Effects of Electrical Stimulation on Proteins Related to Signal Transduction, c-Src and Focal Adhesion Kinase in Fibroblasts

Kazuo Katoh

Laboratory of Human Anatomy and Cell Biology, Faculty of Health Sciences, Tsukuba University of Technology, Japan;
katoichi@k.tsukuba-tech.ac.jp

CELLS 2023 Cells, Cells and Nothing but Cells: Discoveries, Challenges and Directions
06–08 March 2023 | online
Chaired by Dr. Alexander E. Kalyuzhny
cells MDPI

Abstract

In the field of acupuncture, electrical stimulation of the skin and muscles is known to locally increase blood flow and metabolism, and maintain the body in a sustained healthy state. However, little is known about the changes in cellular morphology and the localization of specific proteins associated with electrical stimulation. In this study, we analyzed the effects of electrical stimulation on the cytoskeletal system in fibroblasts.

When electrical stimulation was applied to cells for about 1 hour, the stress fibers (SFs) in the cells became thicker and the cells showed contraction. When the cells were subjected to periodic electric current for 20 hours, the SFs increased in thickness. In addition, the focal adhesion (FA) increased after 2 hours of continuous stimulation, and both the SFs and the FAs became thicker and larger after 20 hours of continuous stimulation. Staining of the cells after electrical stimulation with anti-phosphotyrosine antibody showed enhanced staining in FAs. The intensities of staining for focal adhesion kinase (FAK) and activated c-Src were also enhanced, indicating that signal transduction-related proteins were affected by electrical stimulation.

Methods

In this study, we used cultured fibroblasts (NIH 3T3) that were thought to receive electrical stimulation from acupuncture. The cells were subjected to single-pulse electrical stimulation using an electrical stimulator (SEN-2201; Nihon Kohden), and the morphological changes in the cells were examined by fluorescence microscopy. The device used to apply the electrical stimulation to the cultured cells was made using a circuit board and platinum wire. The pattern and duration of electrical stimulation were adjusted for the cells in the culture system because the culture environment was different from that of cells in vivo. Based on our preliminary research, the cells were stimulated at 50 volts 60 times per minute in a cyclic manner. Morphological changes in the cells and changes in tyrosine-phosphorylated proteins were analyzed in the cells exposed to electrical stimulation, and the results were compared with unstimulated controls. In particular, the intracellular skeletal structures, stress fibers, and focal adhesions are expected to be involved in the transmission of various cellular stimuli that connect the outside environment to the inside of the cell. Based on the hypothesis that stress fibers and focal adhesions function as receptors for various stimuli, we focused on the localization and morphological changes of the proteins after electrical stimulation.

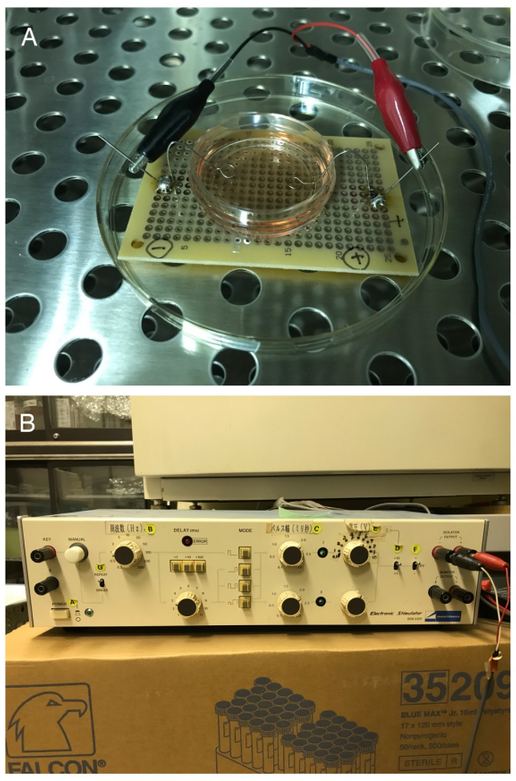


Figure 1. Apparatus for providing single-pulse electrical stimulation to cells and simple stimulation device.
We developed a device using an electronic circuit and platinum wire to apply electrical stimulation to cultured cells, and confirmed that it could apply electrical stimulation to the cells (A). The morphological changes in the cells were examined under a microscope by applying electrical stimulation with a simple simulator (SEN-2201) (B).

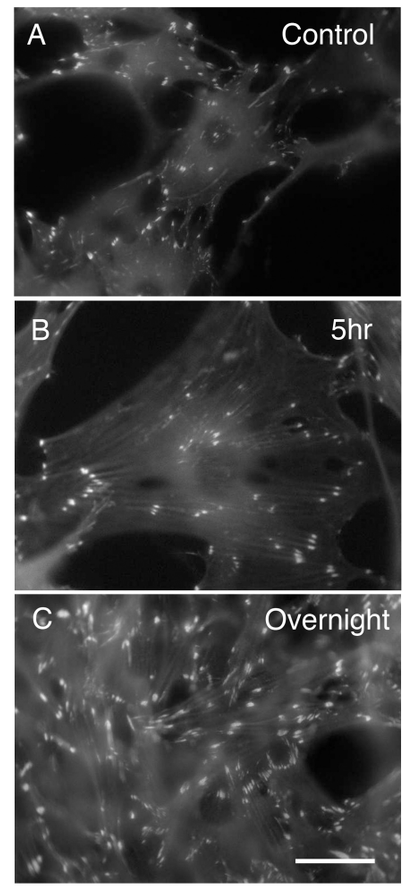


Figure 2. Changes in focal adhesions after electrical stimulation of cells.
Fibroblasts were transfected with GFP-paxillin, and the focal adhesions were examined after periodic electrical stimulation. After 20 hours of periodic electrical stimulation, focal adhesions became larger (A: control; B: 5 hours; C: control): Bar: 20 µm.

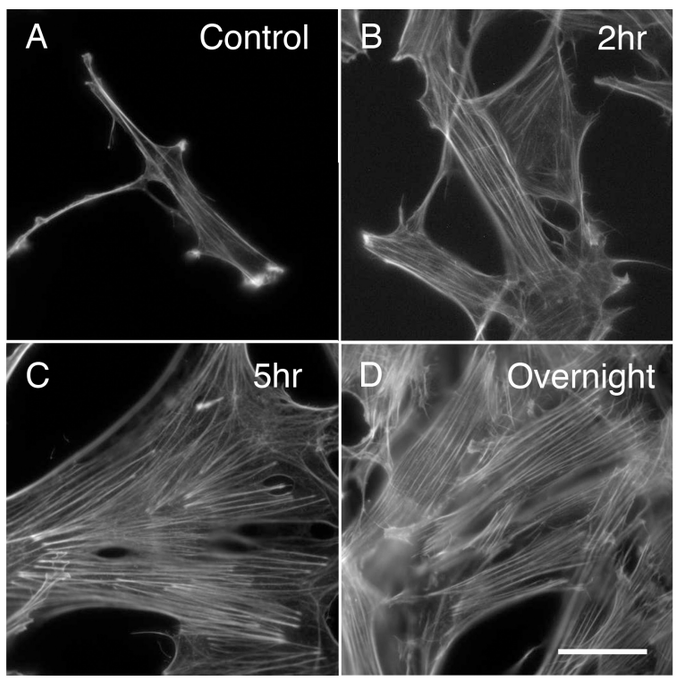


Figure 3. Changes in stress fibers during electrical stimulation of cells.
The cytoskeletal structure (stress fibers and focal adhesions) of the fibroblast cells was significantly altered with application of periodic electrical stimuli of 50 volts at 60 times/min. Electrical stimuli were applied to the cells for 30 minutes, 1 hour, 2 hours, 6 hours, and 20 hours (overnight). The stress fibers began to increase in number after 30 minutes to 1 hour of electrical stimulation. After about 2 hours of electrical stimulation, the stress fibers became thicker, and the cells were observed to contract. After 20 hours of periodic electrical stimulation, the number of stress fibers remained the same, but the thickness of the fibers increased (A: control; B: 2 hours; C: 5 hours, D: overnight). Bar: 20 µm.

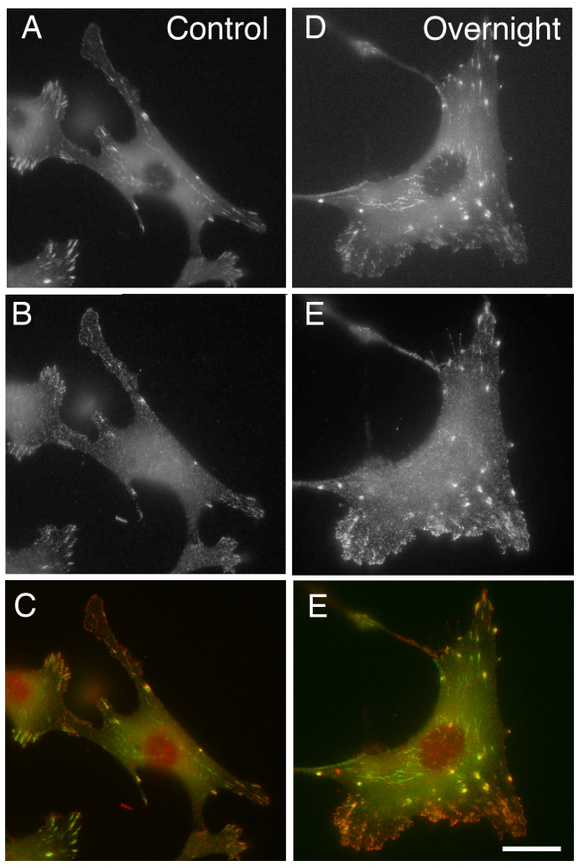


Figure 4. Increase in focal adhesions and accumulation of tyrosine-phosphorylated proteins by electrical stimulation.
After 2 hours of electrical stimulation of GFP-paxillin-transfected cells, the focal adhesions started to enlarge, and after 20 hours of periodic electrical stimulation, focal adhesions showed intense staining with anti-phosphotyrosine antibody (PY-20), indicating that the focal adhesions were tyrosine-phosphorylated.
A – C: Control (no electrical stimulation)—A: GFP-paxillin. B: Anti-phosphotyrosine antibody (PY-20). C: Merge. D – F: 20 hours of electrical stimulation—D: GFP-paxillin. E: anti-phosphotyrosine antibody (PY-20). F: Merge. Bars: 20 µm.

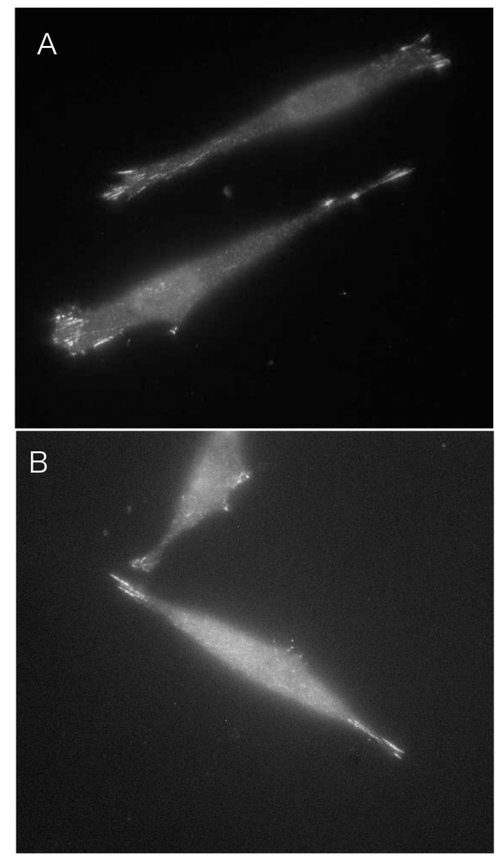
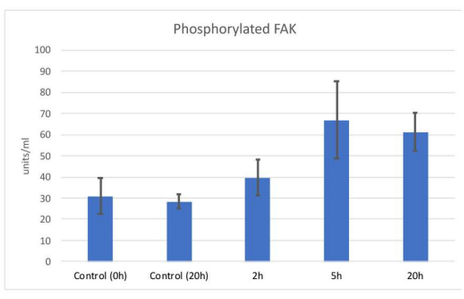


Figure 5. Staining of the active form of FAK and c-Src in cells with periodic electrical stimulation.
Cells stimulated for 20 hours were stained with anti-phosphorylated FAK (pY397) antibody (A) or anti-phosphorylated c-Src (pY418) antibody (B). Intense staining for both tyrosine-phosphorylated proteins was observed in focal adhesions. Bar: 20 µm.

A



B

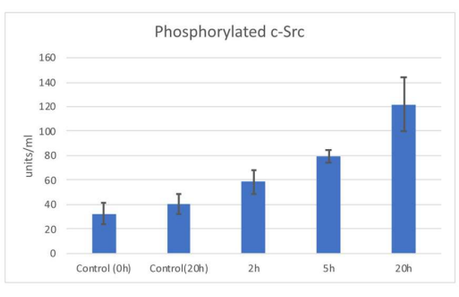


Figure 6. Changes in phosphorylated proteins, tyrosine-phosphorylated FAK (A), and tyrosinephosphorylated c-Src (B) were examined by ELISA.
Both the tyrosine-phosphorylated FAK (A) and tyrosine-phosphorylated c-Src (B) levels increased gradually according to the electrical stimulation time. The level of FAK phosphorylation remained almost the same between 5 and 20 h of electrical stimulation (A). The level of tyrosine-phosphorylated c-Src increased by about 3.7-fold compared to controls at 20 h of electrical stimulation (B).

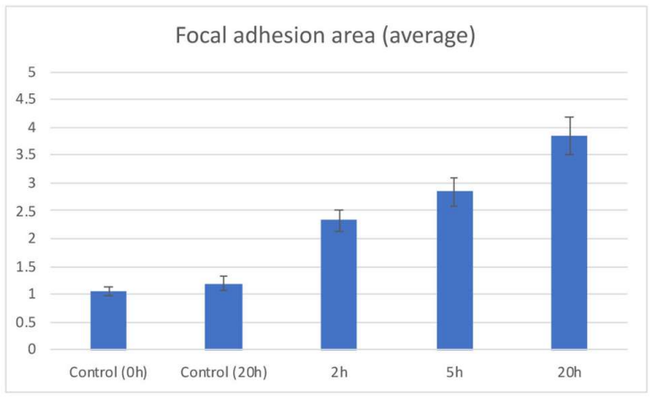


Figure 7. Quantification of focal adhesion area.
The mean areas of focal adhesions are shown for the unstimulated control (Control 0 h), unstimulated control without electrical stimulation for 20 h (Control 20 h) 2, 5, and 20 h of electrical stimulation. Number of vertical bars is area of the focal adhesions (m2). Error bars represent the SEM.

Summary

The morphological changes in cultured cells with application of electrical stimulation were clarified. After 2 hours of electrical stimulation, the stress fibers, which are the contractile apparatus distributed in the cells, became thicker, confirming that the cells were contracting. After 20 hours of periodic electrical stimulation, the number of stress fibers did not change, but their thickness increased. In addition, the focal adhesions became larger after 2 hours of electrical stimulation, and both the stress fibers and focal adhesions became thicker and larger after 20 hours of periodic electrical stimulation.

References

Katoh, K. Effects of Electrical Stimulation of the Cell: Wound Healing, Cell Proliferation, Apoptosis, and Signal Transduction. *Med. Sci.* **2023**, *11*, 11. https://doi.org/10.3390/medsci11010011

Katoh, K. Effects of Electrical Stimulation on the Signal Transduction-Related Proteins, c-Src and Focal Adhesion Kinase, in Fibroblasts. *Life* **2022**, *12*, 531. https://doi.org/10.3390/life12040531

Katoh, K. FAK-Dependent Cell Motility and Cell Elongation. *Cells* **2020**, *9*, 192. https://doi.org/10.3390/cells9010192

National University Corporation
Tsukuba University of Technology