Quantifying Drug-induced Nano-mechanics and Mechanical Effects to Single Cardiomyocytes for Optimal Drug Administration to Minimize Cardiotoxicity

AUTHORS: *Tao Yue[1], Ki Ho Park[2,3], Benjamin E. Reese[1], Hua Zhu[2,3], Seth Lyon[1], Jianjie Ma[2,3], Peter J. Mohler[2], Mingjun Zhang[1,2,4*]*

[1]Department of Biomedical Engineering, The Ohio State University

[2]Dorothy M. Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center

[3]Department of Surgery, The Ohio State University Wexner Medical Center

[4]Interdisciplinary Biophysics Graduate Program, The Ohio State University

[*]Correspondence to: zhang.4882@osu.edu

Supporting Information:

Materials and Methods

Primary mouse cardiomyocytes

Cardiomyocytes were isolated from mice in the age of 8 ~12 weeks. The mouse was injected with 70 μL heparin (70 USP units/mouse) intraparitoneal (i.p.) 10 min prior to the sacrifice. The heart was removed from a mouse under anesthesia. After cannulating the aorta of the heart with 22 gauge feeding needle (Fine Science Tools, Item No. 18061-22), the heart was perfused via the left ventricle for 4 min with perfusion buffer (in mM: NaCl, 120; KCl, 5.4; $MgSO_4$, 1.6; NaH_2PO_4, 1.2; glucose, 5.6; $NaHCO_3$, 20; taurine, 5; 2,3-butanedione monoxime (BDM), 10; aerated with 5 % CO_2/95 % O_2; pH 7.2) using gravity in a langendorff system. The heart was then perfused for 4 min with isolation solution (perfusion buffer containing 1 mg/mL collagenase Type 2 (Worthington, Cat. No. LS004176, CLS-2), 0.02 mg/mL protease Type XIV (Sigma-Aldrich, Cat. No. P5147)) and then perfused for 8~12 min with 50 M $CaCl_2$ contained isolation solution. Next, the heart was transferred to a 35 mm dish with 2.5 mL of 50 M $CaCl_2$ isolation solution. The right ventricle, aorta, atria, and great vessels were trimmed away from the heart with fine surgical scissors. The remaining heart was gently teased into 10-12 small pieces with two fine-tip forceps. The heart pieces were then gently transferred to a 15 mL polypropylene conical tube and added 7.5 ml of 50 M $CaCl_2$ isolation solution and the cardiomyocytes were disassociated by a 7 mL transfer pipette. After removing debris by 250 m nylon mesh, centrifugation of 1 min at 45 x g was conducted. The supernatant was removed and the pellets were re-suspended in 10 ml calcium solution containing 100 μM $CaCl_2$ and 2.5 % FBS. The cells were allowed to settle down for 10 min at room temperature, then again the supernatant was removed and the pellets were re-suspended in the same solution one more time to stop enzyme activity completely. The cardiomyocytes with a proper cell density were used immediately after

isolation. The following medium, which contains Minimal Essential Medium Eagle, 25 mM $NaHCO_3$, 10 mM HEPES, Insulin-Transferrin-Sodium Selenite, 1 % penicillin/streptomycin, pH7.4, 2.5 % FBS, was used in the experiments. The cells were placed on glass dishes with 1 mL of this medium and incubated at 37 ^{o}C for 15 min to allow cells to attach. Finally, the solution was replaced with the same medium without 2.5 % FBS before characterization.

Spontaneous and electric stimulated contractions

In order to select the proper contraction conditions, both of spontaneous contraction and electric stimulated contraction of primary mouse cardiomyocytes were evaluated as shown in **Figure S1**. Force curves of spontaneous contraction showed much larger variations on force value and beating frequency, which disturbed the acquisition of proper data. Controlled stimulation provided stable contraction force from the desired cells to facilitate the data acquisition, while the spontaneous beating was unpredictable. Therefore, in all experiments including control and drug treated groups, the electric stimulation was utilized to obtain stable and regular surface beating patterns.

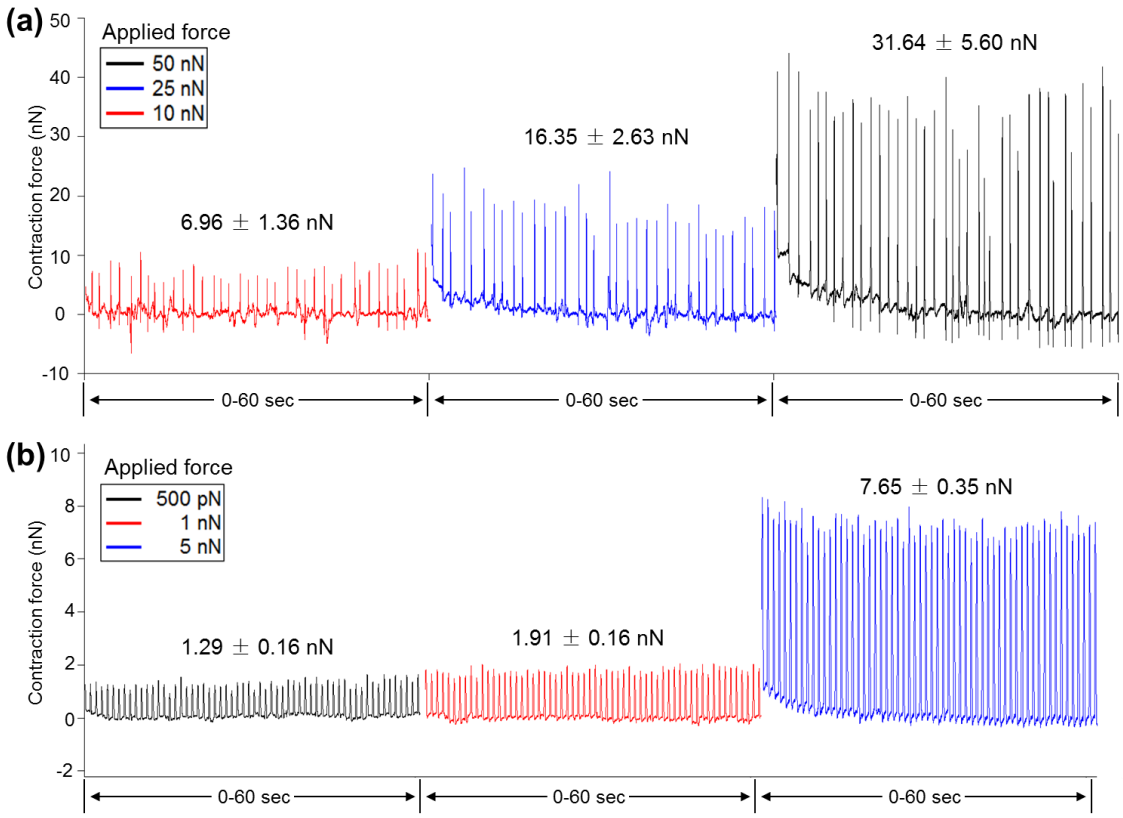

Figure S1. Comparison of the response from a spontaneously active cell versus an electrically stimulated cell. (**a**) The force of surface beating at varying loads showing a high standard deviation. (**b**) The results from a stimulated cell showing a much lower standard deviation relative to the spontaneous cell in (**a**).

Base line nano-mechanics characterization

Without any drug treatment, the time course changes of electric stimulated cells, including cell stiffness, beating force and FWHM were characterized. Results are shown in **Figure S2**.

In normal culture condition without any drugs, we compared the cell stiffness changes without or with electric stimulation. As shown in **Figure S2**(a), the cell without stimulation showed stable stiffness value during the 90 min monitoring. The stimulated ones had a gradually increase, eventually up to 40%. Without stimulation, the resulting stiffness values were more consistent and stable over the same time period. As shown in **Figure S2**(b), the stimulated cells had a relatively stable FWHM, coupling with the gradually decreased surface beating force. The stimulated results might be caused by a gradual increase of intra-cellular calcium irons due to potential electroporation or damage to the cell membrane. They could also be due to potential cytoskeletal remodeling or enhanced sarcomeric organization occurring in response to continuously eliciting contractions.

These results demonstrated the capabilities of our platform as well as a real-time quantitative approach for single cardiomyocyte studies on mechanobiology in response to the drug exposures. These base line characterization data serviced as control comparing with drug treated groups.

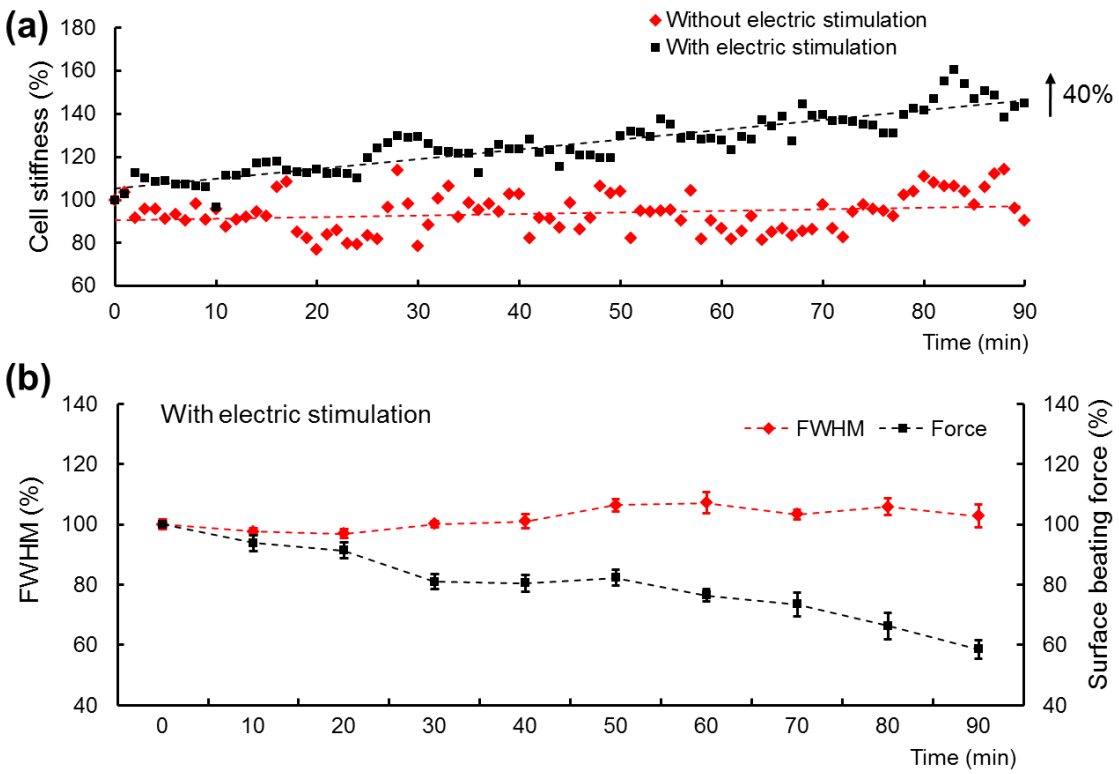

Figure S2. Base line nano-mechanics characterization. (**a**) Stiffness comparison for cardiomyocytes without and with electric stimulation. For non-stimulated cells, stiffness data showed a stable trend. For stimulated cells, data showed a gradual increase. (**b**) For stimulated cells, FWHM showed a relatively stable value, coupled with the gradually decreased surface beating force.

Cardiomyocyte selection criteria

In order to evaluate the influence of sarcomeric organization to the cell stiffness and beating force, two types of cells with significant difference of size and sarcomeric organization were characterized under different stimulation force generated by AFM cantilever. Results are shown in **Figure S3**. Cell A (the big one) had lower beating force than Cell B (the small one), while Cell A had higher stiffness. Based on the magnified images of both cells, there were no clear striations on Cell A, while Cell B had clear sarcomeric organization which was thought to be related with the cell stiffness and beating capability.

The results were able to show that sarcomeric organization had greater influence on the force of beating compared to size. The no clear striations on Cell A was resulted from the damage caused during the isolation. Cell B was able to generate nearly twice the amount of force compared to Cell A. A positive correlation between the applied force and the resulting force of beating was clearly observed. The cells with sarcomeric organization similar as Cell B were considered as the ones received light damage during the isolation, and they were selected as proper samples in the following experiments.

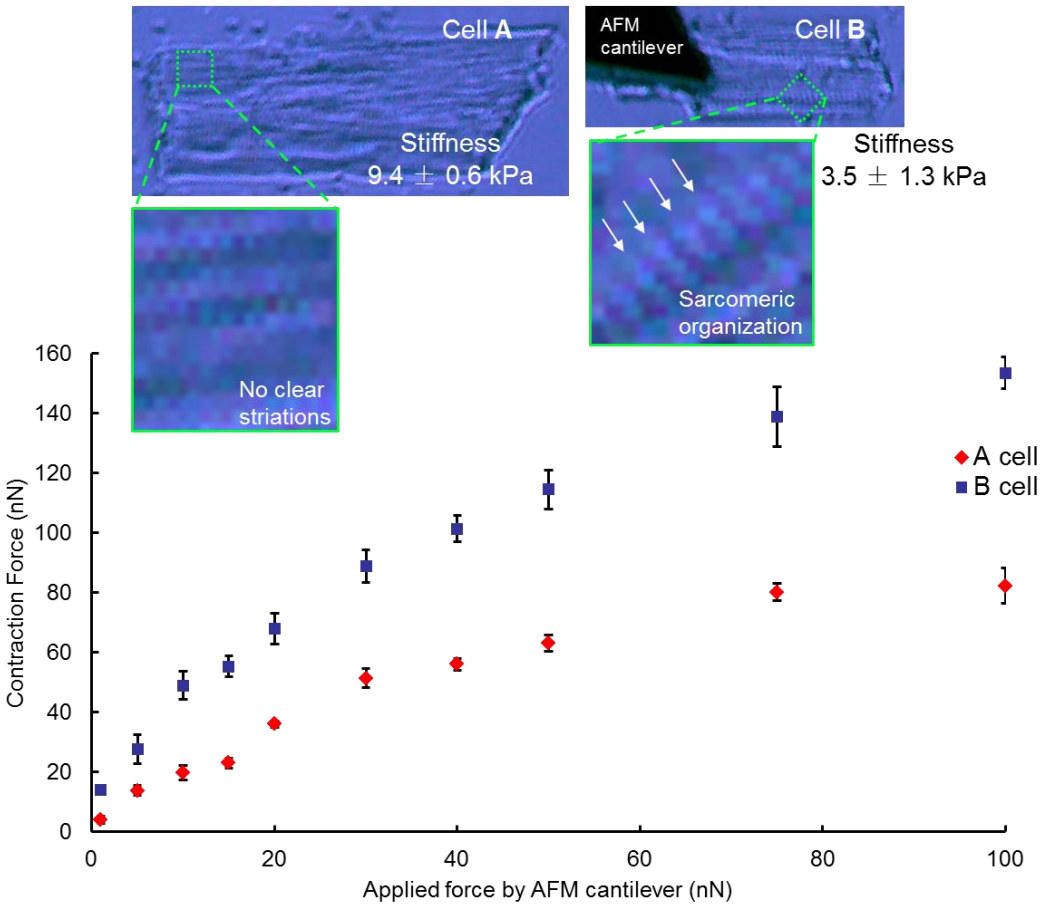

Figure S3. The trend of applied load versus the resulting beating force of 2 different cells. Cell A has no clear striations, while Cell B has much better sarcomeric organization. Cell A has higher stiffness but lower beating force comparing with Cell B.

The DHM phase time-dependent monitoring of the region of interest (ROI)

After the ROI was defined, the phase data (transferred from radian to length unit nm) of each frame was calculated and the phase changes (difference) in the next frame was recorded. The time course data was obtained from these frame to frame phase dynamic changes, as shown in **Figure S4**. It was presented as a waveform graph, containing the surface beating level (considered as same as the amplitude) and the duration FWHM.

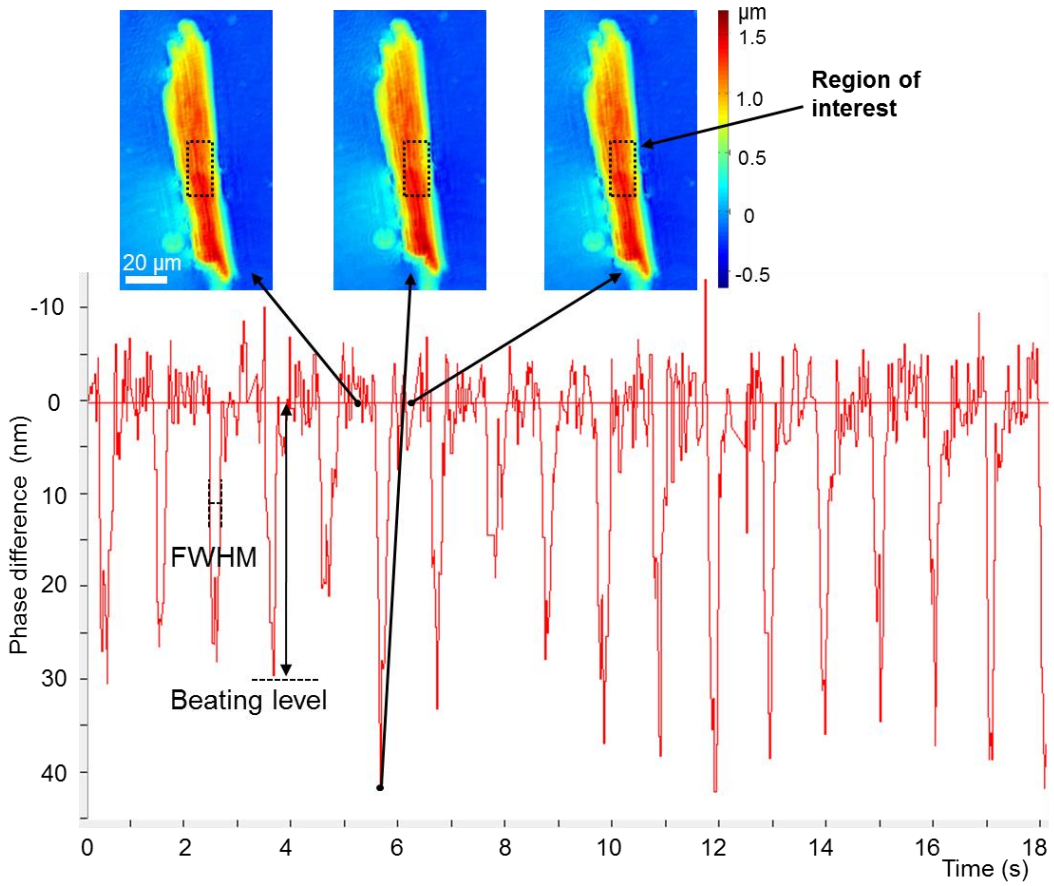

Figure S4. Time course data of the DHM phase monitoring for a cardiomyocyte. The surface beating level and duration FWHM are obtained from the phase difference of a ROI.

The multi-peak analysis for AFM "dwell" curve

Data extraction from the AFM "dwell" curves was performed using a multi-peak analysis package in the Igor software. The last 30 peaks were selected as the stable beating pattern and fit individually. As shown in **Figure S5**(a), the original curve (in the middle) was processed to obtain the fitting peaks (in the bottom) and deviation (in the top). The magnified graph was shown in **Figure S5**(b) and two representative peak statistics were shown in **Figure S5**(c). The "height" means the surface beating force.

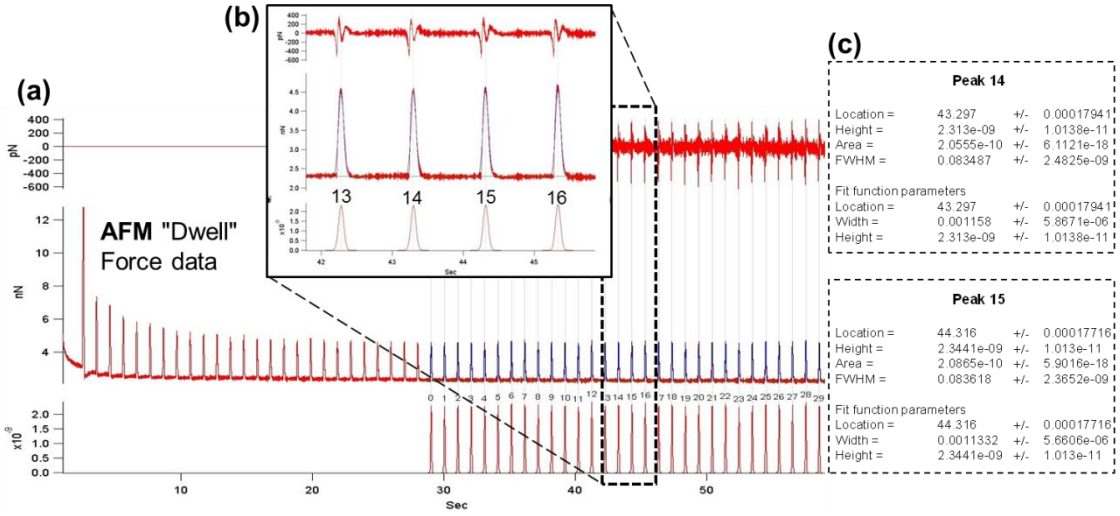

Figure S5. Time course data extracted from dwell curves performed by AFM. The multi-peak analysis of beating curves was performed to obtain the beating amplitude and duration FWHM.

Shape changes of cardiomyocytes during experiments

As additional data to show the drug-induced effects, the shape changes of single cardiomyocytes were evaluated. **Figure S6**(a) shows the representative optical micrographs of cardiomyocytes at different time points under various treatments. **Figure S6**(b) and c show the cell length and width measurement data respectively. Doxorubicin treated group had significant differences comparing with other groups. For the cell length, doxorubicin treated samples increased constantly, and "Dex - Dox" group maintained a stable level. Other groups decreased slowly. For the cell width, doxorubicin treated samples had the largest decrease. Mostly, other groups had less decreases and the control group maintained a relatively stable width. These data demonstrate that doxorubicin has significant effects on cell shape, which might indicate its different functionality on cell functions comparing with other groups.

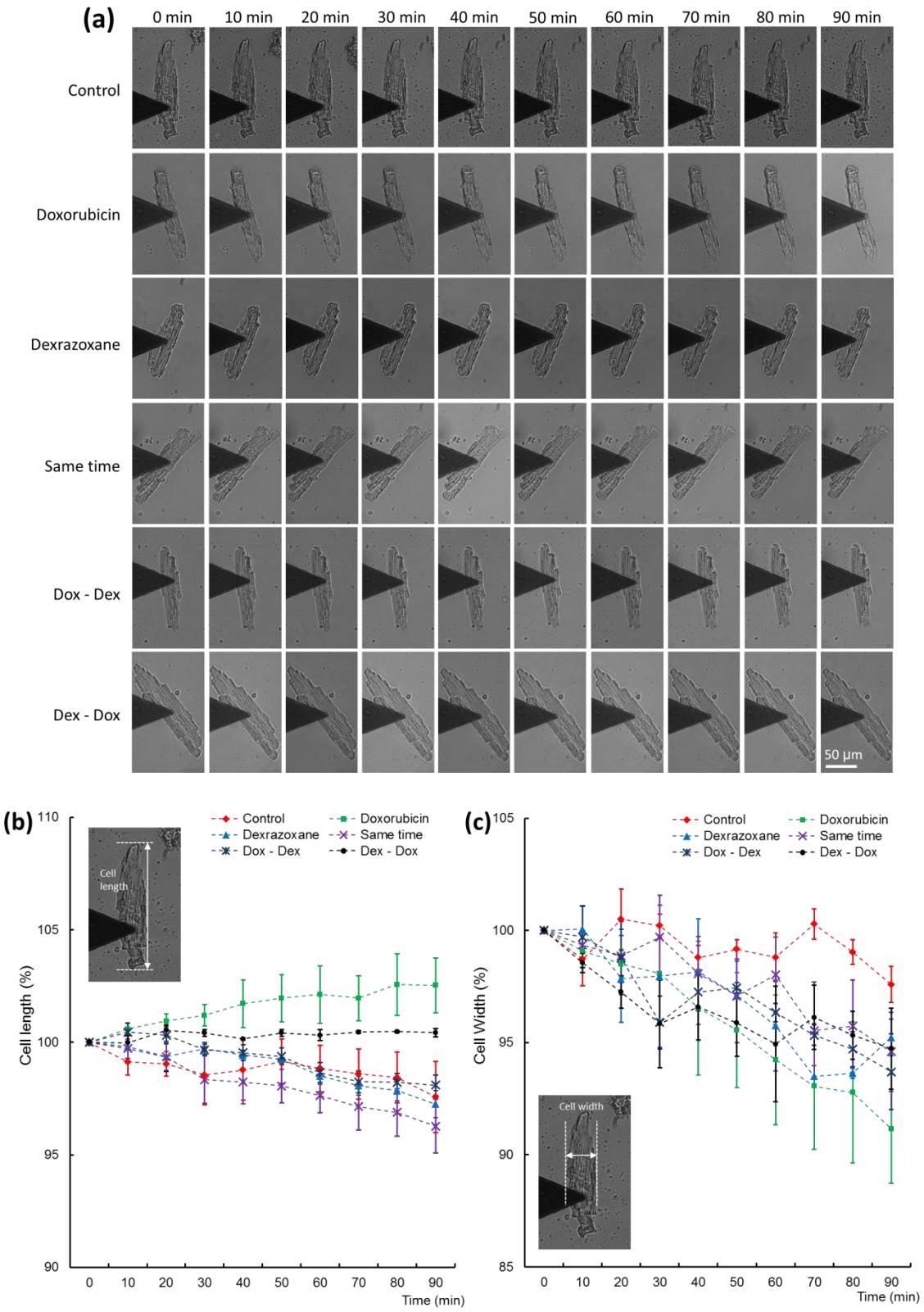

Figure S6. Shape changes of single cardiomyocytes during 90 min experiments. Doxorubicin treated group has significant differences comparing with other groups.