Contractility detection of isolated mouse papillary muscle using myotronic Myostation-Intact device

Hong Lian1,2 | Zhuyun Qin1,3 | Mengge Wu4 | Peipei Zuo4 | Lina Bai1 | Minjie Lu1 | Lulu Li1,3 | Haitao Zhang3

1State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2Beijing Key Laboratory of Preclinical Research and Evaluation for Cardiovascular Implant Materials, Animal Experimental Center, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
3Adult Surgical Intensive Care Unit, Fuwai Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
4National Health Commission Key Laboratory of Cardiovascular Regenerative Medicine, Fuwai Central-China Hospital, Central-China Subcenter of National Center for Cardiovascular Diseases, Zhengzhou, China

Abstract

Background: To understand the relationship between myocardial contractility and external stimuli, detecting ex vivo myocardial contractility is necessary.

Methods: We elaborated a method for contractility detection of isolated C57 mouse papillary muscle using Myostation-Intact system under different frequencies, voltages, and calcium concentrations.

Results: The results indicated that the basal contractility of the papillary muscle was $0.27 \pm 0.03$ mN at 10 V, 500-ms pulse duration, and 1 Hz. From 0.1 to 1.0 Hz, contractility decreased with an increase in frequency ($0.45 \pm 0.11$–$0.10 \pm 0.02$ mN). The voltage-initiated muscle contractility varied from 3 to 6 V, and the contractility gradually increased as the voltage increased from 6 to 10 V ($0.14 \pm 0.02$–$0.28 \pm 0.03$ mN). Moreover, the muscle contractility increased when the calcium concentration was increased from 1.5 to 3 mM ($0.45 \pm 0.17$–$1.11 \pm 0.05$ mN); however, the contractility stopped increasing even when the concentration was increased to 7.5 mM ($1.02 \pm 0.23$ mN).

Conclusions: Our method guaranteed the survivability of papillary muscle ex vivo and provided instructions for Myostation-Intact users for isolated muscle contractility investigations.

Keywords: calcium concentration, frequency, isolated papillary muscle, muscle contractility, Myostation-Intact, voltage
Heart failure (HF) indicates that the heart is damaged and its pumping function is impaired, and thus the heart is not capable of delivering sufficient blood and oxygen to peripheral organs and tissues. HF remains one of the leading causes of premature death and ill health. Various cardiovascular diseases, for example, cardiomyopathies, can lead to HF, which usually weakens

*FIGURE 1* Schematic graph of Myostation-Intact system
the cardiac and myocardial contractility. Thus, detecting isolated myocardial contractility is essential to understanding the physiology of muscle contractility properties, which can be an adjunctive method to diagnose cardiomyopathies in animal research. Presently, myocardial contractility detection has been widely used in scientific research. 2–5 Because of the anatomical structure of the heart, papillary muscle is usually used as a representative part of the heart for the measurement of myocardial contractility. 6–8

Ex vivo muscle contractility has been detected in many studies for a long time. An organ bath is a traditional ex vivo testing system used for contractility detections; however, the solution in the detection chamber must be replaced at certain periods to ensure sufficient oxygen in the solution. Another new similar system is the Rodent Oscillatory Tension Setup to study arterial compliance, however, whether this setup contains a heating system remains unknown. Myostation-Intact (Myotronic, Heidelberg, Germany, Figure 1) is a recent system used for the detection of ex vivo muscular tissue function. Previously, the practicality of this system has been proved in studies on skeletal muscles. 9–12 Here, we adopted this system to test the contractility of the cardiac muscle. Myostation-Intact possesses some superior properties: it is tiny and convenient to fix the tissue; solutions used in this method can be ventilated outside the device to maximally reduce signal disturbance and to circulate pure oxygen. Thus, Myostation-Intact is sensitive and precise for the contractility detection of isolated muscle and can produce reliable results in muscle contractility research. In this paper, we first elaborated our method using Myostation-Intact system for contractility detection of isolated papillary muscle. Using this method, we tested frequency-, voltage-, and calcium concentration-dependent contractility of isolated mouse papillary muscle.

### 2. MATERIALS AND METHODS

#### 2.1 Animal experiments and ethics

Three male mice (C57BL/6J, aged 8 weeks, body weight: 20–25 g) were tested in this experiment. The mice were purchased from Vital River Laboratory Animal Technology Co. Ltd.. Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC), Fuwai Hospital, Chinese Academy of Medical Sciences; all animal experiments followed the guidelines of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, United States (IACUC issue no.: FW-2021-0007).

#### 2.2 Preparation of solutions

Two solutions, preparation buffer (PB) and circulation solution (CS), were used for this study (Table 1). PB was used during the preparation period, whereas CS was used for the experiment. Ingredients of PB (Sigma-Aldrich Co.) included 120 mM sodium chloride (NaCl, S6191), 5.4 mM potassium chloride (KCl, P9541), 1.2 mM monosodium phosphate (NaH2PO4, S8282), 5.6 mM glucose (G7021), 5 mM taurine, 1.2 mM calcium chloride (CaCl2, C7902), and 30 mM 2,3-butanedione-monoxime (BDM, B0753, which protects dissected muscle from damage and maintains mechanical properties). Ingredients of CS included 120 mM NaCl, 5.4 mM KCl, 1.2 mM NaH2PO4, 5.6 mM glucose, 5 mM taurine, 1.5 mM calcium chloride (CaCl2), 1.5 mM MgCl2, and 1.5 mM CaCl2. All components were dissolved in 500 ml of ultra-pure, 18.2 MΩ H2O, and sterile filtered using a 0.22-μm filter unit (SLGU033RB, Merck Millipore Ltd.). The pH of the solution was adjusted to 7.35–7.45. All solutions were prepared on the same day.

| Ingredients | Molecular weight (g/mol) | Preparation buffer (mM) | Circulation solution (mM) | Weight (mg/500 ml) | Source | Category number |
|-------------|--------------------------|-------------------------|--------------------------|--------------------|--------|----------------|
| NaCl        | 58.44                    | 120.0                   | 120.0                    | 3506.4             | Sigma-Aldrich | S6191          |
| KCl         | 74.55                    | 5.4                     | 5.4                      | 201.285            | Sigma-Aldrich | S9541          |
| NaH2PO4     | 156.01                   | 1.2                     | 1.2                      | 93.6               | Sigma-Aldrich | S8282          |
| Glucose     | 180.16                   | 5.6                     | 5.6                      | 504.45             | Sigma-Aldrich | G7021          |
| HEPES       | 238.3                    | 10.0                    | 10.0                     | 1191.5             | Sigma-Aldrich | S4547          |
| MgCl2       | 203.3                    | 3.4                     | 3.4                      | 162.65             | Sigma-Aldrich | M8266          |
| Taurine     | 125.15                   | 5.0                     | 5.0                      | 312.875            | Sigma-Aldrich | T8691          |
| CaCl2       | 110.98                   | 1.5                     | 1.5                      | 83.25              | Sigma-Aldrich | C7902          |
| pH          | —                        | 7.35–7.45               | 7.35–7.45                | —                  | —      | —              |
| Ventilation | —                        | 100% O2                 | 95% O2+5% CO2            | —                  | —      | —              |
| Temperature | —                        | 4°C                     | 32°C                     | —                  | —      | —              |

Abbreviations: BDM, 2,3-butanedione-monoxime; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
of utilization to avoid the precipitate formation. If the solutions are prepared in advance, they must be stored at 4°C but only for less than a week. PB was cooled to 4°C or placed in ice and was oxygenated with 100% O\(_2\) for 20–30min before papillary muscle was isolated to ensure the PB solution had sufficient oxygen. CS was preheated to 32°C and ventilated with 95% oxygen (O\(_2\)) and 5% carbon dioxide (CO\(_2\)) throughout the detection process.

2.3 | Preparation of device

The temperature of the device was set to 32°C before application, and the transducer-micrometer system was flipped upside. Some bubbled CS was transferred into both the mountain aid and cuvette (4 ml) of the Myostation-Intact device using a burette or a pipette, and the CS was circulated via an external pump during the whole experiment.

2.4 | Killing of mice

Mice were injected with a bolus of tribromoethanol (concentration: 17.5 mg/ml, dosage: 0.1 ml/10 g) for narcosis. To test the sedative effect on mouse, one of its hind-leg toes was pinched; if there is no reflexed twitch, it means that the mouse is fully sedated. Next, the mouse was fixed on the surgery plate using syringe needles and disinfected with 75% ethanol, and then the skin and subcutaneous tissues were dissected. The ribs along the two sides of the sternum were cut off from the xiphoid, until the heart was completely exposed. The heart was dissected, and the auricles and excessive vascular tissue were removed; then the heart tissue was transferred into a paraffin-embedded petri dish containing cooled and bubbled PB for exsanguination.

2.5 | Isolation of papillary muscle

The following procedures were performed under a stereoscope. The right ventricle of the heart was pinned on the petri dish. The right ventricular wall and the middle of the interventricular septum were carefully dissected to expose the two papillary muscles attached to the left ventricular wall. The papillary muscles and additional left ventricular wall muscles were removed for subsequent analysis (the longer and thicker the isolated muscle is, the easier it is to maintain its vitality for the analysis). The papillary muscle was held using ophthalmic forceps and gently mounted onto the mounting aid containing bubbled

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**FIGURE 2**  Schematic flow chart of isolated papillary muscle contractility detection experiment. (A) Isolated intact clean heart. (B) Two exposed papillary muscles attached on left ventricular wall. (C) Isolated papillary muscle. (D) Mountain aid containing circulation solution with the isolated papillary muscle. (E) Isolated papillary muscle fixed on hooks of Myostation-Intact. (F) Magnification of fixation of the isolated papillary muscle.
CS. One loop below the stainless-steel hook of the device was penetrated using microsurgical forceps; either side of the papillary muscle was held on the mounting aid and stretched out. The string was pulled outward to fix the papillary muscle; the other side of the papillary muscle was fixed similarly (Figure 2). The fixed tissue was immersed in the cuvette filled with CS for detection. Of note, to ensure the vitality and to prevent damage to the papillary muscle, the tissue must not be stretched or touched. Open Myodat software, and orderly select “ClickandMove” and “On” to reveal “stimulation” window (Figure 3). The force was adjusted to zero using the “coarse” and/or the “fine” micrometer screw on the bridge amplifier.

2.6 | Equilibration and basal contractility detection

The fixed papillary muscle was given a single electric stimulation (10 V) to ensure its vitality; equilibration was conducted for 20–40 min, and then the detection study was initiated. The conditions for basal contractility detection were as follows: voltage, 10 V; frequency, 1 Hz; and pulse duration, 500 ms; and the amplitude value was regarded as the contractility of the isolated papillary muscle.

2.7 | Stimulations and contractility detection

We explored the relationship among papillary muscle contractility, frequencies, electrical intensity (voltages), and calcium concentrations. The detection of frequency-dependent contraction force was studied at 1 Hz. The frequency was started from 0.1 Hz and incrementally increased by 0.1 Hz until 1 Hz was reached. For voltage, electrical stimulation was increased by 1 V until the twitching amplitude stopped increasing, where the tissue reached the stimulation adaption. In addition, to detect contractility in different calcium concentrations of CS, we first ventilated the solutions; then the external pump was stopped, and the ventilated solution of different calcium concentrations (from 1.5 to 7.5 mM) was replaced using a burette.

2.8 | Data analysis

Myoviewer (Myotronic) was designed for data analysis; the data were exported in Excel format (Microsoft Excel, version 16.54). Graphpad (Prism 8, Graph Pad Software Inc.) was used for plotting. Data are described as mean ± standard error of the mean.

3 | RESULTS

3.1 | Equilibration and basal contractility

Equilibration helps the ex vivo papillary muscle adapt to the solution environment and conserve energy. The equilibration process lasted 20–40 min. Basal contractility was detected at 10 V, 1 Hz, and
500 ms pulse duration. The stimulation was stopped when the ampli-
tude \((\text{Force}_{\text{max}})\) remained at a relative constant value \((0.27 \pm 0.03 \text{ mN}; \text{Figure 4A})\).

### 3.2 | Frequency-dependent contractility

To investigate the relationship between contractility and frequencies below 1 Hz, we altered the frequency under similar conditions. The frequency was started at 0.1 Hz and terminated at 1 Hz. Therefore, contracting amplitude of the isolated papillary muscle showed an overall successively decreasing tendency when the frequency was increased \((0.1-1 \text{ Hz vs. } 0.45 \pm 0.11-0.10 \pm 0.02 \text{ mN}; \text{Figure 4B})\).

### 3.3 | Voltage-dependent contractility

To find how voltage influences contractility, we studied the voltage from the beginning of contractility of the isolated papillary muscle to 10 V. Electrical stimulation that initiated muscle contractility varied from 3 to 6 V, which was regarded as the stimulation threshold. Then, we incrementally increased the voltage by 1 V until it reached 10 V, and the amplitude augmented accordingly \((6-10 \text{ V vs. } 0.14 \pm 0.02-0.28 \pm 0.03 \text{ mN}; \text{Figure 4C})\).

### 3.4 | Calcium concentration-dependent contractility

To study contractility at various calcium concentrations, we changed CS with different calcium concentrations (from 1.5 to 7.5 mM). The result showed that the contraction force of the isolated papillary muscle increased when the calcium concentration was increased from 1.5 to 3 mM \((0.45 \pm 0.17-1.11 \pm 0.05 \text{ mN}); \text{however, after } 3 \text{ mM, the contraction force did not continue to increase even if the calcium concentration was increased to } 7.5 \text{ mM (1.02 \pm 0.23 mN}; \text{Figure 4D})\).

### 4 | DISCUSSION

Myostation-Intact is a new device used for physiological and pharmacological study of ex vivo muscular tissues, including skeletal, cardiac, and smooth muscles. It is tiny, exquisite, and sensitive for the detection of muscle contractility. We developed a method for contractility detection of ex vivo papillary muscle using this device. Our results revealed that...
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ttractility detection. First, the nature of the isolated papillary muscle
activates contractility when the threshold is reached.16 Our results indi-
lation below a certain threshold cannot activate muscle contractility and
the calcium concentration was increased from 1.5 to 3 mM. However,
myocardial contractility, of which we investigated frequency, voltage,
and calcium concentration. Consistent with previous studies, increased
frequency can lower papillary muscle contractility below 1 Hz.14,15
Mammalian muscular tissue has a voltage-gated channel; voltage stimu-
lation below a certain threshold cannot activate muscle contractility and activates contractility when the threshold is reached.16 Our results indi-
cated that the threshold of isolated mouse papillary muscle was at 3– 6 V.
Increasing calcium concentration can in turn enhance myocardial con-
tractility.15 We observed that myocardial contractility increased when
the calcium concentration was increased from 1.5 to 3mM. However,
the contraction force stopped increasing after 3mM, which is possibly attributed to the reduction in myofilament calcium sensitivity.17

In our study (Figure 5), some points are vital for successful con-
tractility detection. First, the nature of the isolated papillary muscle is crucial; the longer and thicker the isolated muscle is, the more pos-
sible it survives in ex vivo conditions. Solutions (PB and CS) can be
critical during the whole detection process. CS should be preheated to
the desired temperature before being perfused into the cuvette to
save time at later stages. Previous studies showed that mild hyper-
thermia (32–35°C) can protect myocardial tissue by increasing myo-
cardial contractility and decreasing body metabolism.18–20 Though
these studies were conducted in vivo, lower temperature can also be
applied on ex vivo myocardial tissues. Thus, in this study, we chose
32°C to maintain the vitality of the isolated papillary muscle and to
increase its survival in ambient conditions.

Because any change in the isolated papillary muscle can facilitate
its devitalization, ex vivo papillary muscle needs sufficient oxygen to
survive. Both PB and CS were oxygenated throughout. The papillary
muscle removed from the body was relatively hypoxemic, so PB was
oxygenated with 100% O₂ so that the tissue acquired sufficient ox-
ygen can be used for subsequent procedures.

An organ bath is a traditional ex vivo testing system used for
physiological research involving tissues.6,21,22 It is reliable in studies
involving contractility detections; however, the solution in the de-
tection chamber must be replaced at certain periods to ensure suffi-
cient oxygen in the solution. Whereas Myostation-Intact is equipped
with a circulation system, which is more convenient in application.
Besides, Myostation-Intact can be oxygenated outside the cham-
ber, prohibiting signal disturbance. Moreover, as the devise consists
of the mountain aid, attached hooks, and loop-grabbers, mounting the
tissue on Myostation-Intact is much easier. Another new similar
system is the Rodent Oscillatory Tension Setup to Study Arterial
Compliance, 23 which is also a reliable system; however, whether this
setup contains a heating system remains unknown.

Regarding the advantages of the Myostation-Intact system,
more future studies on isolated cardiac muscle contraction proper-
ties need to be conducted to study the diagnosis and mechanisms
of some diseases. For instance, ex vivo cardiac muscle contractility
can be studied in cardiomyopathy animal models to compare the
discrepancy between focal myocardial contractility and contraction
function of the entire heart, which can be helpful for further study
of cardiomyopathy mechanisms and treatments.

This study has some limitations. Myostation-Intact can mea-
sure only one tissue at a time, and each detection is time con-
suming. In addition, our method can be applied only to adult mice
papillary muscles; it is sometimes difficult to maintain the vitality
of isolated neonatal or juvenile mouse papillary muscle after being
isolated even if it is untouched or not stretched. Future studies
can be focused on these points when using Myostation-Intact.

In conclusion, we developed a method for the contractility de-
tection of isolated mouse papillary muscle using the Myostation-
Intact system. This method can ensure the survivability of ex vivo
papillary muscle and produce reliable results in contractility detect-
tions of isolated muscles.

**AUTHOR CONTRIBUTIONS**
Zhuyun Qin was involved in drafting and revising the manuscript, data
acquisition and analysis, methodology (lead), and investigation (lead);
Mengge Wu, Peipei Zuo, Lina Bai, Minjie Lu, and Lulu Li assisted with
methodology; Haitao Zhang reviewed the manuscript; Hong Lian was
the lead supervisor and was equally involved in review and editing. All
the authors read and approved the final manuscript.

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CONFLICT OF INTEREST
The authors declare that they have no competing financial interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author on reasonable request.

ETHICS APPROVAL STATEMENT
The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC), Fuwai Hospital, Chinese Academy of Medical Sciences; all animal experiments followed the guidelines of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, United States.

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