

Experimental Method for Recording Epicardium Potentials and Cardiac Myocyte Shortening

Gustavo S Marchini¹, Daniel S U Tamashiro², Helena T Oyama³, Lucas Cortella³, Ismar N Cestari³, Idágene A Cestari^{1,3}

¹ Polytechnic School of University of São Paulo, São Paulo, Brazil

² University of Campinas, São Paulo, Brazil

³ Bioengineering Division - Heart Institute (InCor), São Paulo, Brazil

Abstract

The hemodynamic changes observed in advanced stages of heart diseases are often accompanied by changes in the electrical and mechanical properties of cardiac myocytes. The objective of this work is to develop an experimental method for recording ventricle epicardium potentials in isolated rat hearts and isolated cardiac myocyte shortening. Briefly, rat heart was removed, aorta was cannulated and coronary arteries were retrogradely perfused with heated and oxygenated buffer solutions. Ag-AgCl electrodes fixed in a silicone pouch placed around the heart were used to measure epicardium potentials. The perfusion was switched to an enzyme-containing solution for digestion of the heart and obtaining isolated cardiac myocytes. Measurements of shortening were made in cells electrically stimulated. The results suggest the possibility of relating the electrical behavior of the whole heart with mechanical properties of cardiac myocytes and may represent an useful tool in basic cardiac research.

1. Introduction

Cardiac excitation contraction coupling and mechano-electric feedback are two processes that relate electrical and mechanical properties of the heart. In the former, electrical depolarization leads to mechanical contraction and, in the last, mechanical alterations can lead to changes in cardiac electrical activity [1].

Changes in both electrical and mechanical properties can occur in advanced stages of heart diseases. To understand and evaluate the effects at the whole heart and cellular scales two techniques may be used, respectively: epicardial potential mapping and isolated cardiac myocyte shortening. The first, allows the analysis of the spreading of action potentials on the epicardium surface and the velocities along and transverse to the fiber axis [2]. The second, gives assessment of cardiac contractility by quantify-

ing contraction and relaxation kinetics (cross-bridge dynamics) allowing the evaluation of mechanical function [3].

This work describes an experimental method for evaluation of electrical and mechanical properties in a same experiment with the recording of ventricle epicardium potentials in isolated rat hearts and measuring of isolated cardiac myocyte shortening.

2. Methods

2.1. Fabrication of electrodes

Electrodes were fabricated manually using silver wire and a portable torch. From the diameter of the electrode it was possible to determine the length of the wire to be melted. Thus wire was slowly melted in the flame of the torch and a small electrode was formed. The silver electrodes were chlorided to form Ag-AgCl that exhibit lower electrode-electrolyte impedance [4]. Six electrodes (0.5 mm² surface area; 0.8 mm interelectrode spacing) were fixed in a silicone pouch to maintain contact with the heart epicardial surface.

2.2. Animal preparation and heart cannulation

Experiments were performed in six-week-old male Wistar rats and were carried out in accordance with the Committee of Ethics in the Use of Animals of School of Medicine, University of São Paulo. Rat was heparinized (5000 U/kg) and, after 30 minutes, anesthetized with sodium thiopental and euthanized by cervical dislocation. Hearts were then excised and placed in ice-cold, oxygenated buffer solution containing (in mmol/l): 134 NaCl, 4.0 KCl, 1.2 NaH₂PO₄, 10 HEPES, 0.5 MgSO₄, 1.25 CaCl₂ and 11 D-glucose (pH 7.4) [5].

2.3. Measuring of epicardium potentials

The heart was mounted on a Langendorff perfusion system with constant flow (Fig. 1) and perfused with the same buffer solution at 37 °C (6 ml/min). The silicone pouch with the electrodes enveloped the heart and a single reference electrode was placed at the aorta (Fig. 2). Unipolar epicardial potentials were analog filtered (bandpass 0.1 to 300 Hz) and amplified (Gould Electronics, Chandler, AZ, USA), digitalized with a sampling frequency of 8 kHz (DI-720, DataQ Instruments, Akron, OH, USA) and stored in a computer for post-processing. A MATLAB (MathWorks, Inc., Nattick, MA, USA) algorithm was used to determine time delays between epicardium potentials based on the minimum values of the time derivative of shortening.

2.4. Cardiac myocyte isolation

The perfusion solution was switched to the same buffer without Ca^{2+} for 4 minutes followed by digestion step performed with perfusion buffer containing collagenase type II (100 U/ml; Worthington Biochemical, Lakewood, NJ, USA) and 20 μM Ca^{2+} until the heart became pale and flaccid (20-30 min)(Fig. 3A). The ventricles were separated from the heart, cut into small pieces and gently triturated with a pipette in the buffer solution with 50 μM Ca^{2+} and 1% BSA (Sigma, St Louis, MO, USA). Following filtration through 200 μm nylon mesh and sedimentation, the cell pellet was washed four times in buffer solution plus 1% BSA with gradually increasing of $[\text{Ca}^{2+}]$ (0.1, 0.2, 0.5 and 1 mM). Fig. 3B shows an example of isolated cardiac myocytes obtained by enzymatic dissociation.

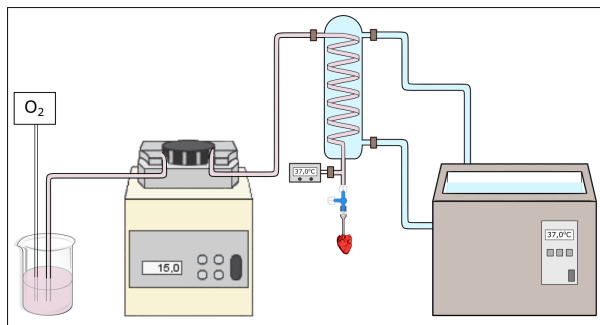


Figure 1. Schematic depiction of the Langendorff perfusion system with constant flow. A peristaltic pump drives the oxygenated solution through a heat exchanger coil connected to a water bath. Heart is cannulated by the aorta allowing retrograde perfusion through coronary arteries.

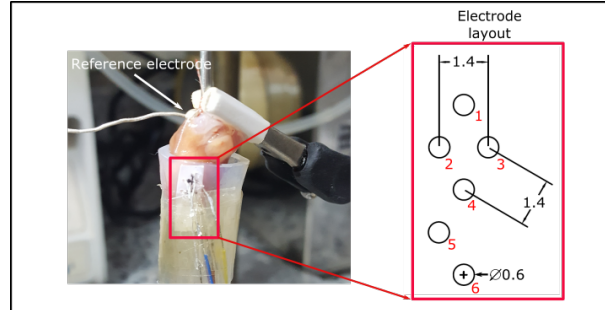


Figure 2. On left, image of the silicone pouch with the electrodes; on right, the electrode layout with the dimensions given in mm. The red numbers are the identification of electrodes.

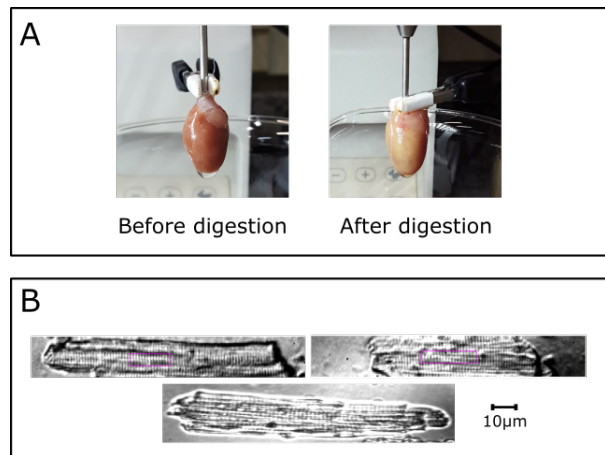


Figure 3. A: on left, cannulated heart just before the start of perfusion with collagenase; on right, the same heart after enzymatic digestion. B: isolated cardiac myocytes obtained by enzymatic digestion.

2.5. Measurement of cardiac myocyte shortening

Shortening of cardiac myocytes by electrical field stimulation (1 Hz) was recorded using a video edge detection system composed by an inverted microscope (Eclipse TS100; Nikon, Tokyo, Japan) with an analog camera (Myocam; IonOptix, Milton, MA, USA). Contraction signals were analyzed using commercially data analysis software (IonWizard; IonOptix, Milton, MA, USA) providing typical contraction parameters including resting cell length, percentage cell shortening (i.e., percentage of resting cell length), shortening and relaxation velocities and time intervals to reach the peak of contraction and 50% resting cell length. The parameters were calculated with the mean of following contractions of a cardiac myocyte. All experiments were conducted at room temperature (23 °C).

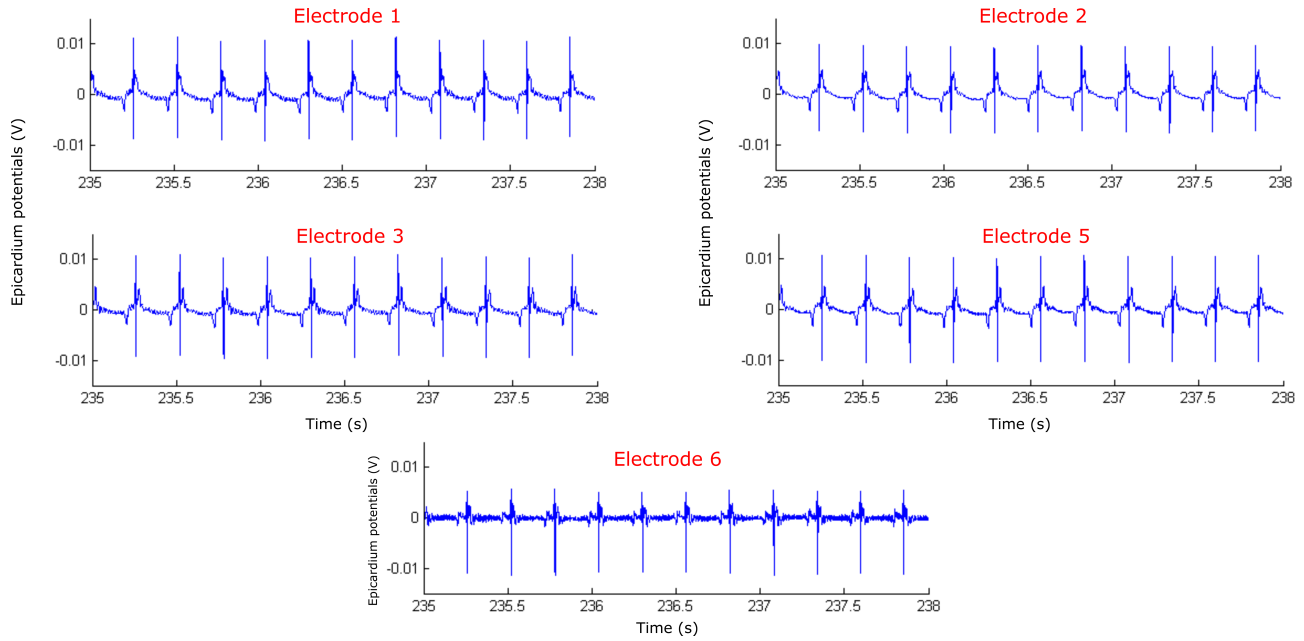


Figure 4. Signals of ventricle epicardium potentials in sinus rhythm recorded in five electrodes in one experiment.

Table 1. Delay values between the epicardium potentials of different electrodes for three experiments. The time intervals correspond to the delay between the most advanced signal and the signaling identified on the respective electrode (fig. 1B).

Experiment 1 (n=27)		Experiment 2 (n=15)		Experiment 3 (n=81)	
Electrode	Mean \pm SD (ms)	Electrode	Mean \pm SD (ms)	Electrode	Mean \pm SD (ms)
6	-	6	-	3	-
5	0.54 \pm 0.08	5	0.58 \pm 0.06	2	0.32 \pm 0.07
1	0.69 \pm 0.06	3	1.50 \pm 0.14	6	0.59 \pm 0.07
3	0.82 \pm 0.06	4	1.63 \pm 0.12	4	0.61 \pm 0.07
2	1.20 \pm 0.08	2	2.02 \pm 0.08	5	0.78 \pm 0.11

3. Results

3.1. Epicardium potentials

Fig. 4 shows an example of ventricle epicardium potentials in sinus rhythm for one experiment. Table 1 shows the time delays obtained in the three experiments. Mean and standard deviation were calculated on time delays obtained from the same epicardium potential on different electrodes. Some electrodes were omitted from the analysis because they did not show signal, probably due to poor contact between electrode and epicardium.

3.2. Cardiac myocyte shortening

Fig. 5 presents an example of a result of cardiac myocyte shortening. The top of the figure shows shortening of an electrically stimulated isolated myocyte and the bottom shows the average shortening. Table 2 summarizes

mean and standard deviation of contraction parameters calculated in isolated cardiac myocytes (n=36).

4. Discussion

The presented method shows the availability of recording ventricle epicardium potentials in Langendorff perfused rat hearts and isolated cardiac myocyte shortening. For epicardium potentials, time delays were used to show one possible analysis for the investigation of whole heart electrophysiology. The analysis can be extended with the increase in the electrode density [6] and interpolation of the signals for a better reconstruction of the electrical activity on the epicardial surface [7]. Different maps for analysis like isochronal, isopotential and phase maps, should be considered as well as the adoption of new structures that improve the contact between the electrodes and the surface of the heart [8].

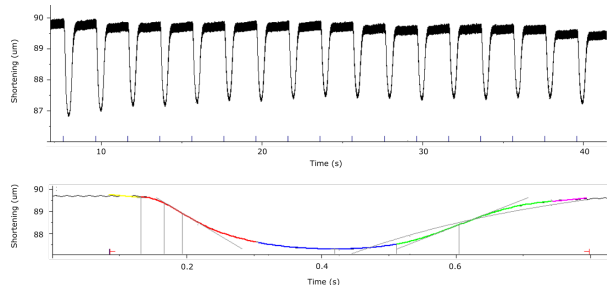


Figure 5. Shortening signal of an electrically stimulated isolated cardiac myocyte (top). Average shortening (bottom). The regions in yellow, red, blue, green and purple are used to calculate, respectively, cell length, shortening velocity, percentage of shortening (and time to peak), relaxation velocity and time to 50% relaxation.

Table 2. Contraction parameters measured in electrically stimulated isolated cardiac myocytes (n=36).

	Mean	SD
Cell length [μm]	87,850	19,846
Percentage of shortening	3,053	1,387
Shortening velocity [$\mu\text{m/s}$]	-29,722	21,424
Relaxation velocity [$\mu\text{m/s}$]	20,612	16,005
Time to peak [s]	0,207	0,043
Time to 50% relaxation [s]	0,346	0,088

For the isolated cardiac myocyte shortening, the measurements were conducted at room temperature. However, the temperature influences contraction parameters such as time to peak and time to 50% relaxation and, also, reduces the biological variability [9] and its control should be adopted in future experiments. Measurements of intracellular calcium can be considered to complement the analysis of contractility of isolated cardiac myocytes.

5. Conclusion

The results obtained in the experiments suggest the possibility of relating the electrical behavior of the whole heart with isolated cardiac myocytes mechanical properties and may represent an useful tool in basic cardiac research.

Acknowledgements

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Address for correspondence:

Gustavo Shimabukuro Marchini
 University of São Paulo
 Bioengineering Division - Heart Institute (InCor)
 Av. Dr. Enéas de Carvalho Aguiar, 44
 So Paulo, SP - 05403-900
 gustavo.marchini@usp.br