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Instrumentation to study myofibril mechanics from static to artificial simulations of cardiac cycle

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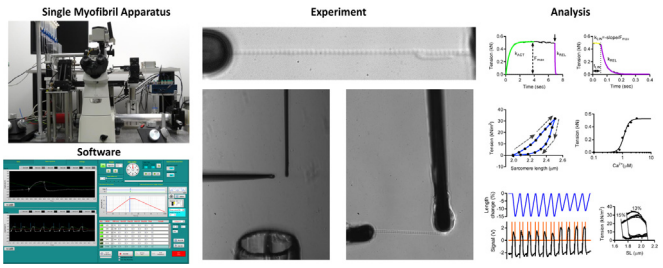
GRAPHICAL ABSTRACT



ABSTRACT

Many causes of heart muscle diseases and skeletal muscle diseases are inherited and caused by mutations in genes of sarcomere proteins which play either a structural or contractile role in the muscle cell. Tissue samples from human hearts with mutations can be obtained but often samples are only a few milligrams and it is necessary to freeze them for storage and transportation. Myofibrils are the fundamental contractile components of the muscle cell and retain all structural elements and contractile proteins performing in contractile event; moreover viable myofibrils can be obtained from frozen tissue.

- We are describing a versatile technique for measuring the contractility and its Ca^{2+} regulation in single myofibrils. The control of myofibril length, incubation medium and data acquisition is carried out using a digital acquisition board via computer software. Using computer control it is possible not only to measure contractile and mechanical parameters but also simulate complex protocols such as a cardiac cycle to vary length and medium independently.

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- This single myofibril force assay is well suited for physiological measurements. The system can be adapted to measure tension amplitude, rates of contraction and relaxation, Ca^{2+} dependence of these parameters in dose-response measurements, length-dependent activation, stretch response, myofibril elasticity and response to simulated cardiac cycle length changes. Our approach provides an all-round quantitative way to measure myofibrils performance and to observe the effect of mutations or posttranslational modifications. The technique has been demonstrated by the study of contraction in heart with hypertrophic or dilated cardiomyopathy mutations in sarcomere proteins.

ARTICLE INFO
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Method details

Many experimental systems have been used for the study of muscle contraction. Single molecule or single filament type assays allow unlimited control over incubating medium and protein composition but do not have the structural organization of a muscle. Chemically skinned muscle cells or fibers allow for the measurement of contractility but response to changes in the medium is limited by diffusion and results may be confused by non-sarcomeric elastic elements. The optimum preparation for studying contractility is the myofibril – the fundamental unit of striated muscle. Myofibrils are $\sim$1 μm in diameter and up to several 100 μm long. Myofibrils are built up from repeating contractile subunits called sarcomeres, composed of actin and myosin filaments arranged in parallel. Powered by energy of ATP, myosin heads push actin filaments alongside causing actin and myosin filaments to slide past each other. Such process results in shortening of sarcomeres, myofibrils and the whole muscle cell. The troponin complex together with tropomyosin, which sterically blocks interaction of myosin with actin at low Ca^{2+} concentration, controls this process [1]. Not much is yet known about the modulation of this process by other sarcomeric proteins, such as myosin binding protein C, titin, nebulin and obscurin. But there is no doubt that this is of physiological importance [2–6]. It was found that the most causes of heart diseases and muscular dystrophies are inherited and caused by mutations in genes of sarcomere proteins [7,8]. What is more, changes in isoform protein expression, phosphorylation [9] and oxidation [10] also affect myofibril contractility in several ways. The muscle diseases not only diminish the quality of life but also are one of the highest causes of death. Comprehensive experimental studies of the mechanisms leading to the diseases are important as for the choice of treatment as well as for the discovery of new ones [11,12].

Myofibrils can be isolated in sufficient quantities from only a few milligrams of fresh or frozen tissue samples or cells meaning that the sources are potentially unlimited. The preparation preserves native organization of sarcomeric and structural proteins. The use of myofibrils in contractile experiments has many practical advantages compared to the use of permeabilised muscle fibers. Because of myofibrils smaller diameter there are no diffusional limitations ($<$1 ms) and rapid solution changes are easy so that the quick processes of contraction and relaxation can be studied. Dynamics of individual sarcomeres can be followed. However, the small size of myofibrils requires special apparatus.

The early apparatus design to measure force in single myofibrils can be dated back to the papers of Iwazumi [13] and Cecchi et al. [14,15] that also provide the most detailed description of the system. Another successful apparatus setup to study single myofibril contractility and elasticity is based on a modified atomic force microscope [16,17]. The later setup did not demonstrate any practical advantages over the original one in terms of system throughput and signal-to-noise ratio. In both cases the principle of the measurement of force in single myofibrils is based on the use of a L-shaped cantilever as a force-sensing element. In the first case the cantilever acts as an obstacle creating a shadow on a photodiode sensor, in the second case the cantilever reflects a laser beam onto a photodiode sensor. In both cases the changes in total proportion of light on the two halves of a split