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In-vitro experiments to characterize ventricular electromechanics

DOI 10.1515/cdbme-2016-0059

Abstract: Computer simulation turns out to be beneficial when clinical data lack spatio-temporal resolution or parameters cannot be measured at all. To derive trustworthy results, these *in-silico* models have to thoroughly parameterized and validated. In this work we present data from a simplified *in-vitro* setup for characterizing ventricular electromechanics. Right ventricular papillary muscles from New Zealand rabbits were isolated and stretched from slack length to l_{max}, i.e. the muscle length at maximum active force development. Active stress development showed an almost linear increase for moderate strain (90–100% of l_{max}) and a significant decrease for larger strain (100–105% of l_{max}). Passive strain development showed a nonlinear increase. Conduction velocity CV showed an increase of ≈10% between low and moderate strain and no significant decrease beyond. Fitting active active stress-strain relationship using a 5th-order polynomial yielded adequate results for moderate and high strain values, whereas fitting using a logistic function yielded more reasonable results for low strain values. Passive stress-strain relationship was satisfactorily fitted using an exponential function.

Keywords: conduction velocity; stress-strain relationship; ventricular mechanics.

1 Introduction

Computational modeling of ventricular electromechanics is considered a promising approach to gain better insight into mechanisms underlying excitation-contraction coupling and mechano-electric feedback at the organ scale. Parameterization and validation of such *in-silico* models based on clinical data is challenging, as numerous parameters cannot be measured at all or only with insufficient spatio-temporal resolution. Recently, we built a standard *in-vitro* experimental setup for characterizing ventricular electromechanics. Using papillary muscles from New Zealand rabbits we measured in a series of stretch experiments auxotonic force transients to construct both passive and active stress-strain curves.

2 Methods

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Four male New Zealand rabbits with a weight of 2.01 kg (median, range 1.68–2.33 kg) were euthanized by the professional team in the Animal Facility of the Medical University of Graz (Certified by ISO 9001: 2008 and approved by the Austrian Federal Ministry of Science, Research and Economy Approval Number: BMWF-66.010/0017-II/3b/2014) with an overdose of Propofol and Fentanyl. Hearts were quickly excised and placed in cooled (8–12°C) and oxygenated Tyrode's solution with low Ca^{2+} and 2,3-butanedione monoxime (BDM). The solution contained (in mmol l^{-1}): NaCl 104.0, KCl 5.4, $CaCl_2$ 0.25, $MgCl_2$ 1.15, $NaHCO_3$ 24.0, NaH_2PO_4 0.42, D-glucose 5.6 and BDM 30.

1–3 papillary muscles from the right ventricle including chordae tendineae (tendons) were removed, transferred to the tissue bath (Mayflower Horizontal Tissue Bath System, HSE, Germany) placed under a microscope (SZX7, Olympus, Japan), and superfused with heated (36.4 ± 0.2°C) and oxygenated BDM-free Tyrode's solution with normal Ca^{2+} (2.5 mmol l^{-1}). The muscles were transfixed on hooks in the tissue bath at slack length with the basal side on a fixed hook and the tendon on a hook connected to the force transducer (HSE-HA F-30, HSE, Germany) as shown in Figure 1.

Preparations were paced at 1 Hz and twice threshold current (WPI A-365, WPI, Sarasota, FL, USA) using a tungsten wire placed at the basal end of the muscle. Unipolar extracellular electrograms were recorded at two positions with thin tungsten wires (50 µm diameter). The reference electrode was a Ag/AgCl-electrode placed in the tissue bath. Electrical signals were amplified

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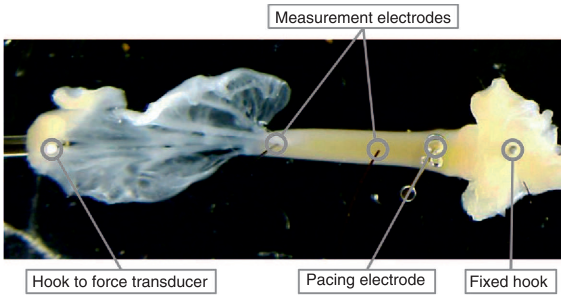

Figure 1: Papillary muscle mounted in tissue bath. The right hook is fixed, the left hook is connected to a force transducer. Muscles were stimulated with a tungsten pacing electrode (1 Hz, twice threshold current). Unipolar extracellular potentials were recorded at two positions with tungsten electrode with respect to a Ag/AgCl-electrode (not shown).

($\times$100) with custom-designed amplifiers, analog filtered (4$^{\text{th}}$-order Bessel lowpass, $f_g = 20$ kHz) and simultaneously digitized (NI-9215, National Instruments, Austin, TX, USA) with 100 kHz per channel.

For documentation of stimulus and recording positions a digital camera (DFW-X700, Sony, Japan) with a resolution of 1024 px $\times$ 768 px was mounted on the microscope. Pixel resolution of the acquired images was 15 µm px^{-1}.

2.1 Experiment protocol

Starting from slack length, load was gradually increased by moving the hooks in steps of 100 µm apart. The preparation was allowed to equilibrate for 2–6 min until a steady-state was reached. Approaching maximum force development, load-steps were reduced to 50 µm and stretching beyond maximum force development was limited to <5% of l_{max}, i.e. muscle length at peak force. At the end of each load step, active and passive force were measured as shown in Figure 2. Whenever feasible the preparation was relaxed to slack length and the measurement cycle was repeated.

For each load-step an image was taken for subsequent determination of muscle length (l_{muscle}), muscle diameter (d_{muscle}), and tendon length (l_{tendon}).

2.2 Data analysis

Stress in this work is given as Second Piola-Kirchhoff stress S, i.e. force per cross-sectional area in the initial configuration (slack length) as follows:

$$S = \frac{F}{A_0} \qquad (\text{mNmm}^{-2}) \tag{1}$$

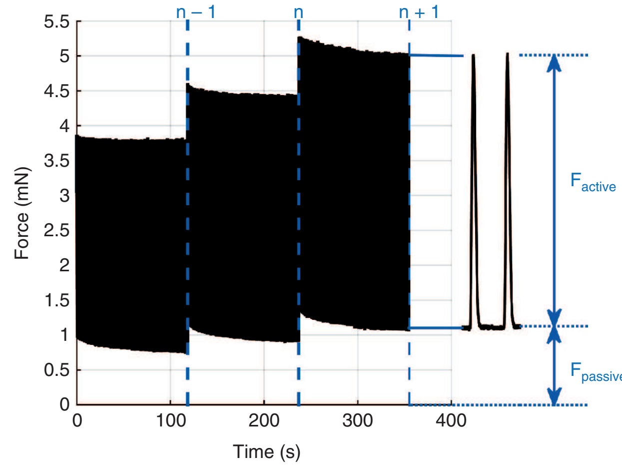

Figure 2: Experimental protocol. For each load-step *n*, active and passive force at steady-state are determined. An enlarged section with actual force transients is shown.

Slack length was defined as the length at the beginning of the experiment protocol and the corresponding muscle diameter was measured in the central section of the muscle. Cross-sectional area was calculated assuming cylindrical shape of the preparation. For each load-step strain was calculated as

$$\lambda = \frac{l_{muscle}}{l_{max}} \tag{2}$$

with l_{max} the muscle length at maximum active force development. Stress-strain plots show λ versus relative stress S_{rel}, i.e. stress normalized to stress at l_{max}:

$$S_{rel} = \frac{S}{S\,(l_{max})} \tag{3}$$

For statistical analysis data points were arranged in bins corresponding to 2% increase in strain. For each bin median active and passive force was calculated as well as the 25$^{\text{th}}$ and 75$^{\text{th}}$ percentiles.

Passive stress-strain data was fitted using an exponential function as follows:

$$S_p(\lambda) = S_{p,0} \cdot e^{\mu\lambda} \tag{4}$$

Active stress-strain data was fitted (i) using a 5$^{\text{th}}$-order polynomial of form:

$$p(x) = p_1 x^n + p_2 x^{n-1} + \cdots + p_n x + p_{n+1} \tag{5}$$

and (ii) using a generalized linear model (logistic function) as described in [1] of form:

$$Q(t) = Q_{inf} \frac{1}{1 + e^{-a(t-t_H)}} \tag{6}$$

with Q_{inf} the function value at infinity, t_H the time of symmetric inflection point, and a the time decay constant.

Conduction velocity CV at each load-step was calculated from the local activation time (LAT) at the two recording positions and the distance between the electrodes measured in image data. LATs were determined from maximum negative deflection of the signal derivative. For presentation CVs were normalized to CV at l_{max}.

3 Results

Eight complete measurement cycles from five papillary muscles were included into analysis. Reasons for exclusion were contracture of the muscle (i.e. continuous increase of passive force) or rupture of tendons. Diameter of the papillary muscles was 0.97 mm (median, range 0.73–1.13 mm). Absolute maximum active force measured was 2.49 mN (median, range 0.91–5.57 mN). Absolute passive force at maximum active force development, i.e. at l_{max}, was 3.53 mN (median, range 0.96–6.95 mN). Resulting stress was 3.45 mN mm^{-2} (median, range 0.91–10.26 mN mm^{-2}) for maximum active stress and 5.10 mN mm^{-2} (median, range 0.97–16.40 mN mm^{-2}) for passive stress at l_{max}. Normalized stress-strain plots for active and passive tension are shown in Figure 3.

Active stress development showed a moderate increase for 80–90% strain (low strain), an almost linear increase for 90–100% strain (moderate strain), and a pronounced decrease for strain beyond l_{max} (high strain). Fitting a polynomial and a logistic function both yielded good results for moderate strain. The logistic function was more reasonably representing low strain but failed to reproduce the decrease of stress beyond l_{max}. Polynomial fitting represented the sharp decrease beyond l_{max} more accurately but low strain was inadequately fitted, i.e. a sharp drop below 80% strain. Overall goodness of fit was fairly poor with R-square values of 0.69 for polynomial and 0.71 for logistic fitting.

Passive stress development showed the expected non-linear behavior. Fitting a simple exponential function yielded accurate representation of the data over the entire range of strain. R-square for the exponential fit was 0.89.

CV was 0.51 m s^{-1} (median, range 0.42–0.58 ms^{-1}). Normalized CV over strain is shown in Figure 4. For low strain CV was between 90–95% of CV at l_{max} and increased for moderate strain. No obvious reduction of CV beyond l_{max} was observed.

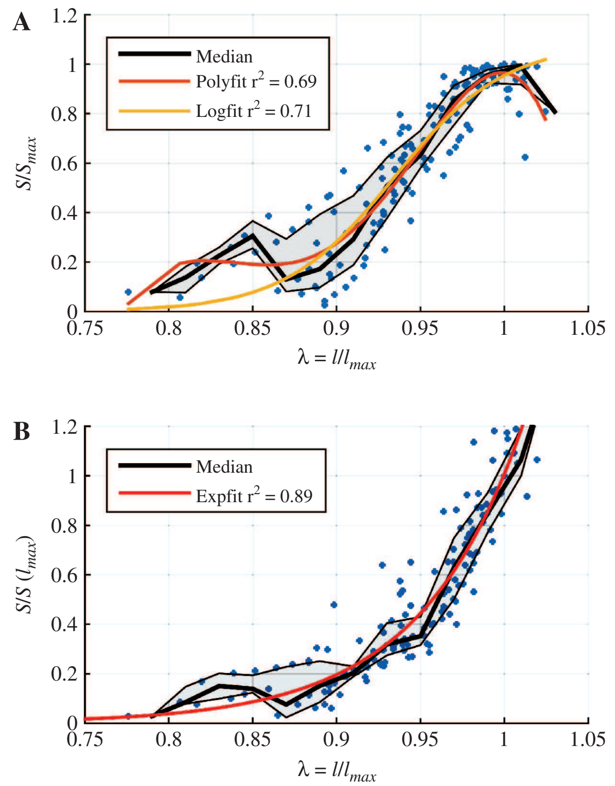

Figure 3: Stress-strain plots of active tension (A) and passive tension (B). Stress was normalized to maximum stress for active stress and to stress at l_{max} for passive stress. Dots represent measured data points. Data points were merged into bins corresponding to 2% increase in strain. Solid black line represents the median values of bins, 25th to 75th percentiles are highlighted as gray area. Active stress was fitted with a 5th-order polynomial (polyfit, $r^2 = 0.69$) and a logistic function (logfit, $r^2 = 0.71$). Passive stress was fitted with an exponential function (expfit, $r^2 = 0{,}89$).

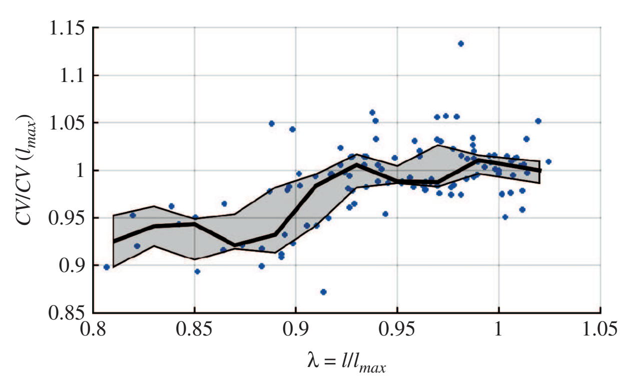

Figure 4: Conduction velocity (CV) as function of strain. CVs were normalized to CV at l_{max}. CV shows a maximum around l_{max} and decreases for strain ≤ 0.9.

4 Discussion

Active and passive stress development shown in this work is qualitatively in good accordance to earlier works as shown in [2] for rat papillary muscle and in [3] for rat trabeculae. Absolute values for peak active stress differ considerably from data shown in the above mentioned works.

This can be attributed to (i) the different species and (ii) to the different experiment protocol (tissue bath temperature 20–25°C, pacing rate $\leq$0.2 Hz). However, it has to be mentioned that the ratio of active to passive stress at l_{max} shown in this work poses the question if the preparations might not be adequately supplied by superfusion. Muscle diameter of our preparations is roughly 1 mm whereas diameters in [2] and [3] where only 0.22 mm. Assuming a diffusion length of 500 μm [4], preparations of 1 mm diameter should be properly supplied.

Interpretation of λ is challenging because sarcomere length, and therefore the "true strain", depends on the configuration of the connective tissue matrix as discussed in [5] and might differ considerably from strain determined from muscle length. This might explain the increasingly large variation of data points at low strain values and the apparently different slack lengths in different preparations. Therefore, fitting the data using a logistic function seems to be more reasonable for low strain. Fitting active stress data with a higher-order polynomial proofed feasible for moderate and high strain and reproduced the decrease of stress for stretching the preparations beyond l_{max}. To accurately represent the data over the entire strain range a more sophisticated model has to be implemented. On the other hand, passive stress data can be reasonably well fitted using a simple exponential function.

Development of CV over strain is in accordance with previous works in rabbit papillary muscles [6] although we did not observe a distinct decrease in CV above l_{max} since we limited strain to $<$105%.

5 Conclusion

Recently, the focus of experimental work on force development shifted increasingly from tissue level to cell level. Hence data from experiments using cardiac tissue is often outdated and additionally ambiguous. However, state-of-the-art *in-silico* models of the whole heart require such data to validate results in the millimeter range. The data gathered from *in-vitro* experiments shown here will foster the description of stress-strain relationship and therefore will support parameterization and validation of modern *in-silico* models. A limitation of our current setup is that only muscle length and not actual sarcomere length can be determined and therefore our setup would greatly benefit from direct sarcomere length assessment, e.g. by laser diffraction measurements.

Acknowledgment: The authors would like to thank Michaela Janschitz, Kurt Feichtinger, Gerald Zach, and Wolfgang Sax for support.

Author's Statement

Research funding: The author state no funding involved. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent is not applicable. Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

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