maximum.

Measurement data from three mice were chosen for demonstration purposes. Fig. 3 shows the relationship between the phase and the magnitude of the measured admittance. When the magnitude of the measured

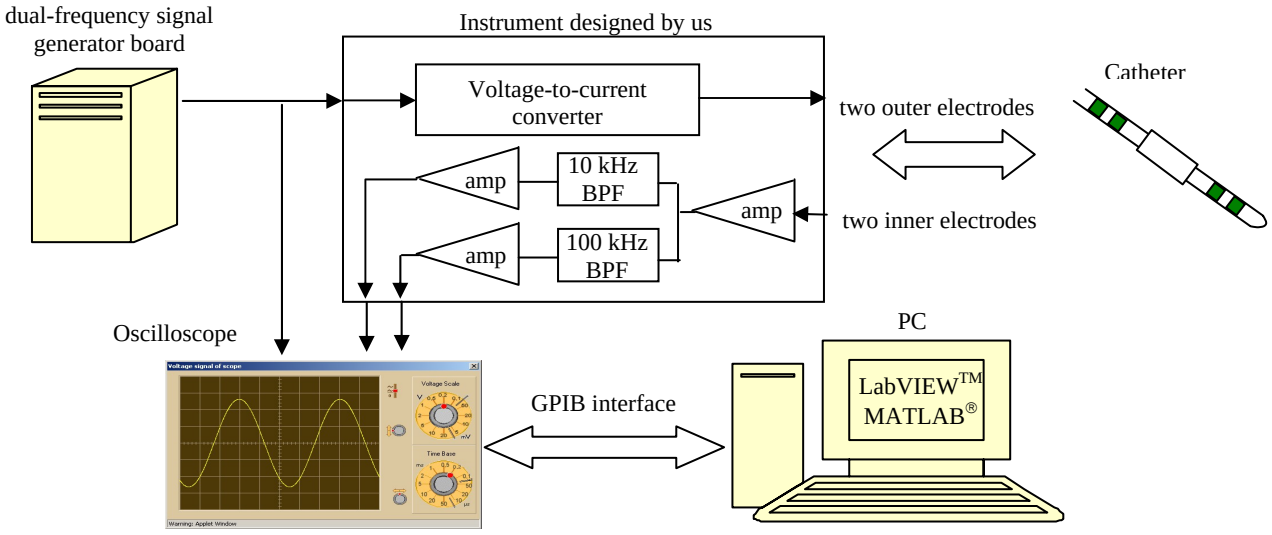

Fig. 1. The block diagram of dual-frequency phase measurement system.

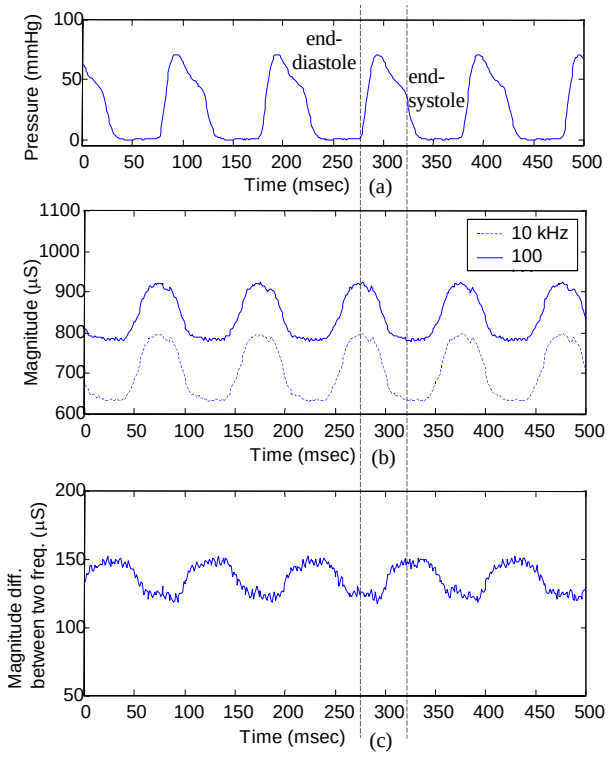

Fig. 2. Waveforms in time domain (a) pressure (b) measured magnitude at 10 and 100 kHz (c) magnitude differences between 10 and 100 kHz.

admittance increases, its phase tends to be smaller. Exponential regression lines are added to emphasize this trend. The correlation coefficients, R^2, are larger at 10 kHz than at 100 KHz.

IV. Discussion

End-diastole refers to the time in a cardiac cycle when the pressure reaches its minimum and the LV volume reaches its maximum. From Fig. 2, it is clear that the measured magnitude of admittance is proportional to the LV blood volume. Therefore, if the phase is plotted versus the magnitude of the measured admittance, as shown in Fig. 3, the timing during the cardiac cycle of the measured phase is evident. The measurements show that the phase measured at 100 kHz is larger than the phase at 10 kHz, and the phase measured at larger volumes (larger magnitudes) is smaller. The fact that phase changes with frequency confirms that the myocardial capacitance is non-negligible compared to the summation of myocardial conductance and blood conductance; otherwise, the phase should be around 0^o at both frequencies. Hence, a mouse LV can be modeled as blood resistance in parallel with myocardial resistance and myocardial capacitance as shown in Fig. 4. Furthermore, since only myocardium has capacitance, not blood, the phase changing within the cardiac cycle comes from the myocardium, which proves that myocardial contribution is not a constant during the cardiac cycle.

As expected, the capacitive effect of the myocardium is more prominent at higher frequency, since the imaginary part of myocardial admittance is proportional to frequency. As a result, the phase measured at higher frequency is larger. On the other hand, as the LV blood volume gets larger, the distance between myocardium and catheter increases and then the effect of the electric field on the myocardium is reduced. This makes the contribution from the myocardium less significant. Therefore, the phase measured at larger LV volumes during the cardiac cycle should be smaller. Also, the same amount of timing error caused by noise makes the phase error at 100 kHz ten times larger than that at 10 kHz. Therefore, the noise sensitivity at 100 kHz is ten times

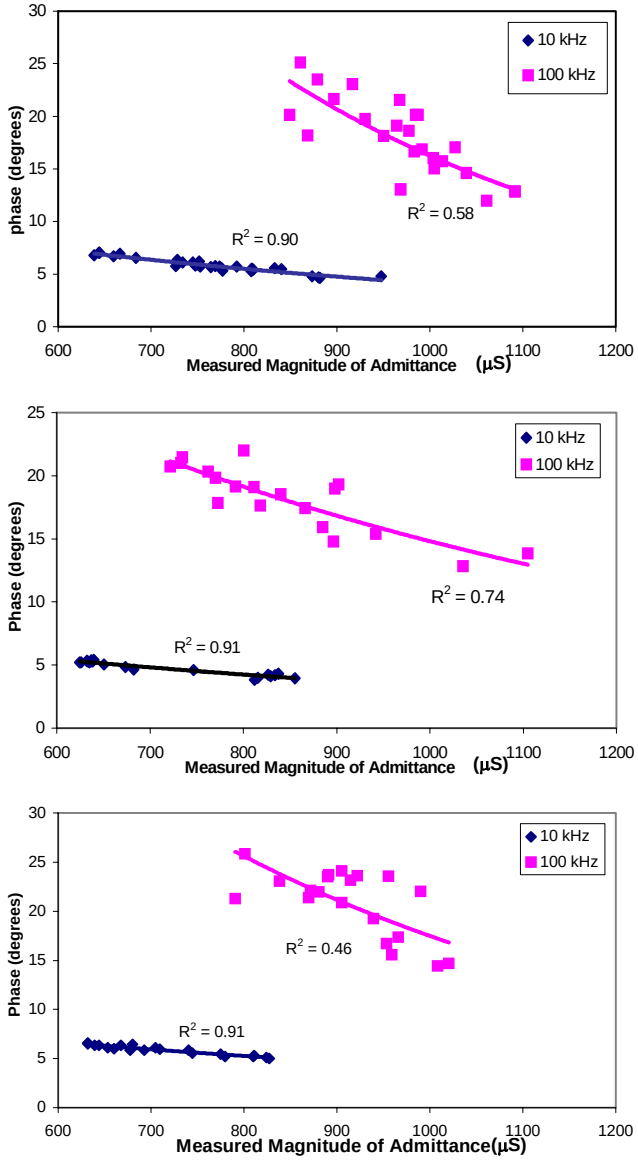

Fig. 3. The magnitude-phase relationships of the LV admittance measured at both 10 and 100 kHz measured in three different mice.

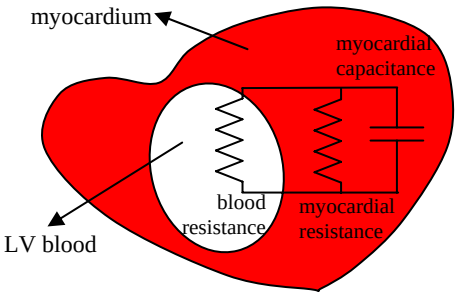

Fig. 4. The equivalent circuit of a mouse LV

larger, which may explain the correlation coefficient at 100 kHz being lower than that at 10 kHz.

From Fig 2, the magnitude differences between two frequencies vary between end-systole and end-diastole. The differences come only from the imaginary part of myocardial admittance, since the real part of admittance is independent of frequency, which further confirms that the myocardial admittance is not a constant during the cardiac cycle. The magnitude differences are larger at end-systole than those at end-diastole. At end-systole, the myocardial wall is closer to the catheter, so the myocardial contribution to the total measured admittance should be more significant, which means that myocardial capacitance, or imaginary part of myocardial admittance, at end-systole is larger. Therefore, the magnitude differences are larger at end-systole than at end-diastole.

V. Conclusion

In vivo phase and magnitude measurements were performed in mice. The hypothesis of non-negligible myocardial capacitance is confirmed by that fact that the phase is increasing with frequency. The hypothesis of time-varying myocardial contribution is supported by the fact that the phase is changing with the magnitude, or the LV volume. Finally, the experimental data also shows that the phase measurement is more sensitive to noise at higher frequency.

References

[1] M. D. Feldman, Y. Mao, J. W. Valvano, J. A. Pearce, and G. L. Freeman, "Development of a multifrequency conductance catheter-based system to determine LV function in mice," *Am. J. Physiol. Heart Circ. Physiol.* 279: H1411-H1420, 2000.

[2] D. Kass, M. Midei, W. Graves, J. Brinker, and W. L. Maughan, "Use of a conductance (volume) catheter and transient inferior vena caval occlusion for rapid determination of pressure-volume relationships in man," *Cath. Card. Diag*., vol. 15, pp. 192-202, 1988.

[3] J. Baan, E. T. van der Velde, H. G. de Bruin, G. J. Smeenk, J. Koops, A. D. van Dijk, *et al.* "Continuous measurement of left ventricular volume in animals and humans by conductance catheter," *Circ*., vol 70, pp. 812-823, 1984.

[4] E. B. Lankford, D. A. Kass, W. L. Maughan, and A. A. Shoukas, "Does volume catheter parallel conductance vary during a cardiac cycle?," *Am. J. Physiol. Heart Circ. Physiol.* 258: H1933-H1942, 1990.

[5] D. Georgakopoulos and D. A. Kass, "Estimation of parallel conductance by dual-frequency conductance catheter in mice," *Am. J. Physiol. Heart Circ. Physiol.* 279: H443-H450, 2000.

[6] T. S. Gawne, K. S. Gray, R. E. Goldstein, "Estimating left ventricular offset volume using dual frequency conductance catheters," *J. Appl. Physiol.* 63(2):872-876, 1987.

[7] D. Georgakopoulos, W. A. Mitzner, C. H. Chen, B. J. Byrne, H. D. Millar, J. M. Hare, and D. A. Kass, "In vivo murine left ventricular pressure-volume relations by miniaturized conductance micromanometry," *Am. J. Physiol. Heart Circ. Physiol.* 274: H1416-H1422, 1998.

[8] C. L. Wei, J. W. Valvano, M. D. Feldman, M. Nahrendorf, and J. A. Pearce, "3D Finite Element Complex Domain Numerical Models of Electric Fields in Blood and Myocardium," *Proc. of IEEE-Engr. In Med. And Biol. Soc., 25th Annual International Conference*, pp.62-65, Sep. 2003.

[9] C. C. Wu, T. C. Skalak, T. R. Schwenk, C. M. Mahler, A. Anne, P. W. Finnerty, H. L. Haber, R. M. Weikle, and M. D. Feldman, "Accuracy of the conductance catheter for measurement of Ventricular Volumes Seen Clinically: Effects of Electric Field Homogeneity and Parallel Conductance," *IEEE Trans. Biomed. Eng.*, vol. 44, no. 4, pp. 266-277, May 1997.

[10] D. K. Cheng, *Field and Wave Electromagnetics*, Addison-Wesley Publishing Company, Reading, MA, 1989, pp. 208-219.

[11] H.P. Schwan, "Electrical properties of blood and its constituents: alternating current spectroscopy," *Blut* 46:185-197, 1983.

[12] P. A. White, C. I.O. Brookes, H. B. Ravn, E. E. Stenbog, T. D. Christensen, R. R. Chaturvedi, *et al.* "The effect of changing excitation frequency on parallel conductance in different sized hearts," *Cardiovascular Research* 38:668-675, 1998.

[13] C. Gabriel, S. Gabriel, and E. Corthout, "The dielectric properties of biological tissues: I. Literature survey," *Phys. Med. Biol.*, vol. 41, pp. 2231-2250, 1996.

[14] S. Gabriel, R. W. Lau, and C. Gabriel, "The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz," *Phys. Med. Biol.*, vol. 41, pp. 2251-2269, 1996.

[15] J. G. Webster, *Medical Instrumentation: Application and Design*, John Wiley & Sons, New York, NY, 1999. pp. 183-230.

[16] D. Georgakopoulos, W. A. Mitzner, C. H. Chen, B. J. Byrne, H. D. Millar, J. M. Hare, and D. A. Kass, "In vivo murine left ventricular pressure-volume relations by miniaturized conductance micromanometry," *Am. J. Physiol. Heart Circ. Physiol.* 274: H1416-H1422, 1998.

[17] M. D. Feldman, J. M. Erikson, Y. Mao, C. E. Korcarz, R. M. Lang, and G. L. Freeman, "Validation of a mouse conductance system to determine LV volume: comparison to echocardiography and crystals," *Am. J. Physiol. Heart Circ. Physiol.* 279: H1698-H1707, 2000.

[18] B. Gopakumaran, J. H. Petre, B. Sturm, R. D. White, and P. A. Murray, "Estimation of Current Leakage in Left and Right Ventricular Conductance Volumetry Using a Dynamic Finite Element Model," *IEEE Trans. Biomed. Eng.*, vol. 47, no. 11, pp. 1476-1486, Nov 2000.

[19] P. Steendijk, E. T. van der Velde, and J. Baan, "Single and dual excitation of the conductance-volume catheter analyzed in a spherical mathematical model of the canine left ventricle," *Eur. Heart J.*, vol. 13, supp. E, pp. 28-34, 1992.

[20] G. Mur and J. Baan, " Computation of the input impedances of a catheter for cardiac volumetry," *IEEE Trans. Biomed. Eng*., vol. BME-31, pp. 448-453, 1984.