課題番号	:F-13-HK-0070
利用形態	:機器利用
利用課題名(日本語)	:
Program Title (English)	: Electric Field Effects on Intracellular Function
利用者名(日本語)	:
Username (English)	: <u>Kamlesh Awasthi</u>
所属名(日本語)	:
Affiliation (English)	:Laboraotry of Molecular Photonics, RIES, Hokkaido University.

## <u>1. 概要(Summary)</u>

An electrode microchamber has been constructed for applying the nanosecond pulsed strong electric fields to living cells. The morphological changes in cells have been observed after the application of electric field having pulsed width 50 ns and strength of 4 MV m<sup>-1</sup>, indicating that apoptosis, which is a programed cell death, was induced by nanosecond pulsed electric fields.

## <u>2. 実験(Experimental)</u>

We have constructed an electrode microchamber by a photolithography method for observing cell response to intense nanosecond voltage pulses and measured the images both of the fluorescence intensity and of the fluorescence lifetime of HeLa cells expressing enhanced green fluorescence protein (EGFP). Nanosecond pulsed electric fields were generated in the electrode microchamber by a pulse generator.

#### 3. 結果と考察(Results and Discussion)

It was seen that application of 50 ns pulsed electric field induced a change in cell morphology, including cell membrane blebbing. The fluorescence lifetime of a HeLa cell expressing EGFP was also reduced by the application of 50 ns pulsed electric fields. The observed cell membrane blebbing is related to the cell death through apoptosis, indicating that the nanosecond pulsed electric fields induce apoptosis cell death. The loss of plasma membrane asymmetry is one of the key features of the early stage of apoptosis, and the redistribution of phosphatidylserin (PS) to the outer layer of plasma membrane is detected through apoptosis with annexin V, that has strong affinity for PS, in the presence 50 ns pulsed field.

It was also noticed that the magnitudes of the field-induced changes both in morphology and the lifetime depend on the size and the physical conditions of the cells and their alignment between the electrodes even with the same strengths of the applied electric field.

# <u>4. その他・特記事項(Others)</u>

"Control of intracellular function by nanosecond pulse electrical field: Observation of apoptosis induction is successful using fluorescence lifetime imaging" is issued through press release and introduced in Hokkaido Medical Newspapers dated 19 October 2012.

### <u>5. 論文·学会発表(Publication/Presentation)</u>

 Awasthi, K. Invited Lecture in Asian International Symposium of Chemical Society of Japan, 2013, 3A1-48.

# 6. 関連特許(Patent)

None