Title	Response of biological cell exposed on burst RF fields
Author(s)	Nomura, Naoyuki; Koga, Y.; Abe, S.; Uchida, Ichiro; Abe, K.; Katsuki, Sunao; Namihira, Takao; Akiyama, Hidenori; Takano, H.; Abe, Shin-Ichi; Kohno, S.
Citation	Digest of Technical Papers-IEEE International Pulsed Power Conference, 2005: 1429-1432
Issue date	2005-06
Type	Conference Paper
URL	http://hdl.handle.net/2298/9720
Right	(c)2005 IEEE. Personal use of this material is permitted. However, permission to reprint/republish t…

RESPONSE OF BIOLOGICAL CELL EXPOSED ON BURST RF FIELDS*

N. Nomura[ξ], Y. Koga, S. Abe, I. Uchida[†], K. Abe[†],
S. Katsuki, T. Namihira, H. Akiyama, H. Takano[†], S. -I Abe[†], S. Kohno[††]
Department of Electrical and Computer Engineering, Kumamoto University
Kurokami 2-39-1, Kumamoto 860-8555, Japan
[†] *Department of Biological Science, Kumamoto University*
Kurokami 2-39-1, Kumamoto 860-8555, Japan
[††] *Department of Electrical Engineering, Ariake National College of Technology*
Higashihagio-Machi 50, Omuta, Fukuoka 836-8585, Japan

Abstract

Burst RF fields (BRFFs) were applied to Chinese Hamster Ovary (CHO) cells, and the cell response to the BRFF of two different frequencies, 20 kHz and 50 MHz, was investigated by means of the fluorescent microscopy. The field strength and duration of the BRFF were fixed at 1 kV/cm, and 100 μs, respectively. Cells were placed in a 100 μm-gap electrode plated on the slide glass for a microscopy. Acridine orange (AO), which mainly reacts to DNA, was used as the fluorescent dye. From the experiment, the BRFF of 20 kHz initiates the increase in the permeability of cell membrane. In contrast, the BRFF of 50 MHz does not act on cell membrane but causes degeneration of DNA or RNA. We have experimentally demonstrated that the burst RF fields with only a small electric field of 1 kV/cm cause not only membrane reaction but also intracellular effects.

I. INTRODUCTION

Intracellular effects on biological cell exposed on intense nanosecond pulsed electric fields (nsPEFs) have been demonstrated by Schoenbach and Beebe [1-5]. The intracellular effects are explained as high frequency components of the nsPEF penetrate into the cell and directly stimulate DNA and/or intracellular organelles. From physics point of view, biological effects should depend on frequency, field strength and energy of electric fields. As far as the frequency is concerned, different frequency might act on each specific part of the cell because each sub-cellular component has its peculiar electrical relaxation time. Since frequencies of rectangular PEF are widely distributed, many of sub-cellular components could be stimulated simultaneously, which makes the physics unclear. For the scientific investigation of frequency effect on the biological system or the selective stimulation of the sub-cellular components, the narrowband electric field is desirable.

Here, we propose the use of burst radiofrequency fields (BRFFs), as shown in Fig. 1, instead of PEFs. The advantages of the BRFFs are; (1) narrow frequency band, (2) close relation in electric field strength between the time domain and the frequency domain, and (3) physical parameters, such as, frequency, electric field strength, and deposition energy can be varied independently. The energy is adjusted by changing the burst duration without change in frequency and field strength.

Figure 2 shows the example waveforms of PEF and BRFF together with their frequency spectra. The upper part of Fig. 2(a) shows a rectangular PEF of 60 ns pulse width, and the lower part is its frequency spectrum, which is wideband. The fundamental frequency of the rectangular PEF is dependent on the pulse width and the high frequency components are associated with the rising and falling parts. Also the rectangular pulse has very low frequency components to DC. In contrast, the 50 MHz BRFF's frequency spectrum as shown in the lower part of Fig. 2(b) is narrow band.

This paper describes cell responses triggered by exposing BRFF of different frequencies, 20 k, and 50 MHz. The electric field strength and the burst duration were fixed at 1.0 kV/cm and 100 μs, respectively, in this experiment. Cell response was morphologically investigated using a fluorescent microscopy.

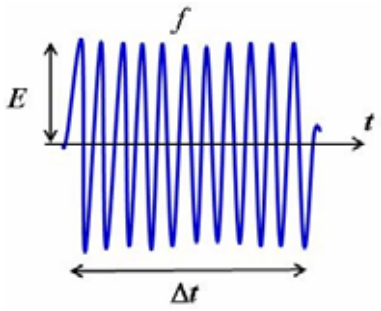
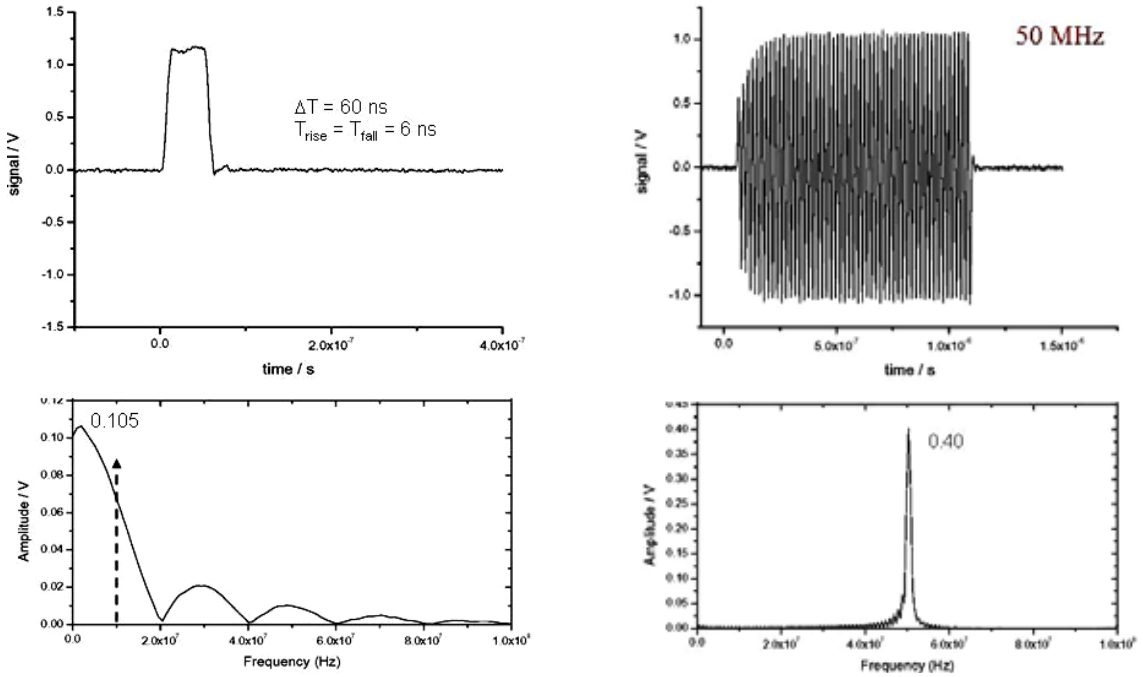


Figure 1. The burst radiofrequency electric field.

* Work supported in part by the 21 century COE program promoted by MEXT, Japan.
ξ email: nomura@st.eecs.kumamoto-u.ac.jp



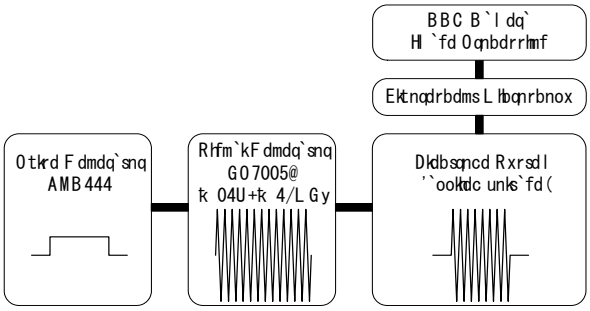
(a) Pulsed electric field and its spectrum (60 ns, 1 V) (b) Burst AC field and its spectrum (50 MHz, 1V)

Figure 2. Comparison between pulsed electric field and the burst AC field for their frequency spectra.

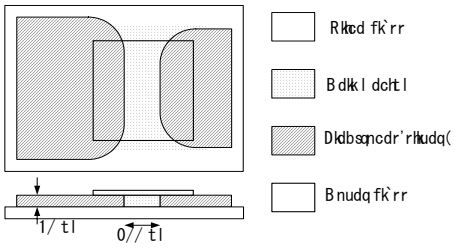
II. MATERIAL AND METHOD

A. Electrical setup

Figure 3 shows the BRFF application system. Sinusoidal wave of a certain frequency is generated by a signal generator (HP8116A, V_{MAX} = 15 V, f_{MAX} = 50 MHz), and the BRFF duration is controlled by the gate pulse from a pulse generator (BNC555). 20 μm thick silver electrodes are metal-plated on the slide glass for a microscope. The electrode gap is 100 μm, which enables to produce the electric field strength of up to 1.5 kV/cm. The electric fields used in this experiment are two-order smaller than that used in the rectangular PEF experiment. The estimated temperature increase during the BRFF shot is 0.3°C. Morphological investigations of cells were conducted by using a fluorescent microscope (Nikon ECLIPSE E600).



(a) BRFF application system



(b) On-slide electrode for microscopy

Figure 3. Experimental setup.

B. Biological

All experiments were conducted using Chinese Hamster Ovary (CHO) cells, cultured with αMEM medium in a 5% CO_2 incubator. Cells were incubated routinely every 2-3 days before the cell culturing condition becomes 90% confluent. The cultured cells were washed by TBS twice, and re-suspended to 1×10^6 cell/ml with αMEM medium. Acridine Orange (AO) is a fluorescent nucleic acid dye that is permeable to living cells. It interacts with double strand DNA (dsDNA) and RNA, which is similar structure to dsDNA and is connected to the base pairs, and fluoresces in green. AO also reacts to RNA and single strand DNA (ssDNA), which fluoresces in red. The intensity of green fluoresces are dependent on the amount of AO bound to nucleic acid, providing quantitative information of nucleic acid. The experimental procedure is as follows:

(1) After pouring AO to the electrode gap, the medium of 10 μl is poured onto the electrode gap. A cover slip is placed on the medium so that the media is diffused evenly.

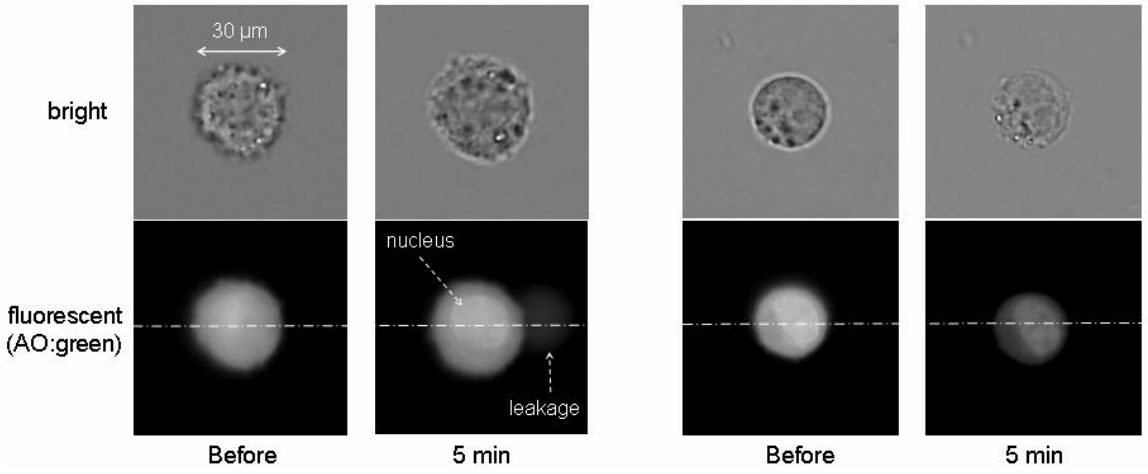
(2) The slide is placed on the holder set fixed to the microscope stage. The electrodes are connected to the BRFF system via a coaxial cable.
(3) BRFF exposure in optional parameter.
(4) Snap shot observation of the cell at 1, 2, 3, 5 and 10 minutes after the exposure.

III. EXPERIMENTAL RESULT AND DISCUSSION

Cells were exposed on the BRFF 3 minutes after they were set to the microscopy. Both bright field and fluorescent images were taken at seven phases. One was taken before the exposure and the other six were after the exposure, each on 1, 2, 3, 5, 7, and 10 minutes. Between the bright sight image and fluorescent image, there is time lag of 30 seconds because of handling the microscope.

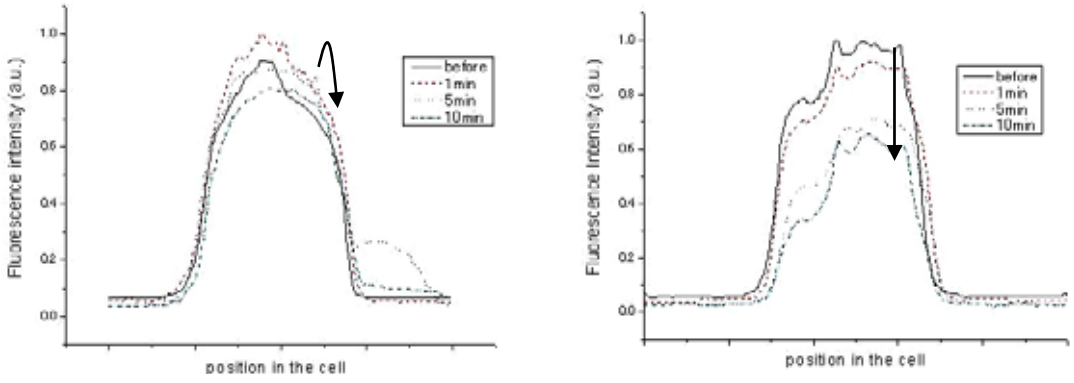
Figure 4 shows the bright field and fluorescent images of CHO cells before and 5 minutes after the BRFF exposure. Figs. 4 (a) and (b) represents the results of 20 kHz and 50 MHz, respectively. DNAs are mostly localized in nucleus, but there exist RNA in the cytoplasm, which has a similar structure to DNA. Therefore, fluorescent comes not only from nucleus but also cytoplasm. In the case of 20 kHz as shown in Fig. 4 (a), no obvious change by the BRFF exposure was observed. However, the fluorescent image at 5 minutes shows a clearer appearance of the nucleus and a leakage of intracellular contents out of the cell. These phenomena strongly suggest that the 20 kHz BRFF might increase the permeability of the plasma membrane. Density of fluorescent molecules in the cell might be decreased due to their leakage out of the cell. In contrast, in the case of 50 MHz, although no change is seen in the bright sight, the dramatic decrease in the intensity was observed in whole region of the cell in the fluorescent image.

Figure 5 shows the temporal variation of the fluorescent intensity along the line in Figs. 4 (a) and (b). The intensity was normalized by the highest part in the nucleus. The temporal variations between 20 kHz and 50 MHz are quite different. A slight increase in the intensity is observed 1 minute after the 20 kHz BRFF exposure. And then the intensity is gradually decreased afterwards. In the case of 50 MHz, the intensity in whole region in the cell is decreased rapidly. The control cell, a sample



(a) 20 kHz, 1kV/cm (b) 50 MHz, 1kV/cm

Figure 4. Microscopic images of CHO cells before and after the exposure of BRFF.



(a) 20 kHz, 1kV/cm (b) 50 MHz, 1kV/cm

Figure 5. Temporal variation of florescence intensity along the line in Fig. 4.

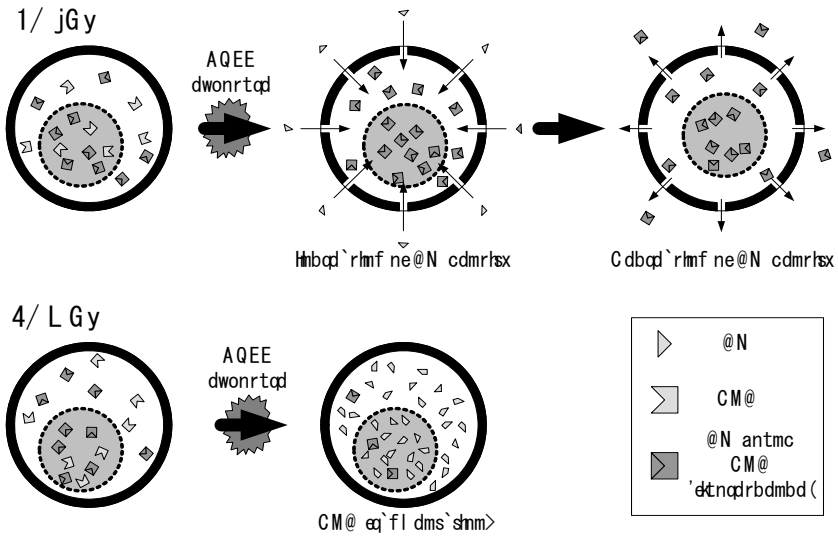


Figure 6. Simple picture of definition in explaining the changes in fluorescence intensity.

without the exposure, did not show any notable changes in terms of their appearance and fluorescent intensity, during observation.

Figure 6 shows the schematic illustration of our understanding of the experimental results. The upper part shows what happens in the case of 20 kHz. The momentary increase in the fluorescent intensity due to the exposure of 20 kHz BRFF could be explained as the increase in the density of AO molecules flowing into the cell through the membrane, of which the permeability is increased by the PRFF exposure. And the subsequent decrease in the intensity could be explained by the leakage of the intracellular contents. This increase in the permeability of the cell membrane by the 20 kHz BRFF is explained as electroporation. On the other hand, the rapid decrease in the fluorescent intensity by the 50 MHz BRFF exposure could be explained as the DNA itself has suffered a damage, which results in the break of the bonding between AO and DNA, or the fragmentation of DNA. The result of 50 MHz BRFF exposure does not seems to cause a significant damage to the membrane.

The frequency of 20 kHz is much slower than the time constant of charging cell membrane, which results in the pore formation on the membrane. On the other hand, 50 MHz, which is faster than the charging time constant, the membrane is not charged up to severe voltage to form pores in the membrane. The BRFF of 50 MHz might resonate large molecules floating everywhere in the cell, which is possible to result in their fragmentation or degeneration.

IV. SUMMARY

We have experimentally demonstrated that the burst RF fields with only a small electric field of 1 kV/cm cause not only membrane reaction but also intracellular effects. Responses of CHO cells to the Burst RF Fields of two different frequencies were shown here. The experiment indicates that the BRFFs of 20 kHz and 50 MHz act on cell membrane and the intracellular molecules, respectively. Further investigation using biochemical analysis would provide us more detailed reactions in biological cells.

V. ACKNOWLEDGEMENT

This is a part of the works of 21st century COE program conducted at Kumamoto University.

VI. REFERENCES

[1] N. Chen, K.H. Schoenbach, J.F. Kolb, R.J. Swanson, A.L. Garner, J. Yang, R.P. Joshi, S.J. Beebe, Biochemical and Biophysical Research Communications, Vol. 317, pp. 421-427 (2004)

[2] K.H. Schoenbach, S.J. Beebe, and E.S. Buescher, Bioelectromagnetics, Vol. 22, pp. 440-448 (2001)

[3] M. Stacey, J. Stickley, P. Fox, V. Statler, K. Schoenbach, S.J. Beebe, S. Buescher, Mutation Research, Vol. 542, pp.65-75 (2003)

[4] S.J. Beebe, P.M. Fox, L.J. Rec, K. Somers, R.H. Stark, K.H. Schoenbach, IEEE Trans. Plasma Sci., Vol. 30, pp.286-292 (2002)

[5] E.S Buescher, K.H. Schoenbach, IEEE Trans. Dielectr. Electr. Insulat., Vol. 10, pp.788-794 (2003)