Frequency change-induced alternative potential waveform dependence of membrane damage to cells cultured on an electrode surface

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Abstract

In the present study, alternative potential stimulation with rectangular pulse, sine and triangular wave forms at 10 and 100 Hz was applied to cells cultured on an ITO electrode. As a result, we found that the alternating potential waveform dependence induced by the frequency on membrane damage of cells cultured on an electrode surface. The cell membrane damage was promoted by a rectangular pulse wave in comparison with sine and triangular waves, when alternating electrical potentials of 0 ~ +1.0 V at 100 Hz were loaded. In contrast, this wave form dependence was not observed when the frequency was 10 Hz. Furthermore, it was found that cell membrane damage was induced at positive potentials more than +0.8 V under the present experimental conditions.

Keywords

Cell; Electrode; Stimulation; Alternative potential; Frequency; Membrane; Damage

1. Introduction

The electrical stimulation of living cells has been attracting increasing interest in the field of cell manipulation. A variety cellular responses elicited by electrical stimulation have been reported. Changes in the membrane structure, permeability and breakdown of the cell membrane are induced by high voltage pulsed stimulation (Powell et al. 1986). It has been demonstrated that cells suspended in solution are easily fused using high voltage pulses (Gerg et al. 1999; Kurischiko et al. 1986; Zimmermann et al. 1982). By using low potential pulses loading, changes in cellular behavior have been observed. It has been demonstrated that the electrical regulation of cell proliferation can be achieved by constant potential loading (Aizawa et al. 1999; Greenberg et al. 1985; Kojima et al. 1991; Kojima et al. 1992, 1996; Yaoita et al. 1990), electrically-activated gene expression for nerve growth factor, *c-fos* and *c-jun* mRNA expression can be induced by the loading of alternative

potentials (Koyama et al. 1996, 1997; Mie et al. 1996), and neural and osteogenic differentiation can be induced by alternative potential stimulation (Kimura et al. 1998; Mie et al. 1996). Furthermore, the combination of electrical stimulation and cisplatin administration on HeLa cells has been investigated using alternative potential application (Manabe et al. 2004; Motohashi et al. 1996). Recently, we reported that the sensitivity to electrical stimulation was much higher in chronically human immunodeficiency virus (HIV-1) infected HeLa cells than in uninfected HeLa cells (Kumagai et al. 2004; Tominaga et al. 2003). This could be considered a novel and useful approach against HIV-1 infection.

The study of the cellular responses to alternative potential stimulation represent an attractive research field for not only a basic understanding of the cell, but also for cell engineering disease therapies. However, the basic cellular responses to electrical stimulation have not been characterized. In the present study, cell membrane damage caused by alternative potentials using rectangular pulses, sine and triangular wave forms loading was investigated. As a result, a waveform dependence induced by the frequency change of the alternative potential for causing cell membrane damage was discovered, for the first time.

2. Materials and methods

MAGI/CCR5 (HeLa-CD4-LTR-b-gal) cells were cloned from HeLa cells (Kimpton et al. 1992; Maeda et al. 2000). The MAGI/CCR5 cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL) supplemented with L-glutamine and 4.5 g dm^{-3} glucose (MP Biomedicals, Inc), and with 0.88 g dm^{-3} Eagle's MEM Amino acids (Nissui Pharmaceutical Co.), 2.0 g dm^{-3} $NaHCO_3$, 1 x 10^5 IU dm^{-3} penicillin, 0.1 g dm^{-3} streptomycin, and 10 % heat-inactivated fetal bovine serum (Gibco-BRL).

A schematic illustration of the experimental setup for the application of the alternating electrical potentials is shown in Fig. 1. A vacuum evaporated indium oxide electrode doped with tin (ITO, Kinoene Optics Co., Japan) was used as the working electrode with a sheet resistance of *ca.*

10 Ω cm^{-2}, which was an optically transparent plate (Kumagai et al. 2004; Tominaga et al. 2003, 1993). The ITO surface was adhered by a glass ring (36 mm in inner diameter, 20 mm in height). The electrode surface was cleaned by sonication in *ca.* 1 % aqueous New-Vista (AIC Corp.) solution, followed by rinsing with pure water and autoclaving. To cultivate the MAGI/CCR5 cells on the ITO electrode, the cells were scraped from a culture dish, and the 9 x 10^4 MAGI/CCR5 cells were seeded onto the ITO electrode surface (10.2 cm^2). The cells were then cultured for 3 days in a 5 % CO_2 atmosphere at 37 ˚C in the absence of electrical stimulation.

Alternating electrical potential was applied to the working electrode with a potentiostat (PS-06, Toho technical research, Japan) through a multifunction synthesizer (Wave Factory 1945, NF Electronic Instruments, Japan) that generated rectangular pulse, sine and triangular waves. An Ag/AgCl (saturated KCl) electrode and a platinum wire electrode were used as the reference and counter electrodes, respectively. All potentials are reported with respect to the Ag/AgCl (saturated KCl) electrode. Cell damage was evaluated by 0.4 % trypan blue dye exclusion for 7 min.

3. Results and discussion

Fig. 2 shows phase-contrast microscope photographs of MAGI/CCR5 cells on the electrode before and after loading alternating electrical potentials of rectangular pulse waves of 0 ~ +1.0 V at 10 Hz for 20 min. Before the electrical stimulation, lengthening and lamellipodia of the MAGI/CCR5 cells on the electrode surface were observed. On the other hand, after the electrical stimulation, a shrinkage of the lamellipodia was observed. The membrane damaged cells were stained by trypan blue. Fig. 3 shows the staining ratio of the MAGI/CCR5 cells as a function of the electrical stimulation loading time. Alternating electrical potentials of rectangular pulses, sine and triangular waves of 0 ~ +1.0 V at 10 Hz were applied to the cells. The staining ratio increased drastically after loading electrical stimulation for 8 ~ 10 min, and quickly reached almost 100 %. This behavior did not depend on the wave form of the alternating electrical potential.

Fig. 4 shows the staining ratio of the MAGI/CCR5 cells as a function of the electrical stimulation loading time, when alternating electrical potentials of rectangular pulsed, sine and triangular waves at 100 Hz of 0 ~ +1.0 V were applied. The results provided us with two pieces of information. First, the cell membrane damage as evaluated by the staining ratio, increased drastically at *ca*. 12 min when the alternating electrical potential was loaded by rectangular pulse waves, which was *ca*. 4 min later in contrast to the 10 Hz alternating electrical potential. This behavior is more typical in the case of sine and triangular waves loading. The cell membrane damage increased at 15 ~ 20 min. Second, a waveform dependence for the cell membrane damage was observed. The cell membrane damage was increased drastically when the rectangular pulse waves were loaded. In contrast, the membrane damage was gradually increased in the case of sine and triangular waves loading.

The upper and lower potential dependence of the cell membrane damage was also investigated. Fig. 5 shows the staining ratio of the MAGI/CCR5 cells as a function of the electrical stimulation loading time, when alternating electrical potentials of rectangular pulses, sine and triangular waves at 100 Hz of +0.8 ~ +0.9 and -0.5 ~ +0.5 V were applied. In the case of the +0.8 ~ +0.9 V application, the cell damage increased drastically at *ca*. 4 min. This behavior did not depend on the wave form, and was about half magnitude as compared with alternating electrical potentials of 0 ~ +1.0 V at 10 Hz. In contrast, cell damage was not observed at a potential loading for 60 min, when -0.5 ~ +0.5 V was applied (Fig. 5). To investigate whether cell membrane damage was induced by a threshold potential, alternating electrical potentials of 100 Hz rectangular pulse waves with -0.2 ~ +0.8, -0.15 ~ +0.85 and -0.1 ~ +0.9 were applied. After electrical stimulation for 30 min, the cell membrane damage at -0.2 ~ +0.8, -0.15 ~ +0.85 and -0.1 ~ +0.9 were evaluated to be 0, *ca*. 30 and *ca*. 80 %. In the case of -0.2 ~ +0.8 V stimulation, no membrane damage was observed, even if the electrical stimulation loading lasted for 60 min. These results indicate that cell membrane damage occurred at a positive potential of more than +0.8 V under the present experimental conditions.

In conclusion, cell membrane damage was induced at positive potentials more than +0.8 V under the present experimental conditions. The alternating potential waveform dependence induced by the frequency on membrane damage of cells cultured on an electrode surface was discovered, for the first time. The cell membrane damage was promoted by a rectangular pulse wave in comparison with sine and triangular waves, when alternating electrical potentials of 0 ~ +1.0 V at 100 Hz were loaded. In contrast, this wave form dependence was not observed when the frequency was 10 Hz. The obtained results represent basic knowledge which may be useful for not only basic researchers, but also cell engineers. We believed that alternative electrical stimulation will be a powerful disease therapy in the near future.

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Figure Captions

Fig. 1 Schematic illustration of the alternative electrical stimulation system.

Fig. 2 Phase-contrast microscopic photographs of MAGI/CCR5 cells cultured on an ITO electrode before (a) and after (b) alternating electrical potential loading using rectangular pulse waves of 0 ~ +1.0 V at 10 Hz for 20 min.

Fig. 3 Staining ratio of MAGI/CCR5 cells as a function of the loading time using alternating electrical potentials with rectangular pulses, sine and triangular waves of 0 ~ +1.0 V at 10 Hz.

Fig. 4 Staining ratio of MAGI/CCR5 cells as a function of the loading time using alternating electrical potentials with rectangular pulses, sine and triangular waves of 0 ~ +1.0 V at 100 Hz.

Fig. 5 Staining ratio of MAGI/CCR5 cells as a function of the loading time using alternating electrical potentials with rectangular pulses, sine and triangular waves of +0.8 ~ +0.9 and -0.5 ~ +0.5 V at 100 Hz

Figure(1-5)

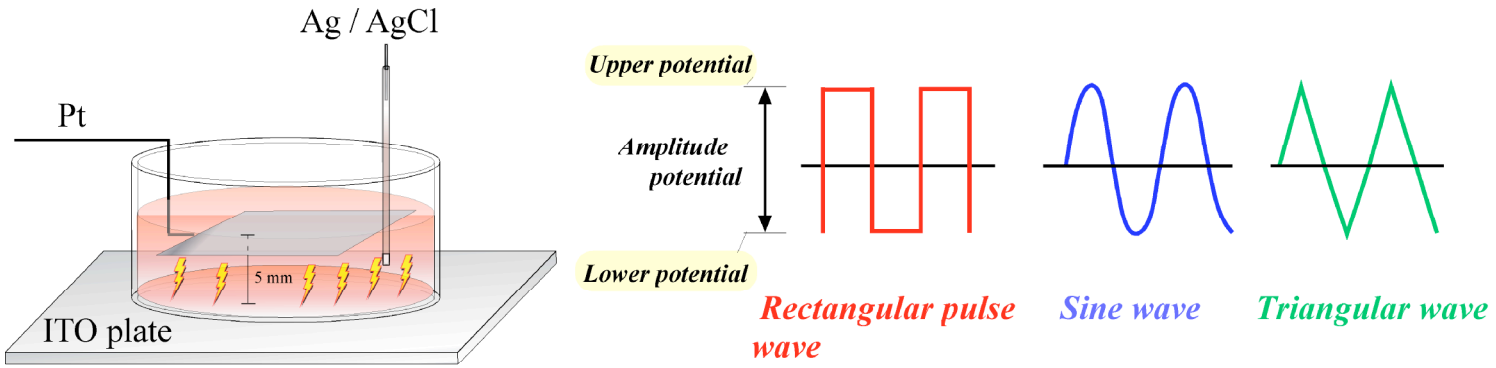


Fig. 1

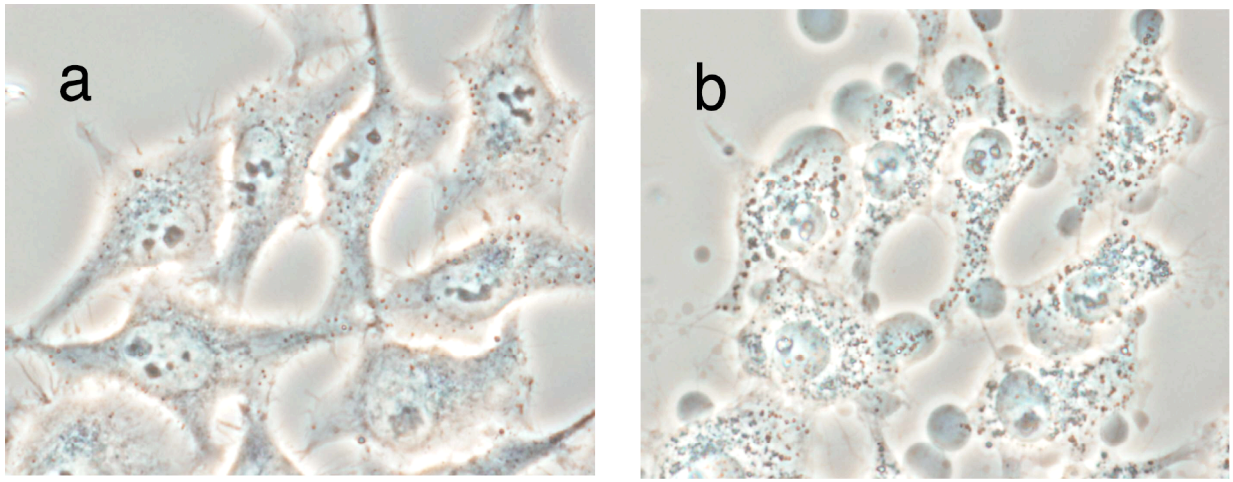
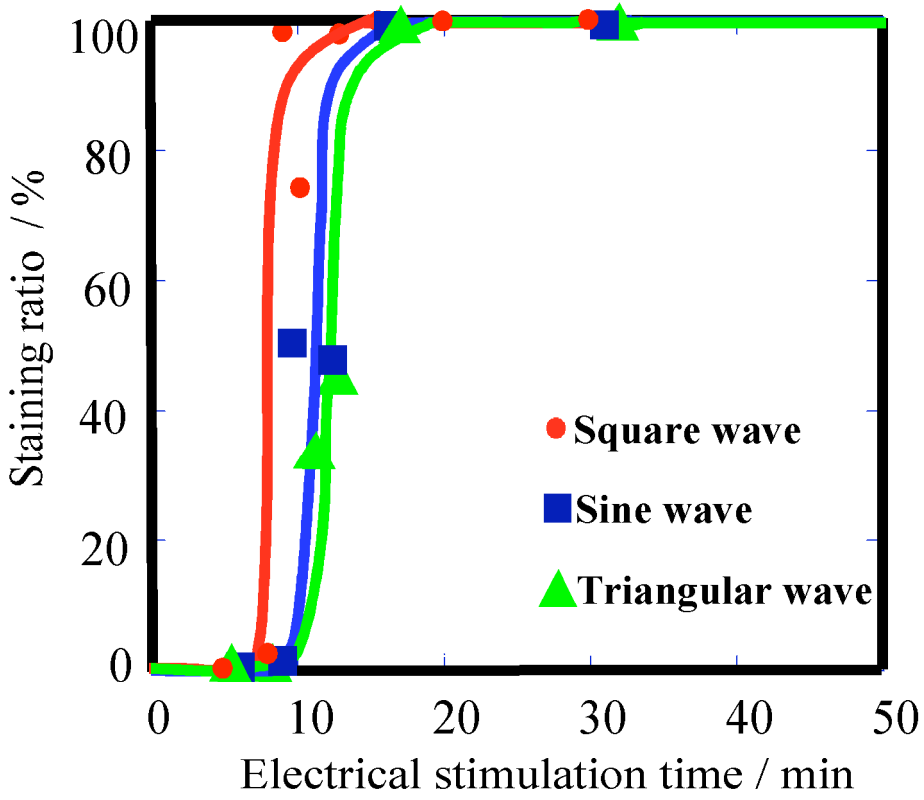


Fig.2


100
80
60
40
20
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10
20
30
40
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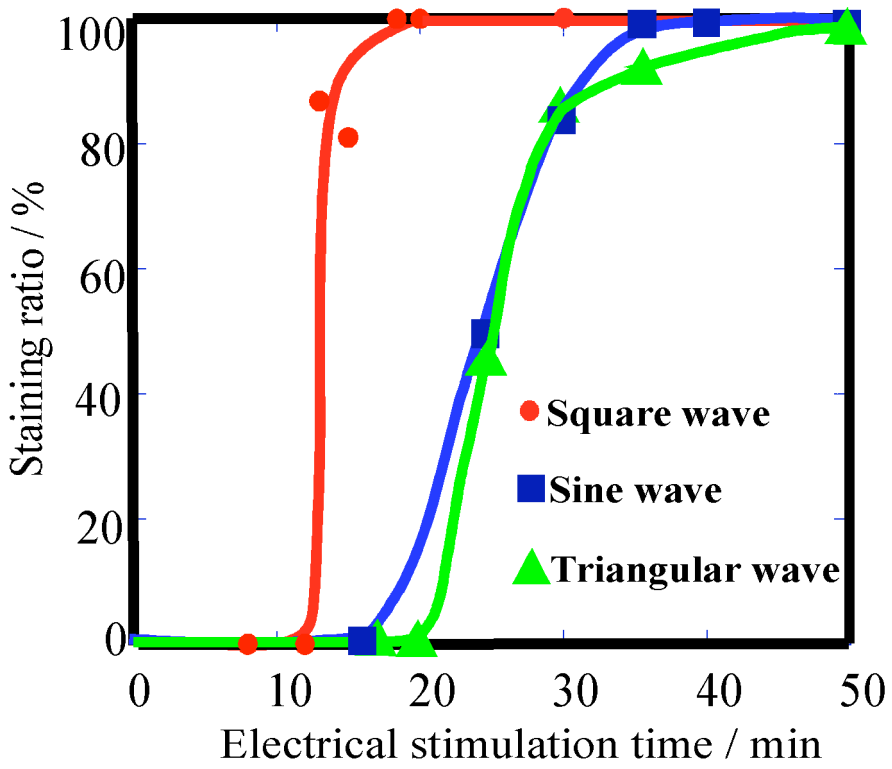
Electrical stimulation time / min
Square wave
Sine wave
Triangular wave

Fig. 3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60
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30
40
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Electrical stimulation time / min
Square wave
Sine wave
Triangular wave

Fig. 4

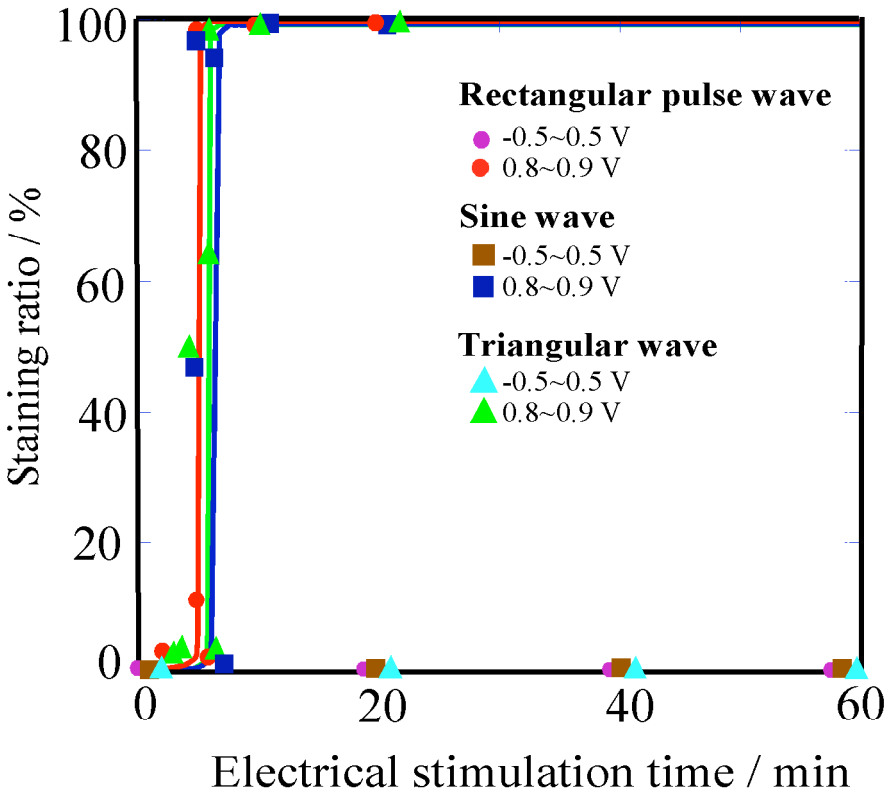
100
80
60
40
20
0
Staining ratio / %
0
20
40
60
Electrical stimulation time / min
Rectangular pulse wave
-0.5~0.5 V
0.8~0.9 V
Sine wave
-0.5~0.5 V
0.8~0.9 V
Triangular wave
-0.5~0.5 V
0.8~0.9 V

Fig. 5