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Experimental study and modelling the evolution of viscoelastic hysteresis loop at different frequencies in myocardial tissue

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Our work involved experimental study of the influence of actomyosin complexes and the main structural components of the myocardial tissue – connective tissue collagen framework and cardiomyocytes – on the characteristics of viscoelastic hysteresis at different frequencies. In this paper a new method was introduced for the analysis of the viscoelastic characteristics of the force hysteresis in the isolated myocardial preparation for the assessment of mechanical energy expenditure in the tension-compression cycle. We established that basic myocardial structures have an impact on the to the characteristics of the viscoelastic hysteresis in many ways. It was shown that in rat's myocardium cardiomyocytes one main factor that define the stiffness and viscosity of the myocardium in the physiological range of deformations, while binding of calcium ions with EGTA and calcium removal of sarcoplasmic reticulum with caffeine reduces viscoelasticity by ~30% and collagen framework is responsible for about 10% of viscoelasticity. It was revealed that in the physiological range of the hysteresis frequencies (3 to 7 Hz) expenditure of mechanical energy per unit of time increases linearly with increasing frequency. We proposed the structural and functional model that adequately describes the characteristics of the viscoelastic hysteresis in myocardial preparation in the range of strains and frequencies being under study.

Key words: *hysteresis, viscoelastic properties, frequency dependence, myocardium, modelling*

Abbreviations

EGTA – ethylene glycol tetraacetic acid,
SDS – sodium dodecylsulfate,
WLC model – Worm-like chain model.

1. Introduction

The functional properties of myocardial tissue are largely determined by the viscoelastic properties of the main structural components – collagen connective tissue matrix and cardiomyocytes [19], [21]. However, the experimental assessment of viscoelasticity influence on the contractile function of heart is a difficult task because of clearly expressed nonlinear elastic and viscous characteristics of tissue, Ca^{2+}-ions inflow into myoplasm during deformation and impact of the long-lived actomyosin complexes under various loads [4], [20]. It is well known that myocardium has a three dimensional composite structure [5]. At the same time it is possible to define the organized groups of cardiomyocytes surrounded by a general connective tissue shell – sheet (lamina) – forming the various layers of the ventricular wall [9]. The typical objects of study used to carry out an adequate assessment of myocardial viscoelasticity characteristics are trabeculae or papillary muscles consisting of several sheets [17], [18]. In experiments on isolated preparations (mainly due to the longitudinal orientation of cardiomyocytes in the papillary muscles) it is possible to define involvent of myocytes and the connective tissue to the viscoelastic properties of myocardium [16] separately. The experiments of viscoelastic force hysteresis as function of the muscle's length enable to obtain dy-

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namic viscoelastic characteristics under various loads and it to carry out a specific assessment of the mechanical work efficiency in the heart muscle. Compliance with the energy expended in the active contraction-relaxation cycle of rat papillary muscle, and the area of force developed by papillary muscle as a function of muscle length (viscoelastic hysteresis) has been showning [2].

It should be noted that it is frequently impossible to analyze processes occurring *in vivo* during biomechanical experiment, particularly processes with different characteristic time. In this case, the only solution is the use of mathematical models that adequately describe phenomenon of interest for complete understanding of the mechanisms of interrelation between structure and tissue function.

Thus, in our work we carried out a comprehensive experimental and theoretical study of characteristics of the viscoelastic hysteresis of the isolated rat myocardial samples at different stress–strain frequencies was carried out. The influence of the major sources of passive tension of myocardium to its viscoelastic properties was determined. Also we proposed a structural and functional model adequately describing the experimentally obtained viscoelastic hysteresis characteristics of the myocardial samples in the considered range of deformations and frequencies was proposed. Numerous experiments were carried out on the model to analyze how changes in the mechanical characteristics of each structural component affects the viscoelastic characteristics of the tissue.

2. Materials and methods

Experiments were carried out on the papillary muscle of the right ventricle obtained from healthy hearts of weiging between 150–250 g and being 4–6 months of age. Animals were treated in according to the principles adopted by the Committee on the humane treatment of animals of Institute of Immunology and Physiology, Ural Branch of Russian Academy of Sciences. Preparations of papillary muscles and the calculations of benchmark parameters of muscle's slack length L_0 (which corresponds to zero passive force) and working length $L_w \sim 0.95L_{max}$ (L_{max} is a length of the muscles, corresponding to the maximum of developed active force) were carried out in accordance with the method used by us previously [16]. In this study viscoelastic characteristics of hysteresis of the papillary muscles in normal saline solution ($N = 7$, $P < 0.05$) was obtained; then the flow of Ca^{2+} ions into myoplasm and excluded the impact of actomyosin complexes on the viscoelastic characteristics in calcium-free solution with the addition of caffeine (10 mmol) and EGTA (5 mmol) (further – Ca-free solution) in the same preparations [7], [8]. Finally, to assess the contribution of connective tissue in the carcass muscle viscoelasticity the removal of cardiomyocytes was carried out – decellularization of preparations with 1% sodium dodecyl sulphate for 60 minutes following the procedure [13] (further – SDS). Viscoelastic characteristics of hysteresis were obtained on papillary muscles near the working length L_w in the frequency range from 0.1 Hz to 10 Hz of the cyclic sawtooth length changes of the preparation and the amplitude of 4% of the initial muscle length (L_0).

The initial recording signals of the myocardial sample force and length were filtered in the experiment for subsequent processing according to the method developed by us previously [14]. Figure 1A shows an example of the viscoelastic hysteresis loop evolution of the rat right ventricle papillary muscle at different frequencies. Next, the dependence of viscoelastic hysteresis loop area per unit time on the frequency was plotted (Fig 1B, data are presented in the logarithmic frequency scale for convenience).

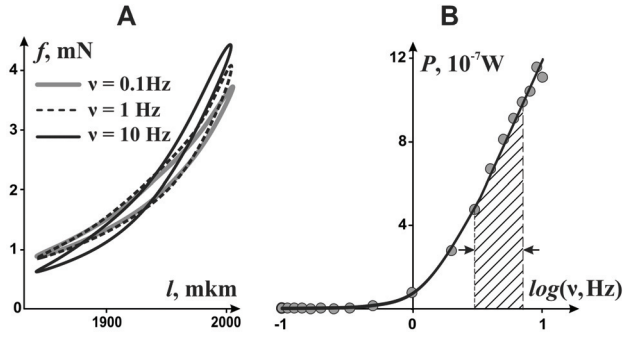

Fig. 1. A representative example of the hysteresis loop for the rat papillary muscles under different periodic deformation (A); The dependence of the mechanical energy dissipated per tension – compression cycle per time unit (power) of rat papillary muscle on frequency of ramp deformation (logarithmic scale). Physiological range of the tension – compression cycle frequency (from 3 Hz to 7 Hz) of rat papillary muscles is marked with hatching (B)

The transition to the relative specific values were done to compare the data of hysteresis loop areas in different experimental groups (Eq. (1)):

$$f\,[\mathrm{mN}] \to \sigma\left[\frac{\mathrm{mN}}{\mathrm{mm}^2}\right]$$
$$l\,[\mathrm{mm}] \to \varepsilon \qquad (1)$$
$$P\,[\mathrm{W}] \to \rho\left[\frac{\mathrm{W}}{\mathrm{m}^3}\right]$$

where:

f – passive force developed by muscle,

σ – mechanical stress of muscle,

l – muscle's length,

ε – relative muscle's strain,

P – power,

ρ – power density per unit volume.

The experimental data were evaluated using the nonparametric Mann–Whitney U-test for data having an abnormal distribution and a small sample. The level of significance was $P < 0.05$.

3. Results

It has been shown that after cardiomyocytes after removal there is a significant decrease (~ 90%) of the amplitude and the area of the viscoelastic hysteresis loop. It is statistically significant in the whole range of tension-compression frequencies of the preparation taken under consideration (Fig. 2). It should be noted that the charts have a different scale on the Y-axes. It is noteworthy that within the frequency range of 0.5 Hz to 10 Hz the area and the amplitude of the viscoelastic hysteresis loop of preparation in Ca-free solution are significantly smaller than in the control solution.

As results show there is no significant change in the amplitude or the square of the viscoelastic hysteresis depending on the tension-compression frequency in any one of the experimental groups discussed.

Modelling of viscoelastic characteristics of hysteresis was carried out using the model approach developed earlier [15]. Since the geometric model is similar to the real geometry of isolated papillary muscles, the initial geometric parameters were slack length and diameter of the papillary muscles in each experiment. The model reflects the main morphological structures providing passive stress of myocardial tissue: connective tissue matrix and titin [3]. The model is described by four constant parameters: 3 for stiffness, 1 for viscosity (Fig. 3). Viscoelastic behavior of the model can be described by system of Eqs. (2).

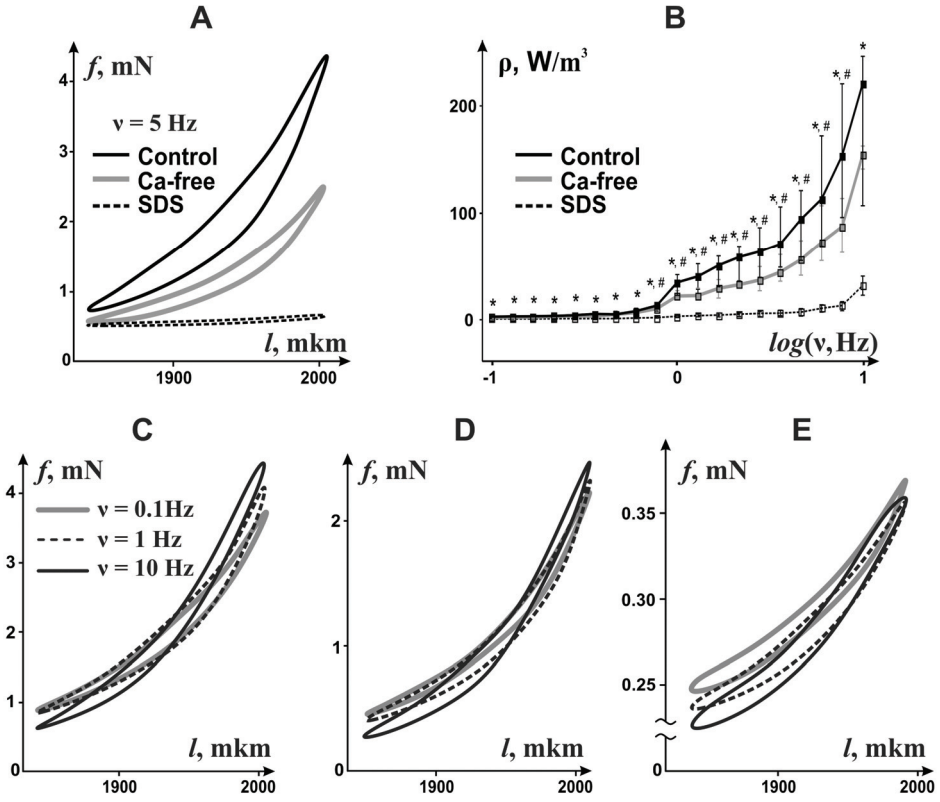

Fig. 2. A representative example of the hysteresis loop for the rat papillary muscles under periodic deformation chosen for modelling (different solutions) at physiological frequency of stretching-shortening cycle (A); the force-frequency dependence of the mechanical energy dissipation (power) for a series of tension-compression cycles of passive rat's papillary muscle. Statistically significant differences between SDS and the others are marked with *. Statistically significant differences between Calaghan and Ca-free are marked with # (B); representative examples of the hysteresis loop for the rat papillary muscles in different solutions: Control (C), Ca-free solution (D), after SDS treatment (E)

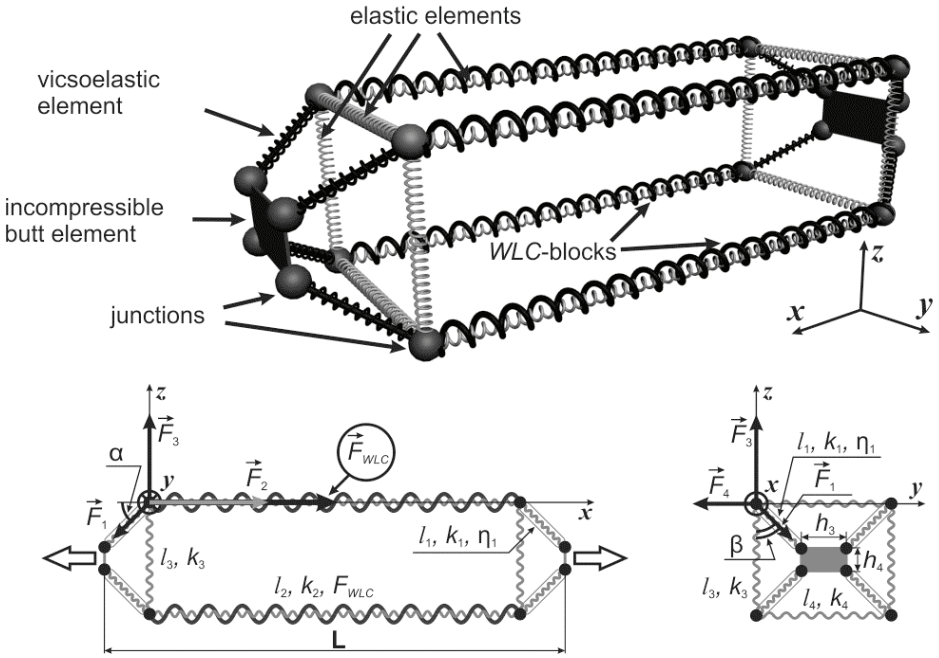

Fig. 3. Three-dimensional structural model of myocardial morphofunctional unit with WLC-blocks. Large empty arrows show the direction of deformation of the model and the direction of whole model force action

$$\begin{cases} 2 \cdot l_1 \cdot (F_2 + \mathrm{F_{WLC}}) - (L - l_2) \cdot F_1 = 0 \\ 2 \cdot l_1 \cdot F_3 - (l_3 - h_3) \cdot F_1 = 0 \\ 2 \cdot l_1 \cdot F_4 - (l_4 - h_4) \cdot F_1 = 0 \\ (l_3 - h_3)^2 + (l_4 - h_4)^2 + (L - l_2)^2 = 4 \cdot l_1^2 \end{cases}$$

where

$$\begin{aligned} F_1 &= k_1 \cdot (l_1 - l_{10}) + \eta_1 \frac{d}{dt} l_1 \\ F_2 &= k_2 \cdot (l_2 - l_{20}) \\ F_3 &= k_3 \cdot (l_{30} - l_3) \\ F_4 &= k_4 \cdot (l_{40} - l_4) \end{aligned} \qquad (2)$$

where F_{WLC} is force developing by titin "WLC-blocks"; l_{10}, l_{20}, l_{30}, l_{40} are initial lengths of model elements; l_1, l_2, l_3, l_4 are current length of the elements; k_1, k_2, k_3, k_4 are elastic coefficients; η_1 is viscous coefficient; F_1, F_2, F_3, F_4, are forces developed by corresponding elements; h_3, h_4 are dimensions of incompressible butt element; L is length of the whole model. The input parameters of the system of Eq. (2) are initial lengths of model elements l_{10}, l_{20}, l_{30}, l_{40} ($l_{30} = l_{40}$); elastic coefficients k_1, k_2, k_3, k_4 ($k_3 = k_4$); viscous coefficient η_1; dimensions of incompressible butt element h_3, h_4 ($h_3 = h_4$); parameters of WLC-model [10]. The output parameters are current length of the elements l_1, l_2, l_3, l_4.

Experimental protocol for real muscle was repeated during numerical experiments on model by corresponding changes of parameters. Figure 4 shows an example of modelling viscoelastic hysteresis loop at a physiological stress-strain frequency of 5 Hz.

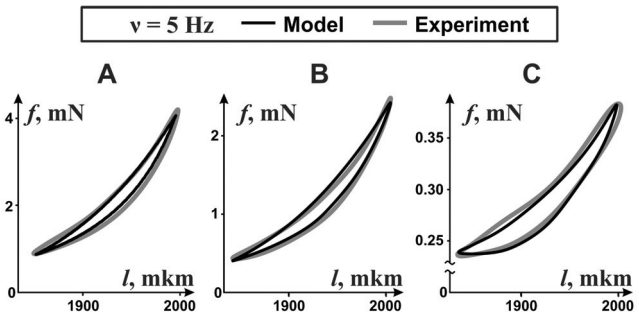

Fig. 4. Comparison the experimental hysteresis loop of the rat's right ventricle papillary muscle at a frequency of 5 Hz and modelled hysteresis loop (Control – (A), Ca-free solution (B), after SDS-treatment (C))

Table 1. Values of structural element parameters of the model (different solutions)

	Control	Ca-free	SDS
k_1, N/m	5.12	5.07	1.21
k_2, N/m	8.24	5.19	0.01
k_3, N/m	1.04	1.02	0.62
η_1, s	120	80	8

The model adequately captures the characteristics of the viscoelastic hysteresis in all experimental groups considered, while the values of model pa-

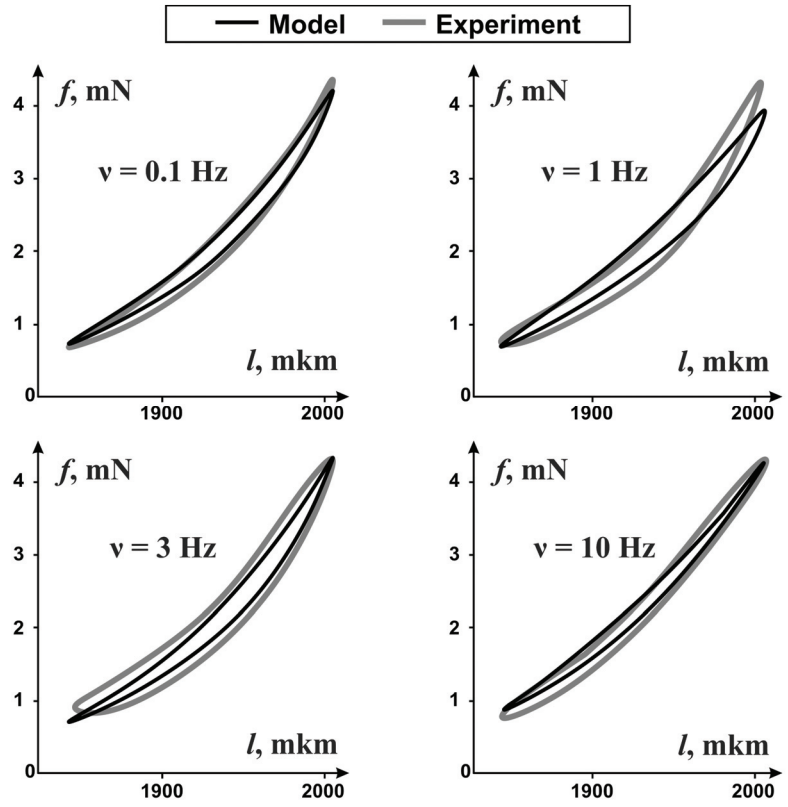

Fig. 5. Comparison of the experimental hysteresis loop of the rat's right ventricle papillary muscle at different frequencies and modelled hysteresis loop (Control)

Table 2. Values of structural element parameters of the model (different frequencies)

	ν = 0.1 Hz	ν = 1 Hz	ν = 3 Hz	ν = 10 Hz
k_1, N/m	6.25	5.87	6.12	6.21
k_2, N/m	5.18	5.94	5.43	6.07
k_3, N/m	0.57	0.61	0.52	0.58
η_1, s	120	140	130	90

rameters (Table 2) reflect the quantitative changes in the amplitude and area of loof for an appropriate solution.

In the next stage out simulation of viscoelastic hysteresis of papillary muscles at different tension-compression frequencies was carried out. The results are shown in Fig. 5.

The model captures the viscoelastic hysteresis characteristics quite well in the entire range of considered tension-compression frequencies. It was noted that for reproduction of the experimental curves obtained for a particular preparation at different tension-compression frequencies it was necessary to adjust the model parameters. However, this adjustment was about 10–15% in all cases and was a random variation of the corresponding values.

4. Discussion

First data about evolution of the viscoelastic hysteresis loop depending on the frequency of the sawtooth deformation were obtained experimentally with real myocardial preparations. This gives certain assessment of the effectiveness of mechanical work of the heart muscle's preparation. No significant changes have been shown in the shape and area of the viscoelastic hysteresis loop in the above range of the stress–strain frequencies (including physiological range). This suggests that the expenditure of mechanical energy during one tension-compression cycle is practically unchanged. In other words, the work of the heart spent on the development of its own mechanical stress is directly

proportional to the frequency of the sawtooth deformation.

In addition, we have experimentally assessed the contribution of the main structural components of the myocardial tissue – connective tissue framework and cardiomyocytes – in characteristics of the viscoelastic hysteresis. Despite the fact that the stiffness of the collagen fibers is higher by two orders than the stiffness of myocytes [1], [11], it was found that the removal of cell elements results in a substantial reduction (almost 90%) of the elastic force developed by the preparation and its viscosity. This can be explained by the fact that the connective-tissue matrix of myocardium has structural elasticity [6], which is most affected by the three-dimensional structure of the matrix. Our results also indicate the presence of residual stresses in the myocardial tissue, which is consistent with literature data [12].

Before this work there was lack of data on the correlation between the concentration of Ca^{2+} ions in the muscle and the viscoelastic properties of the myocardium. We have shown that the calcium removal from the test solution and the preparation results in a decrease of the amplitude and area of the viscoelastic hysteresis loop of about ~ 30%. This, in part, can be explained by long-lived actomyosin complexes or the presence of some yet unknown mechanism of the effect of concentration of Ca^{2+} ions in the force developed by cardiomyocytes in a passive state.

We suggested a structural and functional model that adequately describes the characteristics of the viscoelastic hysteresis of myocardial preparation in the investigated range of strains and frequencies of physiologically range. During the verification of the model parameters from experimental curves of viscoelastic hysteresis was the reduction of all model's parameters (stiffness and viscosity of the structural elements) could be observed in the Ca-free solution, and then after SDS treatment. In the process of modelling the characteristics of the viscoelastic hysteresis of single preparation at different tension-compression frequencies, an adjustment of stiffness and viscosity of the model's structural elements was necessary for the most accurate reproduction of the experimental curves. However, there was no regularity of changes depending on the frequency, and the amplitude of the changes was 10–15%. Careful analysis of this fact has confirmed the assumption of the random nature of the deviations caused by the variation of the biological properties of the object and a measurement error in the experiment.

Therefore the developed approach enables us to quantify a mechanical energy expenditure on the development of mechanical stress in the myocardium depending on the load. And the proposed model allows us to calculate the contributions of the main structural components of the myocardium in its viscoelastic characteristics. It is noteworthy that since the reported results were obtained for relatively healthy animals, our method of analysis of morphofunctional characteristics can be used as an estimation of pathological condition of the heart, as in pathology myocardial remodelling of structures.

Conflict of interest statement

The authors have no conflict of interest.

Acknowledgements

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