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Electrically Modulated Cellular Biodevices

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Living cells contain a whole set of intracellular information networks, including signal transduction pathways that control gene expression, intracellular ion influxes and cellular excitability. Stimulations by extracellular molecular messenger or physical stimuli activates these intracellular information networks, modulate physical functions. According to the special characteristics cells could be used as a part of a biological information network within biodevices. Recently, the studies about bioelectronic access to such biological informations as cellular and biomolecular signals were accelerated. This paper describes new concepts of electrochemical methods using cell-based biodevices.

Key Words : Electrical stimulation, Cellular biodevice, Gene expression, Tissue alternatives

1. Introduction

Recently living cells are highlighted as special biomaterials for synthesizing recombinant proteins, hormones, and physiologically active substances *in vivo* and *in vitro*. Especially stem cells, having differential potential, are key materials for tissue engineering and cellular therapy. The other perspective, living cells contain a whole set of intracellular information networks, including gene information networks and signal transduction pathways. On the base of the biological information networks, living cells have successfully been implemented as a unit of biological information networks in biodevices.

To use living cells as biodevices, it is important to construct a control method for drawing out the specific function including cells. Extracellular, molecular messengers such as hormones, cytokines and neurotransmitters are usually recognized by receptors embedded on the cell membrane surface, resulting in the activation of the intracellular information networks mostly to modulate the gene information networks. In addition, it was found that cellular responses to environmental stresses have gained keen attention. Recently the cellular responses to physical stresses, for example to heat, light, electronic or electro-magnetic field, mechanical stresses and so on, are investigated and found that various signal transdaction pathways were activated by stimulating these influence. These results may suggest that some type of physical stress could modulate living cell's function as same as molecular messengers.

In our studies, we have found that the electric stimulation, one of the physical stresses, modulates cellular function. By applying slight heat or an electrical potential to cells cultured on an electrode surface, cellular function, such as the rate of proliferation or protein synthesis, can be reversibly modulated (Figure 1). Based on these findings, we have deveroped a new concepts of cell-based biodevices for assessing environmental stresses or as tissue alternatives.

2. Experimental methods

2-1 Thermal stimulation of cultured cells

Mouse astroglial cells, 3T3-L1 cells, N1E-115 cells and PC12 cells were grown in six-well tissue culture dishes in DMEM supplemented with 10% donor horse serum, 100U/ml penicillin and 100 μg/ml streptomycin. The cells were grown for 1 to 3 days at 37℃ in a water saturated air environment containing 5% CO_2. For thermal stimulation, the cultured dishes were placed in the incubator at 42℃. After 1 h they were returened to 37℃ incubator.

2-2 Electrical stimulation of cultured cells]

Each kind of animal cell was cultured on the surface of an optically transparent electrode, which was fixed at the bottom of a culture dish (Figure 2). A counter electrode and an Ag/AgCl

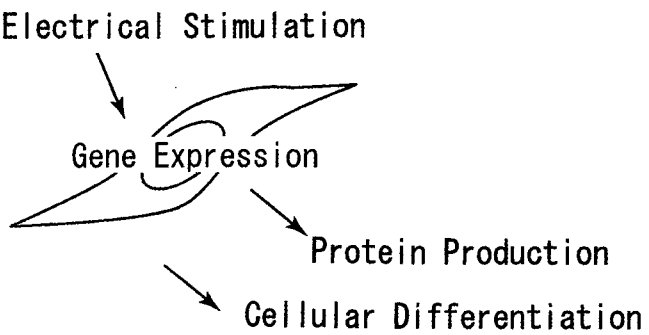


Figure 1. Diagram of electrical modulation of animal cell functions.

reference electrode was also installed in the culture dish, which was filled with a culture medium. A potentiostat set the electrode potential at a fixed value with respect to the reference electrode. Cells were cultured for several days in serum-free Dulbecco's modified eagle medium (DMEM) and then subjected for another 1 h culture along with the application by 10Hz of sine wave electrical potential at varying voltages. After the electrical stimulation, the cells were cultured for reasonable time to analyze the effects of electrical stimulation.

3. Results and discussion

3-1 electrical production of NGF

In our study of the electric effects to animal cell, one of the most exciting findings involves astroglial cells in the brain[1], which support neuronal cells by secreting nerve growth factor (NGF). In primary cultures of astrocytes, it was investigated that NGF gene expression and secretion are activated by various factors, including glial growth factors and cytokines. As an alternative to NGF stimulation by chemical growth factors, we have developed an electrically controlled culture system (Figure 3(a)).

Astroglial cells were cultured for seven days in serum-free Dulbecco's modified eagle medium (DMEM) were subjected for 1 h culture along with the application by 10Hz of sine wave electrical potential at varying voltages. After the electrical stimulation, the cells were cultured for another 24 h, the levels of secreted and intracellular NGF were determined. Intracellular levels of NGF remained unchanged by electric stimulation. In contrast, NGF secretion was extremely enhanced, reaching a maximum at an upper potential of +0.3V.

The level of NGF, *c-fos*, and *c-jun* mRNA in the electrically stimulated astroglial cells were quantified by competitive reverse transcription polymerase chain reaction (RT-PCR) assay[2]. The results clearly show that *c-fos, c-jun* and NGF gene expression are enhanced in astroglial cells (Figure 3(b)). This suggest that the electrically stimulated NGF expression and excretion is triggered by the protein kinase C-AP-1 pathway.

3-2 hsp70 promoter activation by thermal and electric stimulation

In the further investigation, hsp (heat shock protein) 70 promoter was found activated by the low frequency of electric stimulation (Figure 4(a)), which led us to design an electrically responsive cells by gene engineering[3]. The level of hsp70 mRNA was measured in the electrically stimulated astroglial cells by RT-PCR assay. As shown in Figure 4(b), we found that hsp70 gene expression was activated by electrically stimulatior. Such an electrically responsive promoter plays a key role on designing cells to be used in biodevices. A gene of interest is linked with hsp70 promoter to form a plasmid, and is inserted in a cell. The engineered cells become electrically responsive, which results in expression of the gene of interest on call by electric stimulation. The hsp70 promoter is electrically activated at first, which is followed by expression of the gene of interest (Figure 5).

To test this hypothesis, we linked firefly luciferase gene to the hsp70 promoter, and inserted it into a mouse 3T3-L1 cells[3]. These cells were cultured on ITO glass electrode surface and applied the 10 Hz frequency, 0-300mV sine wave potential for 1h. As the result, electric stimulation of the cells resulted in activation of luciferase expression, then confirmed to produce an increase in light emission in the luciferase assay. Furthermore, the promoter was activated depending on the potential and duration of the stimulation applied. From these results, this engineered cell line act as a biomaterial where in electrically stimulation induced light.

Next we investigated the regulation of neural differentiation by electric stimulated gene expression of NeuroD2, which is one of

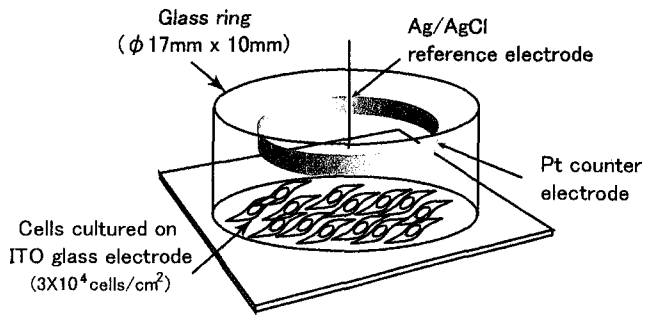


Figure 2. Diagram of an electrically controlled culture system.

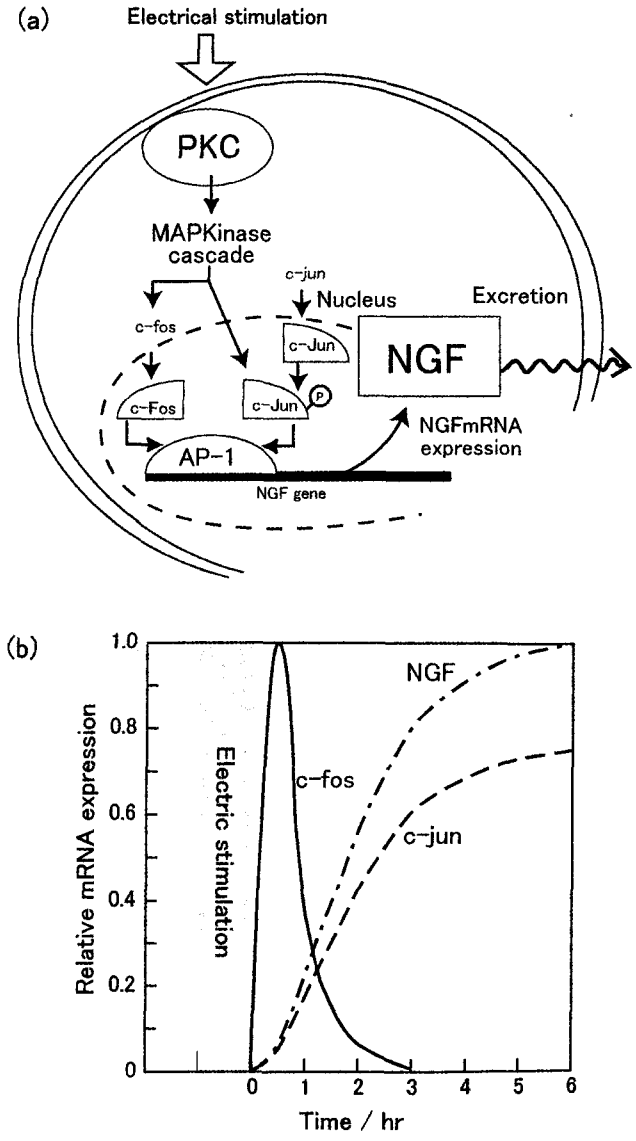


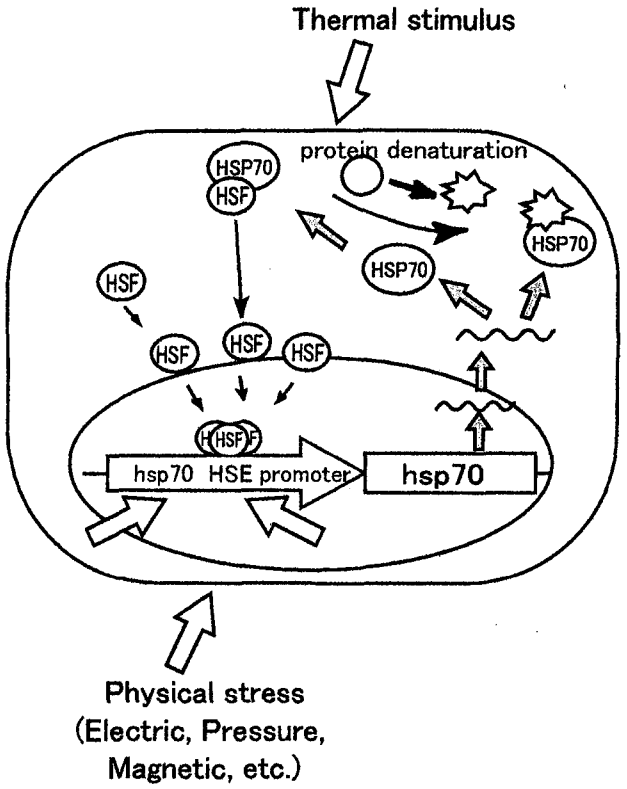
Figure 3. (a) Electrically stimulated activation of astrocytes in NGF gene expression. (b) Time courses of NGF, *c-jun* and *c-fos* mRNA after electric stimulation.

the neural bHLH transcription factors and be able to convert mouse N1E-115 neuroblastoma cells into differentiated neurons[4]. N1E-115 cells were stably transfected with expression vector containing mouse NeuroD2 cDNA under the control of hsp70 promoter. The stably transfected cells were cultured on the electrode surface. Based on cell morphology, we have found that electrical stimulation, induced NeuroD2 expression and neural differentiation.

Using property of hsp70 promoter activation to be synchronized by electrical stimulation, we also investigated whether we could use the hsp70 promoter to construct the protein production system. We constructed an expression vector containing secreted alkaline phosphate (SEAP) cDNA under the control of hsp70 promoter. When transfected C2C12 myoblast cells were cultured under heat shock condition, (42℃, 90min), the activity of SEAP contained in culture medium was increased at 24 h after stimulation. SEAP activity was also increased at 24 h after an electric stimulation. Based on these results, the engineered cells can be used to design cellular biodevices for use as tissue alternatives and as electrically controlled drug excretion on demand.

3-3 Regulation of PC12 cell functions by altering influx of calcium ion

PC12 cell is a cell line derived from pheochromocytoma in the rat adrenal medulla, and differentiates like sympathetic nervous



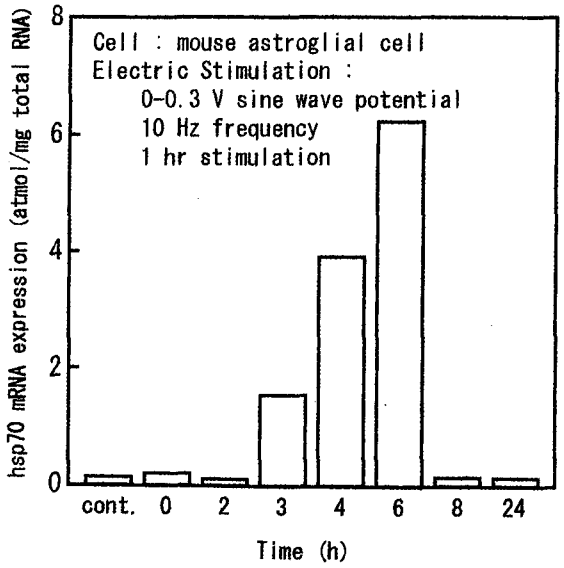


Figure 4. (a) Diagram of activation of cellular hsp70 function by thermal or physical stress. (b) *hsp70* mRNA increased by electrical stimulation in astroglial cells.

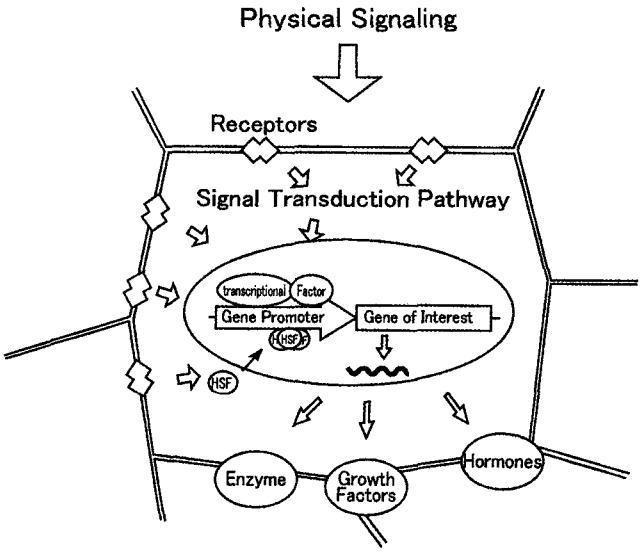


Figure 5. Construction of cellular biodevices. Physical signals induce specific recombinant gene expression and protein production.

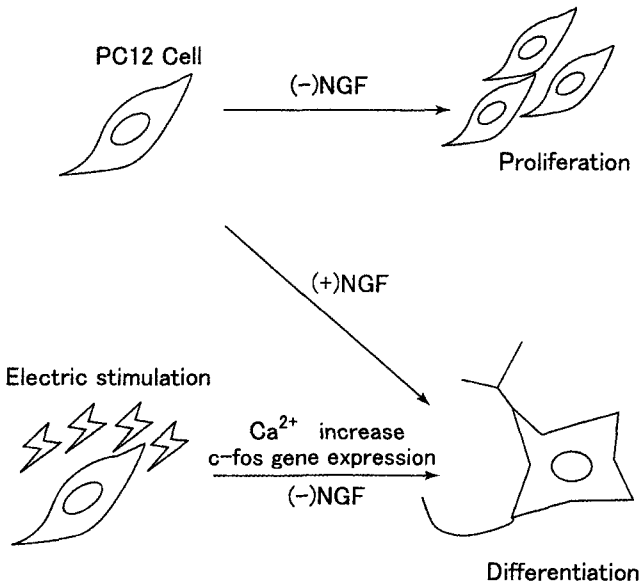


Figure 6. Electrical stimulation promotes the differentiation of PC12 cells.

cells with NGF treatment, extending long neurites that have been used as a good marker to investigate its differentiation. PC12 cells were subjected to a peak-to-peak electrical potential of 100mV with a frequency of 100Hz for 30min every 24 h, for 3 days. These cells were then incubated for additional 2 days under normal culturing conditions. We found that the PC12 cells were changed their morphology (Figure 6), causing them to grow neurites in the absence of NGF in the cultured medium[5]. And the level of *c-fos* mRNA, the gene marker of differentiation, was increased, which was essential for differentiation of PC12 cells[6]. From the fluorescent measurement of intracellular calcium ion, the electrical stimulation caused a gradual increase in the level of intracellular calcium ion in PC12 cells, which is known to be required for potassium ion-induced differentiation. Moreover, the electrically induced differentiation would require a calcium influx via an L-type channel, such as SA channels because the SA channel blocker perfectly inhibited the electrically stimulated calcium influx and differentiation.

Exocytosis in PC12 cells was also induced by electric stimulation[7]. This allowed direct visualization of dense-core granules in living cells. Transfected PC12 cells expressed fusion protein of neuropeptide Y (NPY) and enhanced green fluorescent protein (EGFP) were constructed. Based on the change of fluorescence distribution at the tip of nurite of transfected PC12 cells, we found that constant potential of -300mV could regulate the movement of dense-core granules at the neurite of PC12 cells. Therefore the electric stimulation could be a useful tool for promoting the secretion of physiologically active substances.

4. Conclusion

In this paper, we showed that electrical stimulation can modulate cellular function and this can be applicable to the design of cell-based biodevice. The electrically stimulated induction of NGF, hsp70 gene expression was processed through the intracellular signal transduction cascades to activate the downstream of transcriptional factors, for example AP-1 constructed by *c-fos, c-jun* gene products. This approach for controlling gene expression by applying a slight alternating potential is promising for use in a wide variety of applications, including cellular, tissue and medical engineering.

Reference

1. S. Koyama, T. Haruyama, E. Kobatake and M. Aizawa; "Electrically induced NGF production by astroglial cells" *Nature biotechnol.*, 15, 164-166 (1997)
2. S. Koyama, Y. Yanagida, T. Haruyama, E. Kobatake, and M. Aizawa; "Molecular mechanisms of electrically stimulated NGF expression and secretion by astrocytes cultured on the potential controlled electrode surface" *J. Cell. Eng.*, 1, 189-194 (1996)
3. Y. Yanagida, A. Mizuno, T. Motegi, E. Kobatake and M. Aizawa; "Electrically Stimulated Induction of hsp70 Gene Expression in Mouse Astroglia and Fibrobrast Cells" *J.Biotechnol.*, 79, 53-61 (2000)
4. M. Mie, H. Ohgushi, Y. Yanagida, T. Haruyama, E. Kobatake and M. Aizawa; "Osteogenesis Coordinated in Stromal Stem Cells by Adipogenesis Dependent BMP-2 Expression System" *Tissue Eng.*, 6(1), 9-18 (2000)
5. K. Kimura, Y. Yanagida, T. Haruyama, E. Kobatake, and M. Aizawa; "Electrically Induced Neurite Outgrowth of PC12 Cells on the Electrode Surface" *Med. Biol. Engin. Comp.*, 36 (4) 493-498 (1998)
6. K. Kimura, Y. Yanagida, T. Haruyama, E. Kobatake, and M. Aizawa; "Gene Expression in the Electrically Stimulated Differentiation of PC12 Cells" *J.Biotechnol.*, 63, 55-65 (1998)
7. A. Mizuno, M. Mie, Y. Yanagida, M. Aizawa, E. Kobatake; "The secretory response through electric stimulation of differentiated PC12 rat pheochromocytoma cells transfected with neuropeptide Y fused with enhanced green fluorescent protein" *Biotechnol. Lett.*, 25, 547-552 (2003)