14

Increased fibroblast stiffness as a result of pharmacological activation of mechanosensitive Piezo1 ion channels

© M.M. Khalisov[1], V.A. Penniyaynen[1], A.V. Berintseva[1], S.A. Podzorova[1], G.G. Zegrya[1,2], B.V. Krylov[1]

[1] Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia
[2] Ioffe Institute, St. Petersburg, Russia
E-mail: khalisovmm@infran.ru

Received June 5, 2025
Revised September 16, 2025
Accepted September 17, 2025

The effects on cardiac fibroblasts of Jedi2 which is a pharmacological activator of mechanosensitive Piezo1 ion channels were studied. Using atomic force microscopy, it was found that Jedi2 at a concentration of 10 μM increases the cell stiffness. Immunofluorescence analysis showed an increase in the *F*-actin fluorescence intensity in cells at the above-specified concentration of Jedi2. Apparently, Jedi2 at a concentration of 10 μM activates Piezo1-mediated Ca^{2+}-dependent signaling cascades, which elevates the *F*-actin level and, hence, leads to an increase in the cell stiffness.

Keywords: Piezo1, Jedi2, fibroblasts, actin, atomic force microscopy.

DOI: 10.61011/TPL.2026.01.62829.20398

In 2021, D. Julius and A. Patapoutian were awarded the Nobel Prize for their discovery of the molecular structure of Piezo-family mechanosensitive ion channels (Piezo1 and Piezo2) [1]. Those channels modulate a number of important physiological functions and are of great interest in searching for potential targets for drugs necessary to correct emerging pathologies at the organism level, e.g. cardiovascular diseases; expression of those channels has been confirmed in cardiac cells, including fibroblasts [2,3].

Wide opportunities for studying Piezo1 channels have been opened up by the method of atomic force microscopy (AFM). It is based on the force interaction between the surface under study and a probe with a nanometer tip attached to the end of an elastic cantilever. Indentation with the atomic force microscope probe allows not only studying the living cells' mechanical properties but also mechanically stimulating them by a precisely controlled force. It was shown that at low forces (1–5 nN) stiffness of cardiac fibroblasts remains almost the same, while at higher forces (6–7 nN) the stiffness increases [4]. This result can be explained by the fact that reaching the threshold value of mechanical stimulation activates the mechanotransduction process leading to an increase in the stiffness of cardiac fibroblasts; at lower levels of mechanical force, this process is not activated. Thus, the initiation of mechanotransduction can induce changes in the cell mechanical properties. Mechanical properties of the cells are related to the composition and arrangement of their cytoskeleton [5]. Efficient studying of various cytoskeleton components may be provided by fluorescent analysis.

The mechanism of Piezo1-mediated mechanotransduction in cardiac fibroblasts remains as yet poorly studied. The understanding may be improved by studies involving substances that are specific modulators of these channels' activity, e.g. activator Jedi2 [6]. In this study, the effect of the Jedi2 activator on the cardiac fibroblasts mechanical properties and *F*-actin content was examined by using AFM and fluorescent staining with subsequent analysis.

The dissociated fibroblast culture was obtained from hearts of 10–12-day-old *White Leghorn* chicken embryos. Control cardiac fibroblasts were cultured for 5 days at 36.5 °C in 40 mm Petri dishes with a culture medium containing Dulbecco's modified Eagle's medium with a low amount of glucose (1 g/l), 10 % of fetal bovine serum and gentamicin (100 U/ml) and 5 % of CO_2 [7]. Jedi2 was added to fibroblasts at concentrations of 10 or 100 μM immediately prior to AFM examination. For the fluorescence analysis, cells were cultured in the presence of Jedi2 at the same concentrations for 3 h, since it was previously shown that at the concentration of 10 μM the maximum increase in fibroblast stiffness is reached after a 2–3 h exposure to Jedi2 [4].

Cardiac fibroblasts were studied by using atomic force microscope BioScope Catalyst (Bruker, USA) under physiologically relevant conditions, namely, in the culture medium at the temperature of 37 °C. To indent the cells, Bruker SNL-C cantilevers with the nominal probe tip radius of 2 nm were used. The cantilever spring constant was calibrated using the thermal tune method [8]. Operating mode of AFM PeakForce QNM was chosen so as to allow simultaneously obtaining the cell surface relief topography (Fig. 1, *a*) and distribution map of the apparent Young's modulus (Fig. 1, *b*). The apparent Young's modulus of fibroblasts was mapped using the Sneddon model (conical tip probe) [9]. Term „apparent" emphasizes that the signal formally describes the indentation force curve [10]. Values of the apparent Young's modulus were adjusted to take into account the influence of the solid substrate proximity [11]. All the cells were imaged with constant imaging parameters (Fig. 1).

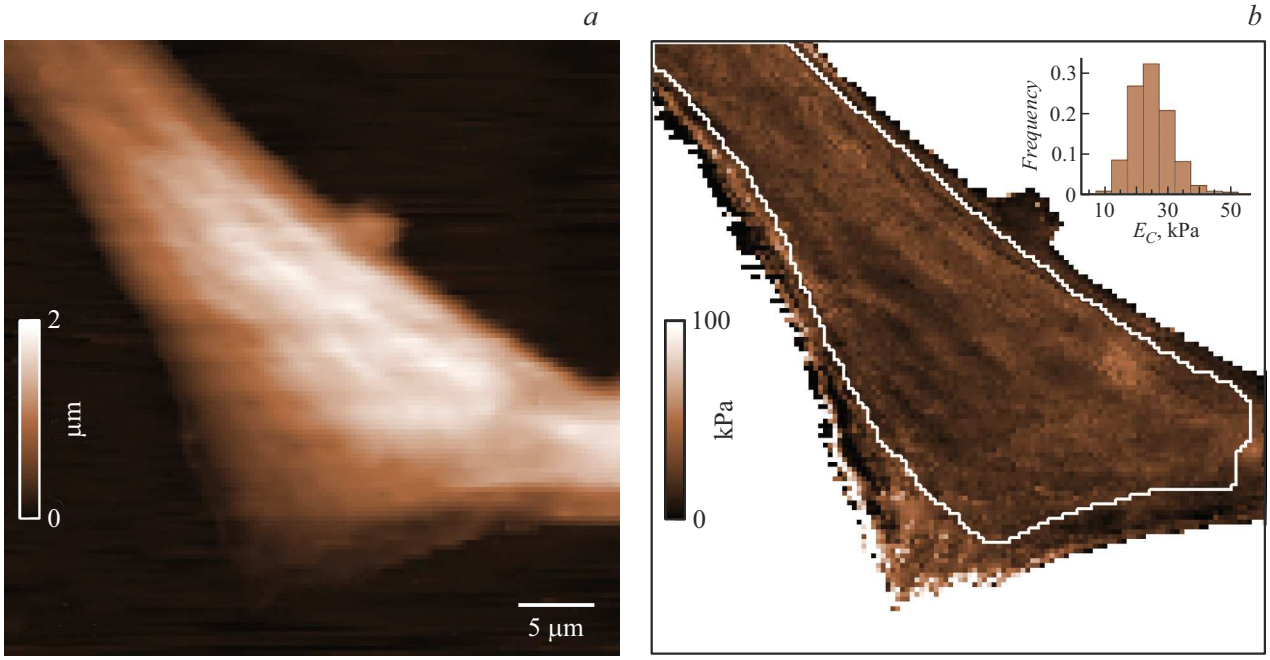

Figure 1. AFM images of a control fibroblast: surface relief topography (a) and map of apparent Young's modulus E_C (b). The white contour indicates the analysis area (the inset presents a histogram of the apparent Young's modulus distribution within the selected analysis area). The AFM imaging parameters are as follows: peak force setpoint — 3 nN, peak force amplitude and frequency — 1000 nm and 0.25 kHz, respectively, scan rate — 0.15 Hz, frame size of 128 × 128 pixels.

Based on the maps of corrected apparent Young's modulus E_C, each fibroblast was characterized by average E_C value. To examine the Jedi2 effect on the fibroblasts mechanical properties, average E_C values for cells exposed to the test substance were compared with those of control fibroblasts. AFM images were processed using program Gwyddion 2.58 [12].

Vital staining of cardiac fibroblasts was performed with a working phalloidin solution conjugated with fluorescent dye Texas Red®-X (Life Technologies, USA). Phalloidin is highly specifically bound to F-actin and allows imaging of the cell actin cytoskeleton.

Fluorescence analysis was performed with confocal laser scanning microscope LSM 710 (Carl Zeiss, Germany) integrated with inverted optical microscope Axio Observer Z1 (Carl Zeiss). The F-actin fluorescence intensity was determined using the ZEN2012 program (Carl Zeiss).

In each fibroblast, the F-actin fluorescence intensity was measured in three randomly selected regions of the same area ($3.6\,\mu m^2$); after that, average intensity was calculated for each cell. The fluorescence intensity distribution in the selected regions matched the normal distribution. The value averaged over the entire group of cells was calculated. The average fluorescence intensity of F-actin of control fibroblasts was taken as 100 %. The study was performed on the equipment of the Confocal Microscopy Collective Use Center (Pavlov Institute of Physiology).

Data analysis was performed in program STATISTICA 10.0 (StatSoft, USA) using the Student's t-test (fluorescence analysis) and Mann-Whitney U-test (AFM). Differences were considered statistically significant at $p < 0.05$.

We have studied the effect of Jedi2, which is a Piezo1 channel activator, on cardiac fibroblasts at the concentrations not affecting the cardiac explants growth ($10\,\mu M$) and completely inhibiting the explants growth ($100\,\mu M$) [4].

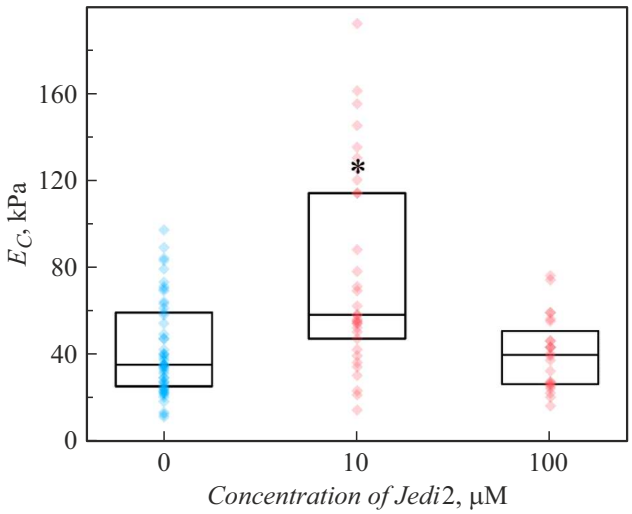

Figure 2. Apparent Young's moduli E_C of cardiac fibroblasts after exposure to Jedi2. The figure shows the median and range from the first to third quartile. The zero Jedi2 concentration corresponds to the control cell group. The number of examined cells was $n = 50$, 33 and 24 at the Jedi2 concentrations of 0, 10 and 100 μM, respectively. The asterisk corresponds to $p < 0.05$.

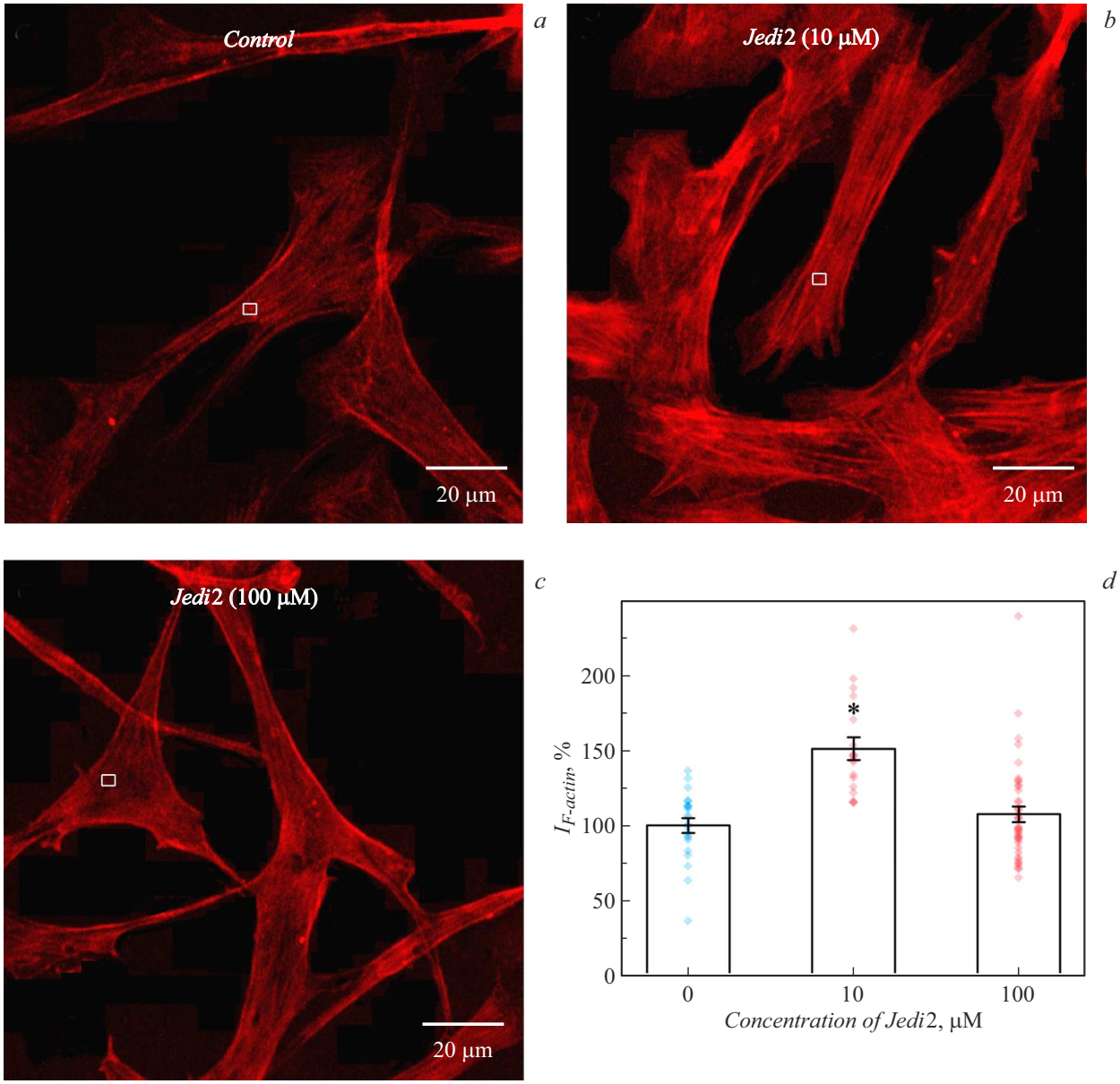

Figure 3. Visualization of the actin cytoskeleton in cardiac fibroblasts in control (a) and after exposure to Jedi2 (b, c). For staining, phalloidin with fluorescent dye Texas Red was used. The rectangle indicates the area of analysis. d — F-actin fluorescence intensity ($I_{F\text{-}actin}$) in cardiac fibroblasts after exposure to Jedi2. Data are presented as mean ± SEM. The zero Jedi2 concentration corresponds to the control cell group. The number of examined cells was $n = 22$, 41 and 18 at the Jedi2 concentrations of 0, 10 and 100 μM, respectively. The asterisk corresponds to $p < 0.05$.

Using AFM, we have found that, being exposed to Jedi2 at the concentration of 10 μM, fibroblasts exhibit an increase in the apparent Young's modulus with respect to the control value, while at the higher concentration (100 μM) the average apparent Young's modulus of the cells remains close to the control value (Fig. 2). The data obtained show that cardiac fibroblasts become stiffer due to exposure to Jedi2 at the concentration of 10 μM. Jedi2 at the higher concentration (100 μM) does not affect fibroblast stiffness.

The fluorescent analysis showed that the F-actin fluorescence intensity increases significantly at the lower Jedi2 concentration (10 μM) but remains almost equal to the control one at the higher concentration (100 μM) (Fig. 3). The results indicate an increase in the F-actin content in cardiac fibroblasts after exposure to Jedi2 at the concentration of 10 μM. At the same time, the higher substance concentration (100 μM) does not cause such an effect.

Activation of the Piezo1 channels by Jedi2 at the lower concentration initiates a cellular response which manifests itself in an increase in the fibroblast stiffness and elevation of the F-actin content relative to control cells. In contrast, Jedi2 at the higher concentration does not affect the fibroblast stiffness and F-actin level. The obtained results suggest that

the revealed increase in the cell stiffness induced by the test substance at the concentration of $10\,\mu$M not affecting the growth of cardiac explants is associated with enhancement of actin polymerization due to Piezo1-mediated Ca^{2+}-dependent signaling cascades, since these channels are permeable to Ca^{2+} ions [2]. Note that another Piezo1-channel chemical activator Yoda1 is also capable of enhancing the actin microfilament polymerization [13]. Thus, pharmacological activation of Piezo1 channels may result in an increase in the *F*-actin content, which leads to an increase in cell stiffness. Fibroblast culturing in the presence of Jedi2 at the concentration of $100\,\mu$M apparently results in overactivation of the Piezo1 channels and, hence, in triggering Ca^{2+}-dependent signaling pathways not associated with the actin polymerization. Overloading with Ca^{2+} ions is known to trigger cascades that ultimately lead to cell death [14]. The possibility of such a result may be indicated by inhibition of growth of explants by Jedi2 at the concentration of $100\,\mu$M [4]. However, testing this hypothesis needs further research.

The effect of the cell stiffness increase due to exposure to Jedi2 at the concentration of $10\,\mu$M is similar to that caused in fibroblasts by mechanical stimulation with an AFM probe with a high force of $6-7$ nN [4]. Therefore, we may assume that the effect of Jedi2 at the mentioned concentration on the Piezo1 channels leads to an increase in the cell sensitivity to mechanical stimulation from the AFM probe; already at the 3 nN force used in the experiments there is observed a cellular response in the form of an increase in the cardiac fibroblasts stiffness. However, the revealed increase in the *F*-actin content caused, apparently, by activation of Ca^{2+}-dependent cascades mediated by Piezo1 in response to Jedi2 alone suggests that fibroblast stiffness may be increased even in the absence of mechanical stimulation. Thus, the study of chemical activation of Piezo1 channels is important for understanding their physiological role and promotes a deeper understanding of regulation mechanisms for these channels. The data obtained in this study contribute to understanding of the effect on cardiac fibroblasts of Jedi2 which is a substance that may have potential for practical application in the treatment of cardiovascular pathologies. Note also that the approach used in this work may be applied to studying the functioning of Piezo1 channels in cells of other types.

Funding

The study was supported by the State funding allocated to the Pavlov Institute of Physiology Russian Academy of Sciences (No 1021062411787-0-3.1.8).

Compliance with ethical standards

All the procedures complied with ethical standards approved by the Russian Federation legal acts and recommendations of the Bioethics Committee of the Pavlov Institute of Physiology of RAS.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] *Nobel Prize in Physiology or Medicine 2021*. [Electronic source]. https://www.nobelprize.org/prizes/medicine/2021/summary/
[2] B. Coste, J. Mathur, M. Schmidt, T.J. Earley, S. Ranade, M.J. Petrus, A.E. Dubin, A. Patapoutian, Science, **330** (6000), 55 (2010). DOI: 10.1126/science.1193270
[3] N.M. Blythe, K. Muraki, M.J. Ludlow, V. Stylianidis, H.T.J. Gilbert, E.L. Evans, K. Cuthbertson, R. Foster, J. Swift, J. Li, M.J. Drinkhill, F.A. van Nieuwenhoven, K.E. Porter, D.J. Beech, N.A. Turner, J. Biol. Chem., **294** (46), 17395 (2019). DOI: 10.1074/jbc.ra119.009167
[4] M.M. Khalisov, A.V. Berintseva, S.A. Podzorova, B.V. Krylov, V.A. Penniyaynen, Integrativnaya fiziologiya, **5** (1), 50 (2024). DOI: 10.33910/2687-1270-2024-5-1-50-59 (in Russian)
[5] R. Emig, W. Knodt, M.J. Krussig, C.M. Zgierski-Johnston, O. Gorka, O. Groß, P. Kohl, U. Ravens, R. Peyronnet, Cells, **10** (3), 663 (2021). DOI: 10.3390/cells10030663
[6] Y. Wang, S. Chi, H. Guo, G. Li, L. Wang, Q. Zhao, Y. Rao, L. Zu, W. He, B. Xiao, Nat. Commun., **9** (1), 1300 (2018). DOI: 10.1038/s41467-018-03570-9
[7] M.M. Khalisov, V.A. Penniyaynen, S.A. Podzorova, K.I. Timoshchuk, A.V. Ankudinov, B.V. Krylov, Tech. Phys., **65** (11), 1853 (2020). DOI: 10.1134/S106378422011016X.
[8] J.L. Hutter, J. Bechhoefer, Rev. Sci. Instrum., **64**, 1868 (1993). DOI: 10.1063/1.1143970
[9] I.N. Sneddon, Int. J. Eng. Sci., **3** (1), 47 (1965). DOI: 10.1016/0020-7225(65)90019-4
[10] K.I. Timoshchuk, M.M. Khalisov, V.A. Penniyaynen, B.V. Krylov, A.V. Ankudinov, Tech. Phys. Lett., **45** (9), 947 (2019). DOI: 10.1134/S1063785019090293.
[11] P.D. Garcia, R. Garcia, Biophys. J., **114**, 2923 (2018). DOI: 10.1016/j.bpj.2018.05.012
[12] D. Nečas, P. Klapetek, Open Phys., **10** (1), 181 (2011). DOI: 10.2478/s11534-011-0096-2
[13] V.I. Chubinskiy-Nadezhdin, V.Y. Vasileva, I.O. Vassilieva, A.V. Sudarikova, E.A. Morachevskaya, Y.A. Negulyaev, Biochem. Biophys. Res. Commun., **514** (1), 173 (2019). DOI: 10.1016/j.bbrc.2019.04.139
[14] S. Orrenius, B. Zhivotovsky, P. Nicotera, Nat. Rev. Mol. Cell Biol., **4** (7), 552 (2003). DOI: 10.1038/nrm1150

Translated by EgoTranslating